Campylobacter Jejuni and Its Antimicrobial Susceptibility Pattern Among Under- Five Children with Gastroenteritis in Northwest Ethiopia

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Abstract

Introduction: *Campylobacter jejuni* is the leading causes of diarrheal disease worldwide among under five years of age. Epidemiological data about *C. jejuni* and drug susceptibility profile of isolates among children with symptom of gastroenteritis has a paramount importance for evidence based decision. However, such information is not available in the study area. Hence, this study was carried out fill this information gap.

Methods: An institution based cross-sectional study was conducted among under-five children (U5C) visiting four primary health centers' pediatric clinic due to acute gastroenteritis from February to May, 2019. Socio-demographic data were collected in a face to face interview using structured questionnaire. Stool specimen was collected and transported to the Bahir Dar University, Medical Microbiology Laboratory. The specimens were inoculated on Charcoal Cefoperazone Deoxycholate Agar. Identification and antibacterial susceptibility test was performed using standard bacteriological methods. The collected data were entered to EpiData 3.1 and analyzed using SPSS version 23.

Results: Among a total of 196 U5C with acute gastroenteritis, 39 (19.9%) were positive for *Campylobacter* species. The proportion of *C. jejuni* was 14.8% (29/196). The multivariate logistic regression analyses revealed that care givers (AOR=3.76, 95%CI: 1.29-10.98), guardians hand washing practice before food preparation (AOR=3.63, 95%CI: 1.15-11.46), and contact with domestic animals (AOR=4.23, 95%CI: 1.46-12.28) demonstrated significant association with *C. jejuni* infection. Among 29 *C. jejuni* isolates, 24 (82.8%), 24 (82.8%) and 18 (62.1%) were resistant for tetracycline, cefoxitine and erythromycin, respectively.

Conclusions: The proportion of *C. jejuni* infection was high. Children who had contact with animals showed association with *C. jejuni* infection. Additionally, majority of *C. jejuni* showed resistance for tetracycline, cefoxitin, ampicillin and erythromycin.

Introduction

Under-five mortality rate is a key indicator countries health system strength and quality [1]. The world has made astonishing strides in decreasing child mortality over the past two decades. For instance, the under-5 mortality (U5M) rate slash from 76 deaths per 1,000 live births in 2000 to 39 in 2018. Sub-Saharan Africa is region with the highest U5M rate [2]. In Ethiopia, between 1990 and 2013, the U5M rate dropped from 203.9 deaths/1000 live births to 74.4 deaths/1000 live births with a total reduction of 64% [3].

Diarrhea is defined as the passage of three and more loose stools per day or more frequent passage than is normal for the individual. Diarrheal disease which is both preventable and treatable is the second leading cause of death in under five years of age. Globally, there are nearly 1.7 billion cases of childhood diarrheal disease and 525 000 deaths among under-five children (U5C) annually [4]. Diarrhea is a leading cause of malnutrition in U5C [4]. Lower respiratory infections, diarrheal diseases, and neonatal syndromes remain the major causes of U5M in Ethiopia [3]. As the COVID-19 epidemic continues to erode

the health systems and disrupt routine services, several thousands of additional U5M are expected following the pandemic [2].

Campylobacter is one of the leading food borne zoonotic causes of diarrheal diseases in Children [5, 6]. *Campylobacter* are small fastidious gram negative curved, rode shaped bacteria. It was isolated by Sebald and Veron in 1963. So far the genus has 31species. However, only 17 of the species are considered as public health and veterinary important [7, 8]. Among the species, *C. jejuni* is the most common causes of gastroenteritis worldwide [9]. *Campylobacter* inhabited in the gastrointestinal tract of animals, birds and their fecal excreta. A gram of bird fecal excreta contain 10^9colony forming unit (CFU) of *Campylobacter*, more than the infective dose; 500 – 10,000CFU [9]. *C. jejuni* can be transmitted through eating of contaminated poultry meat, water, unpasteurized milk [10, 11], contact with domestic animals (cattle, hen, dog and cat) and bottle feeding [12, 13]. It was shown that, Campylobacter infection is mainly associated with children, occupation, immunosuppression (AIDS, cancer, and diabetes mellitus) and frequent use of anti-acids [14, 15]. The pathogenesis mechanism of *C. jejuni* is not fully understood. However, it was known to colonize the distal ileum, jejunum and colon of human intestine. The most commonly recognized virulence mechanisms were motility, toxin production and invasion [16]. *C. jejuni* infection is symptomatically characterized as gastroenteritis, meningitis, endocarditis, septic arthritis, and osteomyelitis. It is also is associated autoimmune disease known as Guillain-Barré syndrome (GBS) and Miller Fisher syndrome, which are an acute demyelinating disease of peripheral nervous system [17, 18].

The prevalence of *C. jejuni* infection among U5C vary geographically. For instance, *the prevalence of C. jejuni was* 4%, 7.7%, 8.4%, 8.9%, 11.9%, 12.9% and 14% in the study from India, Brazil, Poland, Jimma Ethiopia, Kenya, and Gondar Ethiopia, respectively [13, 19–24].

Antibacterial treatment is necessary for severe campylobacteriosis like infections especially in immunosuppressed patients and long-lasting infections. Currently, macrolide groups such as erythromycin and azithromycin are the antibacterial agents of choice for Campylobacteriosis. Moreover, Tetracycline and fluoroquinolones (ciprofloxacin) have also been suggested as alternative treatment [25, 26]. However, globally multidrug resistance is becoming an increasing public health concern [27, 28]. Added to this, the practice of unsupervised veterinary drugs administration to domestic animals is common which has the potential for increasing drug resistance development for veterinary and medical important pathogens [29, 30]. Hence, periodic surveillance and antibiotic stewardship program is required [31, 32]. Based on point prevalence study in Ethiopia, drug resistance rate of *C. jejuni* was 80% for amoxicillin and 55% for erythromycin. Multiple drug resistance was identified in 95% of the *Campylobacter* isolates [33]. In the study from Jimma Southern Ethiopia, the rate of ampicillin and trimethoprim-sulfamethoxazole drug resistance were gauged at 76.3% and 68.4%, respectively [23]. Multiple drug resistance was observed among 78.9% of isolates [23]. This study determined the resistance rate of *C. jejuni* isolates against common antibiotics recommended for Enterobacteriaceae and found resistance rate of 76.3% for ampicillin, 68.4% for trimethoprimsulfamethoxazole, 39.5% for tetracycline, 31.6% for chloramphenicol, 26.3% for clindamycin and 23.7% for doxycycline [23]. Based on

a similar study from Gondar, the Ampicillin resistance rate was reached to 68.2% and the resistance rate of tetracycline and trimethoprim-sulfamethoxazole was 56.8% and 54.5%, correspondingly [13].

Based the study from Ethiopia, exposure to domestic animals, latrine usage, water source, bottle feeding and nutritional status showed significant association with *C. jejuni* infection [13]. From a study in Brazil, significant association was reported between Lower nutritional status and *C. jejuni* infection [20]. Epidemiological information about the prevalence, drug resistance and factors associated with *C. jejuni* infection in U5C is none in one of the most populous city of Ethiopia, Bahir Dar and its surrounding suburban area. Hence, the aim of this study was to fill this information gab.

Methods

Study design, period and setting

Institution based cross-sectional study was conducted in selected four health centers in Bahir Dar city administration, Northwest Ethiopia. The study was conducted from February to May, 2019. The four selected health centers include: Shimibit, Han, Menilik II and Shumabo. These health centers provide basic primary health care services such as outpatient department, emergency, family planning, extended program of immunization, antenatal care, postnatal care, delivery, TB and HIV diagnosis, treatment and support.

The health centers are found in urban slum area where most of the communities lead their life through livestock agriculture. Their main source of income in these slum area is the animal products including milk, chicken, egg and cow dung which can be used for fuel and building material. Majority of the city animal products (Milk, egg) are come from these slum agriculture business. The areas are places for which open field animal slaughtering activities are performed. Moreover, the waste disposal system is very poor which pose major health risk for both animal and villagers. The practice of unsupervised veterinary drugs administration to domestic animals is common in the study area. Such practice has the potential for fueling drug resistance in veterinary and medical important pathogens.

Population and Eligibility

Under-five children with acute gastroenteritis were the study population. Children who took antibacterial agents within the last two weeks were excluded. Moreover, children who were on treatment at the time of data collection were excluded.

Variables

While the dependent variable is the *C. jejuni* infection, the independent variables are age, sex, occupation of care giver and care givers (mother, father, other), residency (urban, rural), health center, hand washing practice, latrine utilization, agents used for hand washing, practice of bottle feeding, nutritional status,

contact with domestic animals and chief clinical symptoms (abdominal pain, fever, vomiting, and type, frequency and duration of diarrhea).

Sample Size and Sampling Technique

The sample size was calculated based on single population proportion formula by taking a 15% prevalence report among U5C in Gondar, Ethiopia [13]. Margin of error was set to be 5% and 95% confidence interval (alpha = 0.05).

$$n = \frac{Z^2pq}{W^2}$$

Where: n = Sample size; p = prevalence of *C. jejuni* in U5C, 15%; q = 1-p = 0.85; z = critical value 1.96, W = precision (margin of error) = 0.05

Thus, setting these value in the formula, the sample size became n=

$$\frac{(1.96)^2 \times 0.15 \times 0.85}{(0.05)^2} = \frac{0.4896}{0.0025} = 195.84 = 196$$

Participants were enrolled consecutively until the required sample size was achieved.

Data collection

A structured Amharic version questionnaire was used to collect data about the demographic characteristics of children and the guardian. Data were collected through face to face interview of the guardian. The weight and height of the children were measured by nurses who administered the questionnaire.

Laboratory procedures

Stool sample collection and processing

Approximately 2grams of stool sample was collected from each child using leak proof and clean stool screw cup with sterile applicator stick. Each sample was labeled with unique code taken from the questionnaire and transported immediately to Bahir Dar University of College of Medicine and Health Sciences Medical Microbiology Laboratory using Cary Blair transport media. The specimen was inoculated on blood free Campylobacter Agar Base, called Charcoal, Cefoperazone, Sodium Deoxycholate agar (CCDA) media (Oxoid,England) [34]. This is a medium in which blood was replaced by charcoal, ferrous sulphate and sodium pyruvate. The peptic digest of animal tissue, beef extract and casein enzymic hydrolysate provides organic nitrogen to the organisms. Sodium chloride maintains the osmotic balance. Sodium deoxycholate inhibits the growth of most gram-positive microorganisms. The selectivity of the media was achieved by using cefoperazone and then through incubation at 42^{o}c [34].

The inoculated media were kept in a 2.5 liter anaerobic jar with Campy-Gen gas generating kit which has microaerophilic atmospheric condition equivalent to 5% O2 and 10% CO2, 85% N_2) (Oxoid CN0025A),

which was inserted to maintain the microaerophilic condition. The jars were incubated at 42°C and the bacterial growth was examined after 24 hrs, then at 48 hrs finally at 72 hrs of incubation (Sigma-Aldrich, 1995). The experiment has been carried out using standard operating procedure [34].

Identification of C. jejuni

C. jejuni was identified using its unique microbiological characteristics such as (1) colonial morphology (moist, grey and flat-spreading, metal sheet like-spreading), (2) Gram's staining result (gram negative curved, rode shaped bacteria), and (3) biochemical tests (oxidase positive, catalase positive and hippurate hydrolysis test positive). Hippurate hydrolysis test used to differentiate *C. jejuni* from other Campylobacter species mainly from *C. coli)* [34].

Antimicrobial susceptibility testing

Antibacterial susceptibility test for *C. jejuni* was performed using disc diffusion technique using Muller Hinton Agar supplemented with 5% sheep blood. The disc diffusion test is a screening test used for epidemiological information. The commonly prescribed antimicrobials were selected and screened as per the recommendations of the Clinical Laboratory Standards Institutions (CLSI) [35]. These antibiotics include ampicillin (Amp:30 µg), amoxicillin with clavulanic acid (AMC: 30 µg), tetracycline (TTC: 30 µg), chloramphenicol (CAF: 30 µg), ciprofloxacin (CIP: 5 µg), cefoxitin (FOX: 30 µg), erythromycin (ERT:15 µg), clindamycin (CLN: 15 µg), trimethoprim- sulfamethoxazole (SXT: 25 µg), tombramycin (TOB: 10ug), cefepime (CFPM:10ug), nalidixic acid (NAL:30 µg) and cephalotin (CEX: 30 µg). Three to four morphologically identical colonies of the bacteria from culture were picked and suspended in sterile normal saline. After 30 minutes, turbidity of the broth culture adjusted to 0.5 McFarland turbidity standards. A loop full of the bacterial suspension was placed at the center of Muller Hinton agar media (Oxoid, LTD) supplemented with 5% sheep blood and evenly spread using sterile cotton tipped applicator stick. After drying, antibiotic discs was placed on the media using clipper and incubated at 42°C for 24 hrs in anaerobic jar using CO2 generating kits. Finally, the diameter of the zone of inhibition around the disks was measured to the nearest millimeter using a ruler and classified as sensitive (S) and resistance (R) [35, 36].

Quality assurance

To ensure the quality and reproducibility of results, all required quality assurance activities have been performed. Briefly, nurses and laboratory professionals who carried out the data collection were trained. The questionnaire was pretested and validated. Standard procedure was followed on the stool sample collection and transportation. In the sterilization process, Geobacillus strearothermophilus (ATCC7953) was integrated to evaluate the effectiveness of the autoclave. Additionally, the sterility of the sterilized media were checked by incubating 5% of the batch at 37^{o}c for 24 hrs and if there were any contamination from the incubated media, the whole batch were discarded. Bio safety class II was used in the dispensation of CCDA, and MHA supplemented with 5% sheep blood, and inoculation of these Medias. The performance of the media and antibiotic disks were probed by inoculating control strains of *C. jejuni* (ATCC33560) and *E. coli* (ATCC 25922) as positive and negative controls respectively. All batch media

that passed the quality check (CCDA, and MHA supplemented with 5% sheep blood) were stored at 4^{o}c and other biochemical tests (1% sodium hippurate and 3.5% ninhydrin solution) was stored at -15^{o}c.

Statistical analysis

The data was checked for its completeness and entered to EpiData version 3.1 and transferred to SPSS version 23. The base line characteristics of the study population were summarized using frequencies, mean and range. To assess the presence of correlation among independent variables, multicollinearity analyses was executed using linear regression analyses. Then, bivariate logistic regression was carried out and variables with a p-value of less than 0.2 were entered in to multivariate logistic regression analysis. A P-value of < 0.05 was considered as statistically significant.

Ethical consideration

The study was approved by Institutional Review Board of Bahir Dar University and a letter which stated the approval of the protocol was written to Amhara regional state research directorate for further support. Then, Amhara regional state research directorate wrote an official letter to Bahir Dar City zonal health departments and the zonal health department wrote another letter to the respective health centers for possible support of the data collection in the selected health centers. After briefing the letter and the study objectives and the data collection procedure, the recruited data collectors (Nurses). Written informed consent was obtained a parent or legal guardian. The purpose and importance of the study was explained to the parents/guardians. Additionally, absence of link between the study and medical service was explained. Participation was entirely voluntary based. The confidentiality of study participants were kept and identification codes was deidentified during analyses. In general, this study was conducted in accordance with the Declaration of Helsinki.

Results

Demographic characteristics of study participants

A total of 196 U5C with diarrhea were included in this study. Of these, 99 (50.5%) were males. The age ranges from 3 months to 5 years and with mean age of 2.07 years. Majority of the participants were from Han Health Center (35.2%) followed by Shimbit (24.5%). Most (36.7%) of the children were in the age range of one to two years. More than half of the guardians of the children were housewives (56.1%). Higher numbers of the participants were urban resident (84.7%) (Table 1).

Table 1
The demographic characteristics of under-five diarrheic children (n = 196) in Bahir Dar city, Northwest Ethiopia, from February to May 2019

Variables	Frequency, N	Percent, %
Sex		
Male	99	50.5
Female	97	49.5
Age in year		
< 1	46	23.5
1–5	150	76.5
Occupation of the caregiver		
Housewives	110	56.1
Farmer	21	10.7
Merchant	18	9.2
Private	25	12.8
Government	13	6.6
Other	9	4.6
Residency		
Urban	166	84.7
Rural	30	15.3
Health center		
Shumabo	33	16.8
Han	69	35.2
Menilik II	46	23.5
Shimibit	48	24.5
Total	196	100

Prevalence of Campylobacter jejuni

Among the 196 children with diarrhea, 39 (19.9%) were culture positive for *Campylobacter species*. The overall prevalence of *C. jejuni* was 29 (14.8%) and other species accounted 5.1%. Proportionally, *C. jejuni* infection was higher in male 17(58.6%) than female 12(41.4%). Similarly, age wise classification showed that, the rate of *C. jejuni* infection was higher in the age range of less than one years (41.4%) than over one years of age. However, none of the variable demonstrated a significant differences (Fig. 1).

Factors associated with Campylobacter jejuni infection

Before analyzing the link between dependent and independent variables, the presence of any positive or negative correlation among independents variables were assessed using linear regression model. Fortunately, the tolerance results were between 0.85 and 0.9, variance inflation factor were between 1 and 1.5) and collinearity diagnostic were < 0.5; confirming apparent absence of multicollinearity among independent variables. Then to identify possible factors associated with *C. jejuni* infection, bivariate and multivariate logistic regressions were performed (Table 2). From all possible factors which were considered; types of guardian (AOR: 3.76, 95%CI: 1.29–10.98), absence of hand washing practice before food preparation (AOR: 3.63, 95%CI: 1.15–11.46, normal body mass index (BMI) (AOR: 0.18; 95%CI: 0.04–0.88, and contact with hen and cow domestic animals (AOR: 4.23; 95%CI: 1.46–12.28 showed significant association. Children who were getting care by neither their mother nor their father were 3.76 times more likely to be infected with *C. jejuni* than those who got care by mothers. Children whose caregivers did not wash their hand before food preparation were 3.63 times more likely to be positive for *C. jejuni* infection than those who wash their hand before food preparation. Body mass index (BMI) of the children were calculated according to Quetelet' index classification [37]. *C. jejuni* culture positivity rate was also associated with nutritional status of the study participants. Based on Quetelet' index classification, children who had normal weight were 0.18 times less likely to be positive for *C. jejuni* than those who were underweight. Having domestic animals in children house were 4.23 times more likely to be positive for *C. jejuni* than those did not had (Table 2).

Table 2
Logistic regression analyses for identifying factors association with *C. jejuni* infection in U5C in Bahir Dar, Northwest Ethiopia, from February to May 2019

Variables	Positive N (%)	Negative N (%)	COR (95%CI)	AOR (95%CI) P-value
Caregiver for children				
Mother	12 (10.3)	104 (89.7)	1	1
Father	4 (20)	16 (80)	2.17 (0.62,7.55)	1.48 (0.33,6.58) 0.61
Other	13 (21.7)	47 (78.3)	2.40 (1.02,5.65)	3.76 (1.29,10.98) **0.02**
Hand washing before food preparation				
No	24 (22.4)	83 (77.6)	4.11(1.50,11.28)	3.63 (1.15,11.46) **0.03**
Yes	5 (5.6)	84 (94.4)	1	1
Hand washing before feeding				
No	10 (28.6)	25 (71.4)	2.99 (1.25,7.18)	1.12 (0.337, 3.734) 0.852
Yes	19 (11.8)	142 (88.2)	1	1
Children latrine utilization				
No	23 (15.3)	127 (84.7)	1.21 (0.46,3.17)	0.87 (0.23,3.33) 0.84
Yes	6 (13)	40 (87)	1	1
Cleaning agent for utensils				
Soap with water	20 (12.6)	139 (87.4)	1	1
Only water	9 (24.3)	28 (75.7)	2.23 (0.92,5.41)	2.07 (0.38,11.11) 0.40
Bottle feeding				
No	22 (15.7)	118 (84.3)	1	1
Yes	7 (12.5)	49 (87.5)	0.77 (0.31,1.91)	1.11(0.27,4.49) 0.89
Nutritional status #				
Underweight	4 (22)	14 (77.8)	1	1
Normal	14 (9)	141 (91)	0.35 (0.10,1.20)	0.18 (0.04,0.88) **0.03**
Risk for Overweight	6 (40)	9 (60)	2.33 (0.51,10.64)	1.22 (0.18, 8.55) 0.84

U5C: Under five children, **COR**: Crude odd ratio, **AOR**: Adjusted odd ratio, **BMI**: Body mass index,
#Underweight < 5th percentile include < 10.3BMI, and normal weight in the range of 5th -85th percentile include 10.3–16.4 BMI, risk for overweight in the range of 85th -95th include 16.40–19.5 BMI and overweight > 95th percentile include > 19.50

Variables	Positive N (%)	Negative N (%)	COR (95%CI)	AOR (95%CI) P-value
Overweight	5 (62.5)	3 (37.5)	5.83 (0.95,35.72)	4.27 (0.41,9.89) 0.22
Contact to domestic animal				
No	10 (9.6)	94 (90.4)	1	1
Cat and dog	4 (10)	36 (90)	1.04 (0.31, 3.54)	0.85 (0.21,3.49) 0.83
Hen and cattle	15 (28.8)	37 (71.2)	3.81 (1.57,9.24)	4.23 (1.46,12.28) **0.008**

U5C: Under five children, **COR**: Crude odd ratio, **AOR**: Adjusted odd ratio, **BMI**: Body mass index, #Underweight < 5th percentile include < 10.3BMI, and normal weight in the range of 5th -85th percentile include 10.3–16.4 BMI, risk for overweight in the range of 85th -95th include 16.40–19.5 BMI and overweight > 95th percentile include > 19.50

The link between *C. jejuni* infection and chief clinical symptoms such as abdominal pain, fever, vomiting and type, frequency and duration of diarrhea were assessed. However, none these variables demonstrated a significant association (data not shown).

Antimicrobial susceptibility profile of Campylobacter jejuni

Antimicrobial susceptibility test was performed for commonly prescribed drugs. As such, C. *jejuni* showed high rate of resistance to tetracycline (82.8%), cefoxitin (82.8%), ampicillin (65.5%) and erythromycin (62.1%). On the contrary, relatively low rate of resistance were observed to ciprofloxacin (31%), Nalidixic acid (34.5%), tobramycin (41.4%) and trimethoprim sulfamethoxazole (44.8%) (Fig. 2). Among the 29 *C. jejuni* isolates, multidrug resistance (an isolate being resistant to three or more class of antibiotics) were detected in 23 (79.3%) of the strains. Among these 15 (51.7%) were resistant to four or more classes of antibiotics.

Discussion

This study addressed three key objectives; determined the point prevalence, assessed the drug resistance profile of isolates and identified factors associated with *C. jejuni* infection. The overall prevalence of *C. jejuni* was calculated at 14.8%. This finding was comparable with other studies reported in Jimma Ethiopia [23] and Gondar Ethiopia [13], kenya [24] with the isolation rates of 11.9% and 14%, 12.9% respectively. However, the present study showed higher prevalence compared to other African studies conducted in Sudan, 2% [38], Burkina Faso, 1.1% [39] and Zambia,3.5% [40]. Additionally, the present study was higher compared with reports from Asia such as from study in India [41], Iran, Asia [42] and Iran [43] which have an isolation rate of 3.8%, 4.1%and 4.5%,respectively. The discrepancy might related with socio economic, the degree contact with animals and animal products, the sample collection, transportation, culturing conditions and the laboratory methods used. For instance, our study used

Preston blood free agar, which has higher sensitivity compared to other [44]. Again, this study demonstrated a lower point prevalence when compared with a study from Pakistan;54.6% [45]. Broadly speaking, Ethiopia is among the top ten countries having the highest number of deaths due to diarrheal diseases among U5C in the world [46]. Community-based cross-sectional study was conducted in Sidama, southern Ethiopia using a total of 537 U5C. The 2 weeks prevalence of diarrhea among U5C was 13.6, 95% CI (10.7, 16.5%)[47]. Another community-based cross-sectional was conducted in Debre Berhan town between 13 and 18 April 2018. The two week prevalence of diarrhea among U5C was gauged at 16.4% (69/351) [48]. Based a systematic review of 31 studies, the pooled prevalence of diarrhea among U5C in Ethiopia was 22% (95%CI: 19, 25%) [49]. The prevalence of diarrheal disease among U5C in Arba Minch Southern Ethiopia and Jabithennan North West Ethiopia showed 30.5% and 21.6% [50, 51], respectively.

In the present study, children who obtain nursery by people other than their mother or father showed significant association for *C. jejuni* culture positivity rate. This might be related with responsibility, knowledge and skill about child care and hygienic practice at the time of food preparation. Hand washing practice before food preparation was showed a significant reduction in *C. jejuni* culture positivity rate [52].

Malnutrition had a significant association with C. *jejuni* culture positivity rate. This result is consistent with previous reports done in Jimma, Ethiopia [12], and Gondar, Ethiopia [13]. This could be due to low immune status of malnourished children. Based on a systematic review of 31 studies, lack of maternal education, lack of availability of latrine, urban residency, and maternal hand washing practices were significantly associated with childhood diarrhea [49]. Based on 2016 Ethiopian demographic health survey (EDHS) data, a total of 10,641 children from 18,008 households were included in a study by Zewudie et al (2016). The under-five child mortality (U5M) was significantly linked with rural residency, none breastfeeding practices, having multiple birth, male gender, having first birth order and having family size six and above [1].

High culture positivity rate was also observed from children who have close contact with domestic animal (hen and cattle). This finding was in line with previous reports done in Jimma, Ethiopia [12, 23], and Gondar, Ethiopia [13]. This is expected because of the fact that, *C. jejuni* is a zoonotic bacteria. According to Abamecha *et al* report[10], *C. jejuni was* isolated from domestic animal feces such as cattle (75.3%), chicken (86.9%), sheep (84.6%) and goat (100%) done in Gambella, Northwest Ethiopia. In Sub-Saharan Africa 31% of children health impact is due to exposure to animal feces and *C. jejuni* was the common pathogen identified from children lived in close proximity with domestic animals [53].

Although molecular and culturing techniques are the recommended diagnostic methods[54] for *C. jejuni* infection, sign-symptoms such as duration of the diarrhea (one to five days), abdominal pain and vomiting can be an indicative for *C. jejuni* infection. However, these sign and symptoms had no significant association in the present study.

In this study, higher sensitivity rate were observed for ciprofloxacin (69%) and nalidixic acid (65.5%) and this finding is in line with similar study done elsewhere in Ethiopia [13] and beyond [55, 56]. Highest

sensitivity rate of nalidixic acid (100%) were reported in Jimma, Ethiopia [12], Sudan [38] and Zambia[40] compared to the present study. The resistance rate of tetracycline (82.8%), cefoxitin (82.8%) and erythromycin (62.1%) were higher in this study compared to results done in Gondar Ethiopia[13] and Jimma, Ethiopia [23]. The resistance rate of ampicillin (65.5%) was in lined with a study done in Gondar, Ethiopia [13] and Jimma, Ethiopia [23].Taken together, the susceptibility profile *C. jejuni* isolates of this study in line with other studies. However, the slight discrepancy could be related with patients and antibiotic stewardship related factors. It must be noted that, disk diffusion technique is a screening test and any resistance report require minimum inhibitory concentration (MIC) test for accurate categorization [35]. The Erythromycin susceptibility report can be used to infer the azithromycin and clarithromycin resistance profile and the quinolone resistance is most reliably detected with nalidixic acid disks [36]. High rate of multidrug resistance was noticed among isolates of this study. The emergency of multidrug resistant pathogens across bacteria, fungi, viruses and parasites is on increasing at a terrifying rate. Despite, drug resistance is a natural evolutionary process, malpractices such as the inappropriate use of antimicrobial drugs, inadequate sanitary conditions, inappropriate food-handling, and poor infection prevention and control practices considered as the driving force for emergence of super bugs [57].

Strength and limitation of the study

This is the first report in the slum area of Bahir Dar City administration focusing on U5C. It assessed several factors and antibiotics. However, this study is not without limitations. The study is conducted on government health facility in which mainly visited by and served for economically disadvantaged community. As such, the finding might not fully inferred to all Bahir Dar City; rather, best inferred for suburban areas of Bahir Dar. The other limitations include the small sample size, failure to recheck the disk diffusion based resistance report with MIC.

Conclusion

Diarrheal disease is leading cause of U5M and mortality. *C. jejuni* continue to be one of the predominant zoonotic bacterial pathogens of U5C. In the present study, higher rate of *C. jejuni* infection was isolated in U5C and more specifically among age groups of one to two years. Types of guardians, hand washing practice, malnutrition and contact with domestic animal showed significant association with *C. jejuni* infection. The most prevalent symptoms of Campylobacteriosis were diarrhea, abdominal pain, and vomiting. Ciprofloxacin, nalidixic acid, and tomboramycin were considered as an effective drugs against *C. jejuni* isolates found in Bahir Dar. On the contrary *C. jejuni* developed high degree of resistance towards tetracycline, ceftriaxone, ampicillin and erythromycin.

The health system actors should concert in tandem with veterinary health officials through one health approach so as break the spill over transmission pathogens from animal to human and the vice versa. While we recommend further study to clearly determine the drug sensitivity profile and antibiotics choices; Ciprofloxacin, nalidixic acid, tobramycin might be considered as first line treatment of choice to the test of

time. Moreover, serotyping and molecular studies should be done to identify the dominant strains responsible for infection and drug resistance.

Abbreviations

AIDS
Acquired Immune Deficiency Syndrome, **ATCC**:American Type Culture Collection, **CDC**:Center for Disease Control, **CFU**:Colony Forming Unit, **CLIS**:Clinical Laboratory International Standard, **EDHS**:Ethiopia Demographic Health Survey, **GBD**:Global Burden of Disease, **GBS**:Guillain-Barré syndrome, **MIC**:minimum inhibitory concentration, **PCR**:Polymerase Chain Reaction, **SOPs**:Standard Operational Procedures, **U5C**:Under five Children, **U5M**:under-5 mortality **WHO**:World Health Organization

Declarations

Methods

We confirm that all methods were performed in accordance with the relevant guidelines and regulations

Ethics Approval and Consent to Participate

The study was approved by Institutional Review Board (IRB) of Bahir Dar University College of Medicine and Health Sciences. Letter of support was obtained from Amhara Public Health Institute; Bahir Dar city Administrative Health bureau and each Health center administrators prior to data collection. The objective of the study was explained and /or clarified and written consent was obtained from the caregivers/guardians of study participants. The guardians were informed about their right to refuse or to withdraw from the study at any time during the study period. Information obtained at any course of the study was kept confidential. Confidentiality was maintained by numeric coding of questionnaires and specimens. Confirmed positive for *C. jejuni* were linked for treatment.

Consent for Publication

Not applicable.

Availability of Data and Materials

The original data for this study is available from the corresponding author.

Funding

Fund for data collection was obtained from Bahir Dar University. No fund was obtained for designing of the study, data analysis and manuscript writing.

Competing Interests

The authors declared that no competing interests exist.

Authors' Contribution

BW imagined and designed the project. BW performed the experiment. BW, DM and AM analyzed the data and wrote the manuscript. All authors have read and approved the manuscript.

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Figures

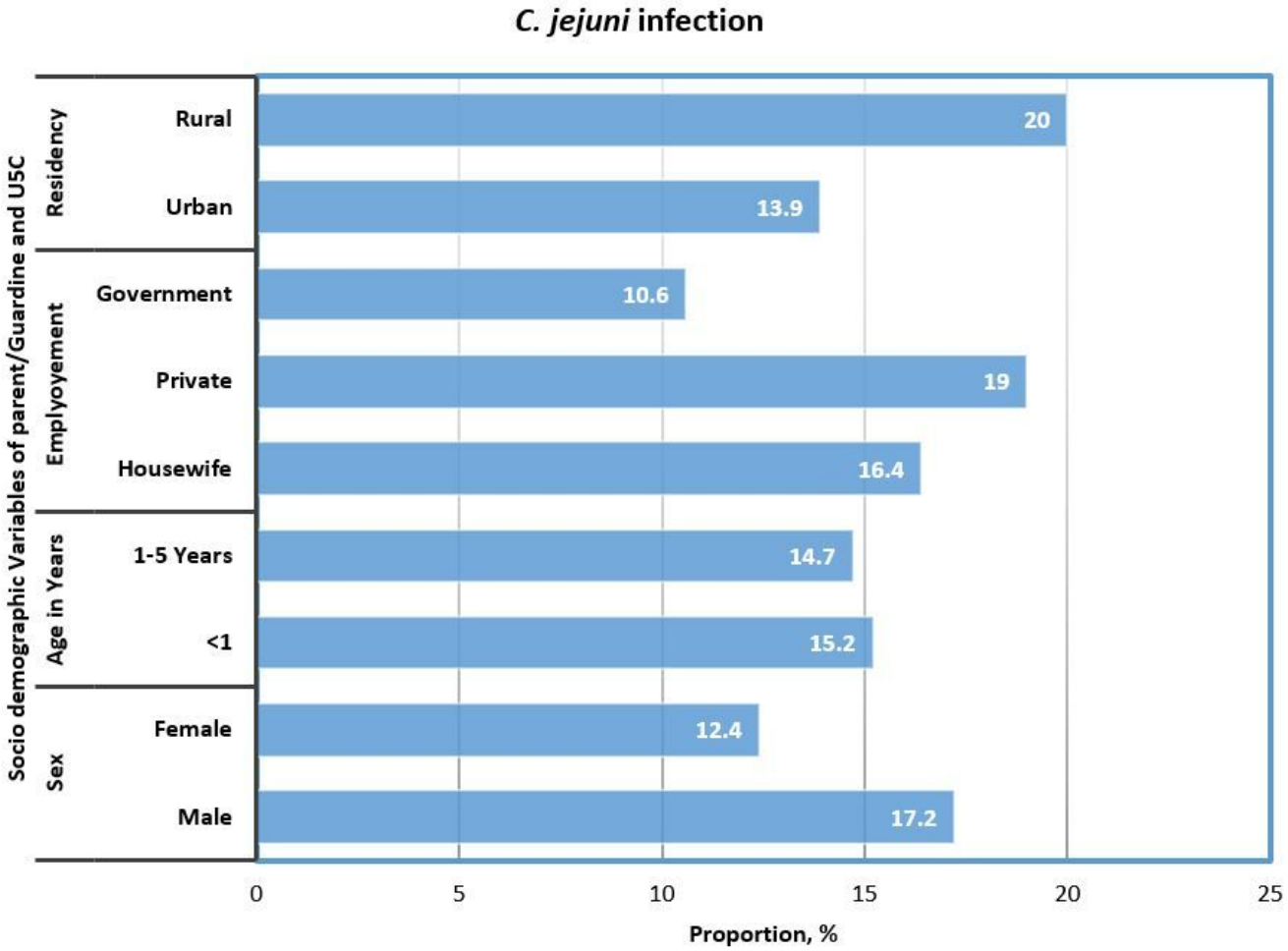

Figure 1

The distribution of C. jejuni infection in under-five children with diarrhea (n=29/196) in Bahir Dar city, Northwest Ethiopia, from February to May 2019

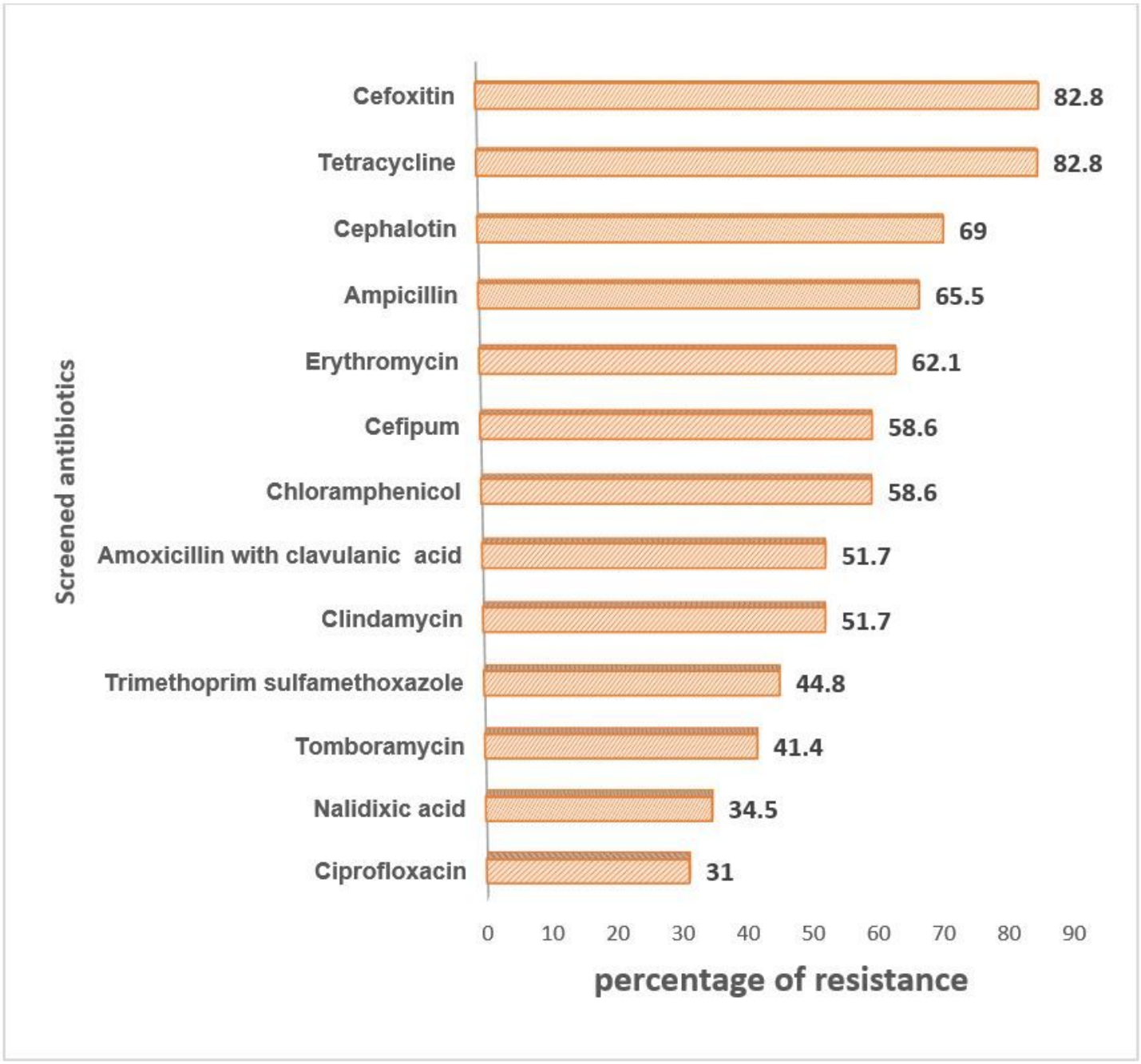

Figure 2

Drug susceptibility profile of C. jejuni isolated in Bahir Dar city, Northwest Ethiopia, from February to May 2019

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