THERMOTOLERANT *CAMPYLOBACTER* SPECIES AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ISOLATES AMONG UNDER-FIVE CHILDREN WITH DIARRHEA AT JIMMA MEDICAL CENTER, JIMMA, SOUTHWEST ETHIOPIA

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JIMMA, ETHIOPIA

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Abstract

Introduction: *Diarrheal diseases are the most common causes of morbidity and mortality among children. Campylobacter species are one of the causative agents of bacterial gastrointestinal infections. Thermotolerant Campylobacter species are responsible for most of Campylobacter gastroenteritis cases among under-five children.*

Objective: *To determine the prevalence, associated risk factors, and antibiotic susceptibility pattern of Campylobacter species among under-five children with diarrhea at Jimma Medical Center from January to April 2020.*

Methods: *A cross-sectional study design was conducted among under-five children with diarrhea. Stool samples were inoculated into Campylobacter agar medium and incubated at 42^{o}C for 48 hrs. Isolation and identification was done using standard bacteriological methods. Antibiotic susceptibility testing was done for isolated strains against selected antibiotics on Mueller-Hinton agar supplemented by 10% sheep blood using disk diffusion method. Associated risk factors were assessed using bivariate and multivariate logistic regression.*

Results: *A total of 214 under-five children with diarrhea were enrolled. The prevalence of thermotolerant Campylobacter species was 8.9%. All isolates were sensitive to gentamycin and azithromycin. Multivariable logistic regression analysis showed that under-five children whose mothers/caretakers do not wash their hand with soap and water before preparing food for a child [AOR: 3.7, 95% CI: (1.2-10.8)], whose family had domestic animals [AOR: 3.6, 95% CI: (1.0-12.7)] and those consumed raw dairy products [AOR: 4.5, 95% CI: (1.4-13.9)] had higher odds of infection with Campylobacter species.*

Conclusion: *Campylobacter species were one of the common bacterial pathogens causing diarrhea among under-five children. Azithromycin and gentamycin were the antibiotics to which all isolates were found sensitive. Consumption of raw dairy products, absence of maternal/caretakers' routine handwashing with soap and water before preparing child food, and the presence of domestic animals in the household were the associated risk factors. This indicates as hygiene and sanitary activities are important factors to be considered to reduce infection with Campylobacter species.*

Key Words: *Campylobacter species, Prevalence, Antibiotic susceptibility pattern, Associated risk factors*

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List of Abbreviations and Acronyms

ART	Anti-retroviral Therapy
AST	Antibiotic Susceptibility Testing
ATCC	American Type Culture Collection
CA	*Campylobacter* Agar
CDC	Center of Disease Control
CLSI	Clinical and Laboratory Standards Institute
DALYs	Disability Adjusted Life Years
EEA	European Economic Area
EU	European Union
FMOH	Federal Ministry of Health
HFA	Height-for-Age
HIV	Human Immune-deficiency Syndrome
JMC	Jimma Medical Center
WFA	Weight-for-Age
WFH	Weight-for-Height
WHO	World Health Organization

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1. INTRODUCTION

1.1. Background

Globally, under-five mortality has reduced from 51 deaths in 2010 to 39 deaths per 1000 live births in 2018. However, a significant number of under-five children are losing their life. In 2018, about 14,500 under-five children were dying every day. Sub-Saharan Africa was the region with the highest under-five mortality rate in the world with an average under-five mortality rate of 78 deaths per 1,000 live births in 2018 (1).

Diarrheal disease is the major cause of mortality and morbidity among under-five children worldwide. In 2016, 9% of all the global under-five mortality was attributed to diarrhea, of which 90% of deaths occurred in Low- and Middle-Income Countries (LMICs). An estimated 290,724 deaths in Sub-Saharan Africa were attributed to diarrhea (2).

Diarrhea can be caused by bacterial, viral, and parasitic pathogens. *Campylobacter* species are one of the common bacterial pathogens associated with diarrhea among under-five children along with Enterohemorrhagic *Escherichia coli* (EHEC), Enterotoxigenic *Escherichia coli* (ETEC), *Salmonella* species, *Shigella* species, *Vibrio* species and *Yersinia* species in different parts of the world (2,3).

Campylobacter species are gram-negative spiral, rod-shaped, or curved bacteria with a single polar flagellum or bipolar flagella depending on the species. They are non-spore forming with a size of approximately 0.2 to 0.8µm width and 0.5 to 5µm in length. Most species grow under micro-aerobic conditions of 5-10% O_2, 5-10% CO_2, and 85% N_2 and have a respiratory type of metabolism; do not ferment nor oxidize carbohydrates but they get energy from amino acids or tricarboxylic acid cycle. *Campylobacter jejuni* and *Campylobacter coli* are the two most common species isolated from humans, although other species like *C. fetus, C. laris, C. concisus, C. upsaliensis,* and *C. hyointestinalis,* are also responsible for human infection. They have a varied temperature requirement of 30-45^{o}C; the most common human pathogens are thermotolerant species and grow best at 42^{o}C (4,5).

Campylobacter species are part of the commensal flora of a wide range of animals including food animals like chickens, pigs, and ruminant animals like sheep, goat, and cattle (6,7). *Campylobacter* gastroenteritis is the most common medical problem caused by infection with

Campylobacter species all over the world. Furthermore, infection with species of *Campylobacter* may result in extra gastrointestinal manifestations like Guillain-Barre Syndrome (GBS), Miller Fisher Syndrome, bacteremia/septicemia, meningitis, and reactive arthritis (8). Due to their ability to colonize most domestic and wild animals, consumption of undercooked meat/poultry, unpasteurized dairy products, contaminated drinking water, and direct contact with farm animals are found to be risk factors for infection with *Campylobacter* species (9).

Infection with *Campylobacter* species is exerting a remarkable effect in both developed and developing countries (10). Worldwide, *Campylobacter* species are responsible for up to 400–500 million infection cases each year (11). In 2016, *Campylobacter* species were responsible for 75,135 global deaths among all ages (2).

The global burden of *Campylobacter* species is not confined only to its ability of infecting humans, rather the emergence of drug-resistant *Campylobacter* species also became the global concern (12).

1.2. Statement of the problem

In 2010, *Campylobacter* species were one of the common bacterial agents of diarrhea and were responsible for about 96 million illnesses, 21,374 deaths, and 2.1 million Disability Adjusted life Years (DALYs) globally. The African regions bore more than half (50.6% of the total DALYs per 100,000 population) of the global *Campylobacter* disease burden. The global morbidity rate of 2010 caused by *Campylobacter* infections was relatively higher as compared with *Shigella* species (1.2 million DALYs), *Vibrio cholera* (1.7 million DALYs), and Enterotoxigenic *Escherichia coli* (2.0 million DALYs) (13).

In 2016, *Campylobacter* species were responsible for about 40,854 deaths among under-five children, making one of the top four bacterial pathogens isolated among under-five children with diarrhea next to *Shigella* species, *Vibrio cholera and* non-typhoidal *Salmonella* species (2).

The burden of *Campylobacter* gastroenteritis is significant in both developed and developing countries. In 2017, about 250,161 confirmed cases were reported in 29 European Union (EU) and European Economic Area (EEA) countries with human campylobacteriosis were common among under-five children (14).

The assessment of the true incidence of *Campylobacter* gastroenteritis in developing countries is found to be difficult. As there are no surveillance systems and routine diagnostic mechanisms in line seen in many developed countries, it has been clear that the incidence of *Campylobacter* gastroenteritis has been underestimated in developing countries (10).

Furthermore, in different parts of the world, large numbers of *Campylobacter* strains are found to be resistant to different antibiotics including the antibiotic classes of fluoroquinolones and macrolides which are considered to be the drug of choice for the treatment of *Campylobacter* infection in immune-compromised individuals, co-morbidities or complicated *Campylobacter* infections (15,16).

Despite the absence of estimates on mortality and morbidity rates of *Campylobacter* infection in Ethiopia, different studies have been conducted in different parts of the country to elucidate that *Campylobacter* species are one of the bacterial pathogens causing gastroenteritis among under-five children (5,17).

The resistance patterns of *Campylobacter* species isolated from children with diarrhea in different parts of Ethiopia have also been identified. Accordingly, most of the isolated strains of *Campylobacter* species developed resistance to most common antibiotics (17–19).

In Ethiopia, the morbidity and mortality rates of *Campylobacter* infection among under-five children are under the shadow. This is due to certain reasons. One is inapplicability of microbiological techniques to diagnose *Campylobacter* species as a causative agent of diarrhea within health facilities. Most of the health facilities were not equipped by the materials required for isolation and identification. Furthermore, acute gastroenteritis is not commonly diagnosed for bacterial pathogens as most of them cause self-limiting diarrhea. In some instances antibiotics will be administered empirically.

On the other hand, the absence of a national surveillance program involving *Campylobacter* gastroenteritis and bacteriological characteristics of the organism (fastidiousness) can also be raised as reasons for absence of morbidity and mortality rates. So, these problems make the rates of *Campylobacter* infection remain hidden unless revealed by different studies. Therefore, the aim of this study was to contribute to the exploration of isolation rate, resistance pattern, and associated risk factors of *Campylobacter* species among under-five children with diarrhea at Jimma Medical Center (JMC).

2. LITERATURE REVIEW

2.1. Prevalence of infection with *Campylobacter* species

Globally, the incidence and prevalence of *Campylobacter* gastroenteritis have been increasing. In EU/EEA countries, the number of confirmed reported *Campylobacter* cases increased by more than 8% in the years 2010-2016 (20).

A study conducted in Poland among less than four years old children with diarrhea has shown 9.3% prevalence of *Campylobacter* species (21). Similarly, in the United Kingdom, *Campylobacter* was the most common bacterial pathogen with the prevalence ranging from 0.2-6% among under-five children with diarrhea (22).

Campylobacter species has a worldwide distribution. Studies conducted in developing countries among under-five children with diarrhea are supportive of this conclusion. The prevalence of *Campylobacter* infection among under-five children with diarrhea was 17.35% in India (23), 7% in Iran (24), 9.37% in Egypt (25).

Sub-Saharan Africa was the region with the highest prevalence of *Campylobacter* infection in 2018 (1). Studies in Sub-Saharan Africa reported a significant rate of *Campylobacter* infection among under-five children including 25% in South Africa (26), 16% in Kenya (27), 9.7% in Tanzania (28), 9.3 % in Uganda (29).

In Ethiopia, studies conducted in different regions revealed as *Campylobacter* species are also a common bacterial pathogen isolated among under-five children with diarrhea. The isolation rates of *Campylobacter* infection among under-five children were 15.4% in Gondar (18), 12.7% in Hawassa (17), and 16.7% in Jimma (5).

The laboratory diagnosis of acute diarrhea among under-five children is not routinely performed in health facilities. This study enables us to reveal the figure of *Campylobacter* gastroenteritis among under-five children.

2.2. Associated factors of *Campylobacter* infection

Campylobacter species are harbored by most domestic and wild animals, and several factors are associated with their transmission from animals to humans. Human *Campylobacter* infection may also be supported by host factors and characteristics of an exposed individual. Once an individual is exposed, there could also be factors encouraging the transmission of *Campylobacter* species between humans (11).

Different studies revealed the associated factors of *Campylobacter* infection among humans, particularly among under-five children. A study conducted in Sweden showed that consuming unpasteurized milk, chicken, or eating pork meats, living or working on a farm, daily contact with chickens or hens were factors associated with *Campylobacter* infection (30).

A study conducted in Norway has shown drinking untreated water was the leading risk factor of *Campylobacter* infection reported by 53% of the cases. Other factors like eating poultry, having occupational exposure to animals, and eating undercooked pork were also identified as risk factors (31).

In European countries, some studies witnessed that *Campylobacter* infection is higher in males than females. Infection with *Campylobacter* species also exhibits seasonality, with a seasonal peak incidence in mid-summer (July) and the lowest incidence between January to April (20,32).

A study conducted in California indicates that human *Campylobacter* infection was associated with the presence of small ruminant animals, dairy cows, and drinking of raw dairy products (33). A similar study conducted in Jordan also identifies that ownership of swine was associated with *Campylobacter* infection (34).

The association between *Campylobacter* infection and consumption of raw dairy products was also indicated by a study conducted in New Zealand (35).

A study conducted in 8 selected low-income countries, lower length-for-age Z-score at 24 months was found to be associated with a high burden of *Campylobacter*. But, exclusive breastfeeding, treatment of drinking water, access to an improved latrine, and recent macrolide antibiotic use were protective for *Campylobacter* infections (36).

In Ethiopia, some studies have identified the associated risk factor of *Campylobacter* infection among infants and under-five children. Accordingly, latrine usage, water source, boiling drinking

water, bottle feeding, nutritional status, and exposure to domestic animals were factors significantly associated with *Campylobacter* infection (18). Similarly, the presence of cattle and animal husbandry practices were also identified as associated risk factors (37).

2.3. Antibiotic susceptibility pattern of *Campylobacter* species

Although the outcome of infection with *Campylobacter* species is mostly self-limiting diarrhea, certain conditions like infections in immune-compromised individuals may require critical medical treatment with appropriate antibiotics. But, the emergence of antibiotic-resistant species of *Campylobacter* is creating a challenging future towards treating not only *Campylobacter* infection but also other bacterial infections. *Campylobacter* species are considered as a member of global priority pathogens group due to their pattern antibiotic resistance (38).

Studies conducted in different parts of the world have a common intersection of findings, concluding that most of the isolates of *Campylobacter* species are resistant to common antibiotics including antibiotics classes of fluoroquinolones and macrolides (39).

In China, a study aimed at identifying the antibiotic-resistance pattern of *Campylobacter* species revealed that about 97.4% of the isolates were resistant to ciprofloxacin. Similarly, about 99.3% were resistant to tetracycline, 80.1% were resistant to erythromycin, and 76.2% were resistant to gentamycin (40).

According to a study conducted in Pakistan, more than 80% of the isolated strains were resistant to seven antibiotics with the highest resistance to ampicillin, erythromycin, and streptomycin in which the rate of resistance was more than 90% (41).

Different studies conducted in different regions of the world also revealed the sensitivity rate of isolated species to gentamycin. A study conducted in Bangladesh indicates that all isolated strains of *Campylobacter* species were sensitive to gentamycin (42). Similarly, more than 91% of the isolates were sensitive to gentamycin according to a study conducted in Korea (43).

According to a study conducted in Macedonia, all isolated strains of *Campylobacter* species were sensitive to erythromycin (44). A study conducted in Korea also revealed that only 3% of the isolates were resistant to erythromycin and azithromycin (43). However, a high rate of resistance was also recorded against cotrimoxazole (91.3%), ampicillin (82.6), and ceftriaxone (65.22%) (44).

A study conducted in South Africa showed more than 60% of the *Campylobacter* species were resistant to ampicillin and amoxicillin/clavulanic acid. About 18% of the isolates were also resistant to fluoroquinolones (45).

The resistance pattern of *Campylobacter* species isolated from under-five children with diarrhea in Ethiopia has also been identified in some regions of the country. According to a study conducted in Hawassa, high rates of resistance of *Campylobacter* isolates were observed against amoxicillin (80%) and erythromycin (55%) (17). Furthermore, the highest drug resistance rates of *Campylobacter* species were against ampicillin, tetracycline, and trimethoprim-sulphamethoxazole with the resistant rates of 68.2%, 56.8%, and 54.5% respectively in Gondar (18), and 76.3%, 39.5%, 68.4% respectively in Jimma (5).

2.4. Conceptual framework

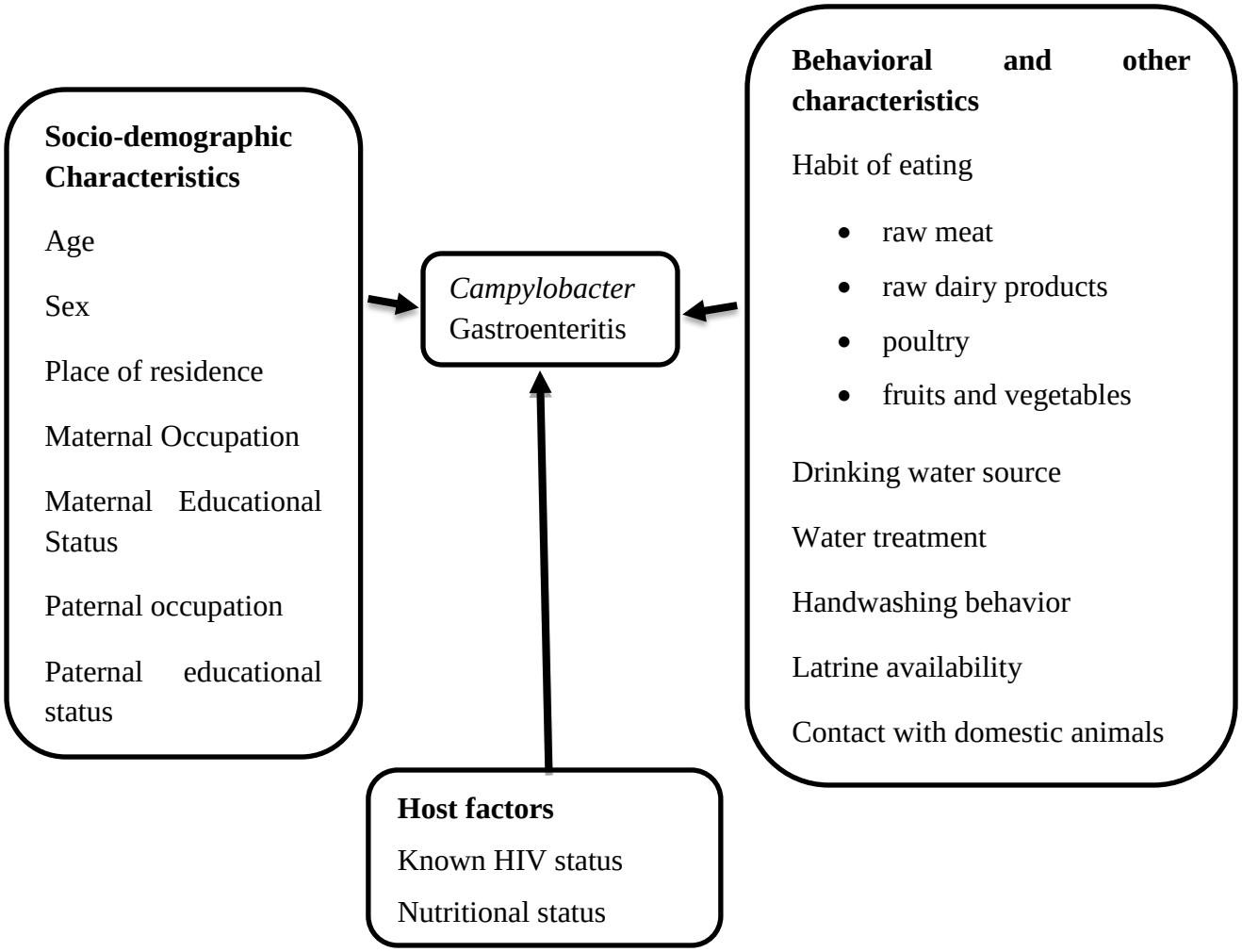

Figure 1: Schematic diagram illustrating the conceptual framework of *Campylobacter* infection and associated factors among under-five children with diarrhea at JMC.

2.5. Significance of the study

This study was aimed at elucidating the prevalence, associated risk factors, and antibiotic susceptibility testing of *Campylobacter* species among under-five children with diarrhea at JMC.

There are limited studies on *Campylobacter* infection among under-five children with diarrhea in Ethiopia. So, this study contributes to revealing the isolation rates of *Campylobacter* species to increase the recognition of *Campylobacter* infection among under-five children. This will give an update on *Campylobacter* gastroenteritis and help the concerned body to give credit, arrange a surveillance program and intervene in the problem to reduce the rate of infection, which has a role in dwarfing the medical problems and complications.

The rate of isolation will also help the medical personnel to consider the importance of culture-dependent diagnosis of gastroenteritis among under-five children with diarrhea.

The antimicrobial profile of isolates will also help in suggesting appropriate drugs for the appropriate treatment of the patient, which further reduces the likelihood of developing resistance.

Furthermore, the identified associated risk factors will also serve as a signal for the family of the study participants to be considered and reduce infection with *Campylobacter* species.

3. OBJECTIVES

3.1. General Objective

The aim of the study was to determine the prevalence, associated risk factors, and antibiotic susceptibility pattern of thermotolerant *Campylobacter* species isolated from under-five children with diarrhea at JMC, Jimma, and Southwest Ethiopia from January to April 2020.

3.2. Specific Objectives

- ✓ To determine the prevalence of thermotolerant *Campylobacter* species among under-five children with diarrhea.
- ✓ To identify the associated risk factors of thermotolerant *Campylobacter* gastroenteritis among under-five children.
- ✓ To detect the antibiotic susceptibility pattern of thermotolerant *Campylobacter* species isolated from under-five children with diarrhea.

4. MATERIALS AND METHODS

4.1. Study area and period

The study was conducted at Jimma Medical Center (JMC) from January to April 2020. Jimma Medical Center (JMC), one of the oldest public hospitals in Ethiopia, was established in 1937. It is one of the teaching and referral hospitals in the southwestern part of the country and serves a catchment population of 15-20 million. The hospital has 600 beds and about 1,620 staff, providing services for more than 218,000 outpatients, 16,000 inpatients, 14,200 emergencies, and 5,900 deliveries annually. The Pediatrics ward of the center has a bed capacity of 110, and admits about 2,030 children per year, and serves 4,680 children per year as an outpatient. The pediatrics clinic has 78 health professionals, of which 12 are pediatricians, 21 are residents and 45 are pediatric nurses. The Medical Laboratory department of JMC has 8 diagnostic areas, of which Medical Microbiology is one. This area is designed for microbiological diagnosis of infectious disease including bacterial culture.

4.2. Study design

A cross-sectional study design was conducted among under-five children with diarrhea at JMC.

4.3. Population

4.3.1. Source population

All under-five children presented with acute diarrhea at JMC pediatrics clinic.

4.3.2. Study population

All under-five children presented with acute diarrhea during the study period at JMC pediatrics clinic.

4.4. Sample size and sampling technique

Consecutive sampling technique was implemented and all under-five children presented with diarrhea during the study period were included. The sample size was determined by using single population proportion formula with the following parameters.

$$n = (Z_{\frac{\alpha}{2}})^2 (\frac{p(1-p)}{d^2})$$

$$n = (1.96)^2 (\frac{0.167(1-0.167)}{(0.05)^2})$$

$$n = 3.84(\frac{0.167(0.833)}{0.0025}) = 3.84(55.64) \approx \underline{214}$$

Where n = the minimum sample size

- P = the previous prevalence of *Campylobacter* species among under-five children at JMC which was 16.7% (5).
- d = Margin of error = 0.05
- $Z_{\alpha/2}$ = 95% confidence interval and 5% level of significance was used.

4.5. Eligibility criteria

4.5.1. Inclusion Criteria

All under-five children presented with acute diarrhea at JMC during the study period were included.

4.5.2. Exclusion Criteria

Under-five children with acute diarrhea whose families/caretakers refused to give an assent.

4.6. Study variables

4.6.1. Dependent variable

- Campylobacter gastroenteritis

4.6.2. Independent variables

I. Socio-demographic characteristics
   - Age of the child
   - Sex of the child
   - Maternal educational status
   - Maternal occupation
   - Paternal educational status

- Paternal occupation
- Place of residence of the child

II. Behavioral characteristics
- Child's habit of eating raw meat
- Child's habit of eating raw dairy products
- Child's habit of eating organic fruits and vegetables
- Drinking water source of the family
- Treatment of water
- Handwashing behavior of the child's mother/caregiver
- Mother's/caregiver's behavior of contact with domestic animals
- Latrine availability in the household
- Presence of domestic animals in the household

III. Host factors/child health-related factors
- Known HIV status of the child
- Nutritional status

4.7. Data collection procedures and sample processing

4.7.1. Socio-demographic and behavioral data

Semi-structured questionnaire was used to collect data concerning socio-demographic and behavioral characteristics of both families/caretakers and under-five children. Data collection procedure was conducted through face-to-face interviews by trained nurses.

4.7.2. Health-related data

Data on the HIV status of the children was taken from either patient record or by directly asking the families/caretakers if the study participant had previously been screened for HIV infection.

The nutritional status of the study participants was assessed using anthropometric measurements. The anthropometric measurements used were height, weight, and age of the child. The nutritional status of the child was presented as indices, including height-for-age (HFA), weight-for-age (WFA), and weight-for-height (WFH). Each index was recorded as a Z-score that describes how far and in what direction the child's anthropometric measurement deviates from the median. The Z-score value of HFA, WFA, and WFH was calculated using WHO-arthro software version 3.2.2. Accordingly, the Z-scores value of <-2 SD for HFA, WFA, and WFH was considered as

stunting, underweight, and wasting respectively. Z-score values between -2 SD and 2 SD were considered as normal, and Z-score values of > 2 SD for WFH were considered as overweight.

4.7.3. Sample collection

Freshly passed stool specimens were collected using a swab and placed in 6ml Cary-Blair transport medium (HKM, China) prepared in a tube. The samples were then transported to microbiology laboratory within 2 hours of collection and processed immediately.

4.7.4. Sample processing, isolation, and identification

4.7.4.1. Isolation and identification of Campylobacter species

Stool samples of study participants were directly inoculated into *Campylobacter* agar base media (HiMedia) supplemented with *Campylobacter* Supplement-I/Blazer-Wang (HiMedia) (having 1.250IU Polymyxin B, 5mg of Vancomycin, 2.500mg of Trimethoprim, 1mg of Amphotericin B and 7.500mg of Cephalothin) and 10% defibrinated sheep blood. An inoculated medium was incubated at 42◦C for 48 hours using a 3.5L anaerobic jar in a micro-aerobic atmosphere of approximately 5-10% O_2, 5-10% CO_2, and 85% N_2 produced using gas generating sachets, CampyGen (Oxoid Ltd, Basingstoke, Hampshire, England).

Grayish, flat and moistened colonies, with a tendency to spread, and having a metallic sheen on *Campylobacter* agar (CA) media were further examined. Saline wet mount was performed and microscopically examined for the characteristic darting/cork-screw motility. Gram staining was also performed and gram-negative rod-shaped, curved, or spiral bacteria having sea-gull wing appearance was considered. Catalase and oxidase-positive organisms were sub-cultured on blood agar and incubated at 42◦C for 48 hours. Colonies having characteristics of non-hemolytic, shiny, and colorless to grayish with irregular or round-edged nature were considered as *Campylobacter* species (46).

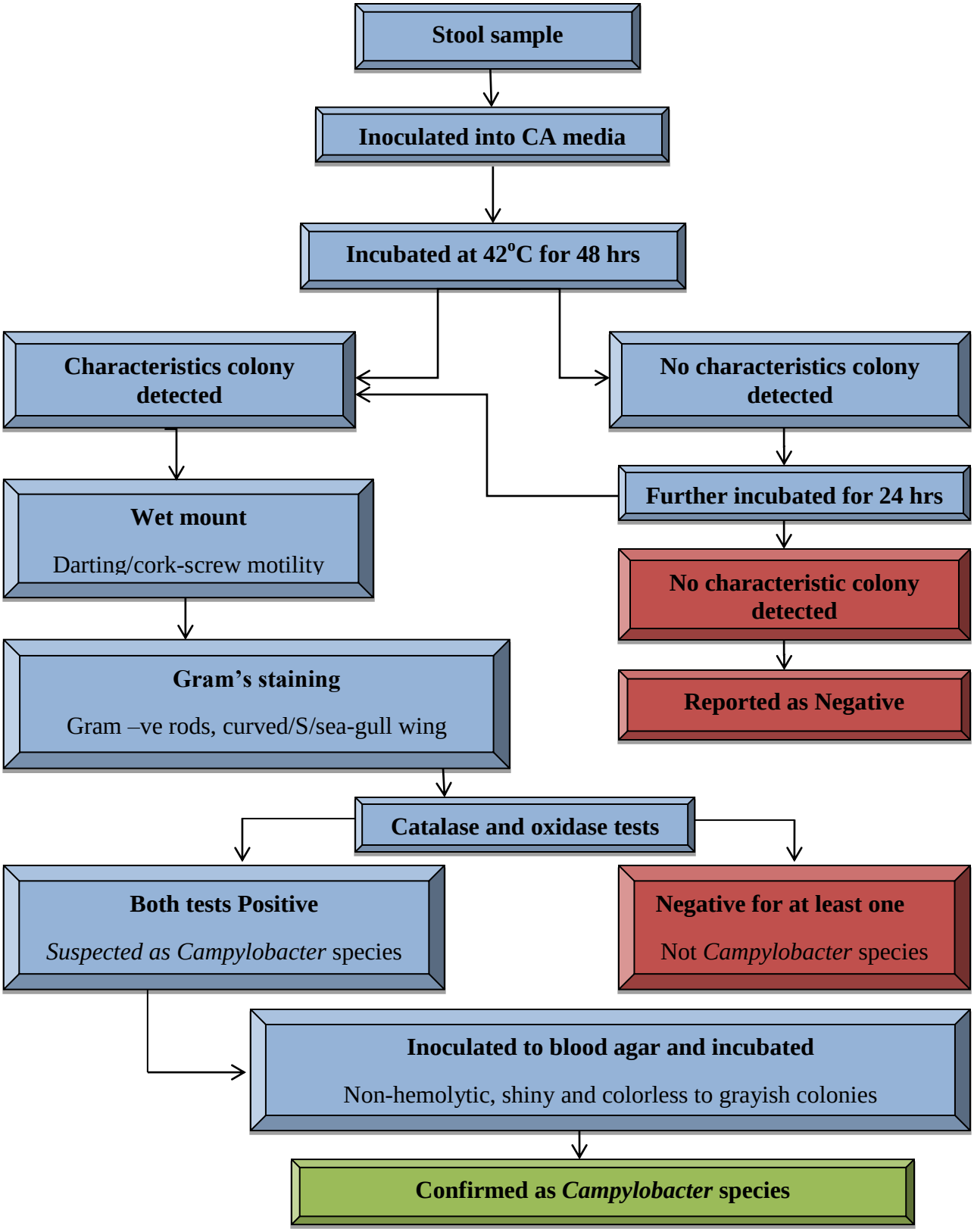

Figure 2: *Campylobacter* species isolation and identification flow chart from stool sample of under-five children with diarrhea at JMC from January to April 2020

4.7.4.2. Species-level identification of isolated Campylobacter strains

The isolated strains of *Campylobacter* species were differentiated/ identified as *C.jejuni/C.coli* from other *Campylobacter* species based on phenotypic classification methods which depends on the H_2S production in triple sugar iron agar and susceptibility to nalidixic acid (30 µg) and cephalothin (30 µg) (47)**.**

Table 1: Biochemical tests for species-level identification of *Campylobacter* strains isolated among under-five children with diarrhea at JMC from January to April 2020

***Campylobacter* species**	**Reactions and characteristics**		
		Susceptibility	
	H_2S production	**Nalidixic acid**	**Cephalothin**
C. jejuni	-	S	R
C. coli	+/-	S	R
C. hyointestinalis	+	R	S
C. laris	-	R	R

+, positive reaction; -, negative reaction; S, susceptible; R, resistant

4.7.4.3. Isolation and identification of Salmonella and Shigella species

Stool samples of each study participant were also assessed for *Salmonella* and *Shigella* species by inoculating into MacConkey agar (HiMedia) and incubating the plates at 37^{o}C for 24 hours. Colorless colonies of non-lactose fermenter organisms were further sub-cultured and incubated at 37^{o}C for 24 hours. Further identification of the genus *Salmonella* and *Shigella* were conducted using colony characteristics, gram's staining, and pattern of biochemical tests which include kligler's iron agar, sulfide indole motility, urea, and citrate and lysine iron agar test (48).

4.7.5. Antimicrobial Susceptibility Testing (AST)

4.7.5.1. AST of Campylobacter species

The antimicrobial susceptibility pattern of the isolated strains was determined by using the Kirby-Bauer disk diffusion technique. Mueller-Hinton agar (HiMedia) supplemented with 5% defibrinated sheep blood was prepared on a 90 mm petri dish. The isolated *Campylobacter* species were mixed into sterile normal saline and bacterial suspension having turbidity equivalent to 0.5 McFarland standards was prepared. Using a sterile cotton swab, a bacterial suspension was streaked all over the surface of prepared Mueller-Hinton agar (HiMedia) supplemented with 5% defibrinated sheep blood.

Antimicrobial disks of ampicillin (AMP) (10µg), amoxicillin with clavulanic acid (AUG) (30µg), gentamicin (GEN) (10µg), ciprofloxacin (CIP) (5µg), ceftriaxone (CTX) (30µg), erythromycin (E) (15µg), chloramphenicol (C) (30µg), meropenem (MEM) (10µg), trimethoprim-sulphamethoxazole (SXT) (25µg) and azithromycin (AZM) (15) (all Liofilchem, Italy) was applied over the inoculated plates and incubated micro-aerobically at 42^{o}C for 24 hours. Susceptibility patterns towards nalidixic acid (NA) (30 µg) and cephalothin (CEF) (30 µg) were additionally used for species-level identification. The panel of antibiotics was chosen in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines from the previous studies and recent reports of resistance of *Campylobacter* species (41,45). Analysis of the diameter of zone of inhibition for erythromycin (E), ampicillin (AMP), ciprofloxacin (CIP), and nalidixic acid (NA) was performed according to the CLSI guideline for *Campylobacter* species from the previous studies (41,49). The rest were done according to CLSI guideline breakpoints for Enterobacteriaceae and were interpreted as sensitive (S), intermediate (I), and resistant (R) (50).

4.7.5.2. AST of Salmonella and Shigella species

The antimicrobial susceptibility testing of *Salmonella* and *Shigella* species was also done by Kirby- Bauer disc diffusion technique. Bacterial suspension having turbidity equivalent to 0.5 McFarland standards was prepared and inoculated into Muller Hinton Agar (HiMedia) by using a sterile cotton swab. Then, each isolate was subjected to antibiotic disks of trimethoprim-sulphamethoxazole (SXT) (25µg), norfloxacin (NOR) (10µg), ciprofloxacin (CIP) (5µg), ampicillin (AMP) (10µg) and ceftriaxone (CTX) (30µg) (all Liofilchem, Italy). The diameters of the zone of inhibition were measured. The isolates were classified as sensitive, intermediate, and resistant according to the CLSI breakpoints for Enterobacteriaceae (50).

4.8. Operational definitions and definition of terms

***Campylobacter* gastroenteritis**: *Campylobacter* infections characterized by diarrhea and vomiting.

Diarrhea: The passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual).

Respondents: Families/caretakers of the children from whom socio-demographic and behavioral data were collected.

Study participants: All under-five children from whom stool samples were collected

Stunting: Height for age Z-score value of less than -2SD

Thermotolerant *Campylobacter* species: *Campylobacter* species having an optimum growth temperature of 42-43^{o}C.

Under-five children: Children aged 0 – 59 months.

Underweight: Weight for age Z-score value of less than -2SD

Wasting: Weight for height/length Z-score value of less than -2SD

4.9. Data processing and analysis

Data were checked for completeness and entered into Epi-data software version 3.1. Then, data were exported to the Statistical Package for Social Sciences (SPSS) program version 25.0. Descriptive statistics like frequency distribution and cross-tabulation were used to describe the socio-demographic characteristics and prevalence of *Campylobacter* species among study participants. Binary and multiple logistic regression analyses were used to assess the presence of a statistically significant association between dependent and independent variables. P-value of ≤0.05 was considered for the presence of statistically significant associations.

4.10. Data quality management

The prepared questionnaire was evaluated by advisors for questions that might be ambiguous or vague. Furthermore, training was given for the data collectors. Epi-data software version 3.1 having inbuilt checks was selected for better data entry.

Campylobacter agar base media was checked for sterility by incubating a dummy medium at 42^{o}C for 48 hours. A sterile campylobacter agar media, campylobacter supplement, and the mechanism of isolation were checked for appropriate functionality using control strains of *Campylobacter jejuni* (ATCC 33560) and *Escherichia coli* (ATCC 25922) for growth and partial or complete inhibition characteristics respectively. Hydrogen peroxide for catalase test was checked using *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 19615) as positive and negative control respectively. Similarly, the performance of the oxidase test was checked using *Pseudomonas aeruginosa* (ATCC 27853) as a positive control and *Escherichia coli* (ATCC 25922) as a negative control. All antibiotic disks and Mueller-Hinton

agar medium were checked for correct functionality by the control strain of *Escherichia coli* (ATCC 25922) (51).

4.11. Ethical consideration

Before data collection, ethical clearance was obtained from Ethical review board of Health Institute, Jimma University, and a formal letter of cooperation was written to JMC. Overview of the study including study purpose, confidentiality, harm, and benefits of the study, and the study procedures were explained for families/caretakers of the children. Then, formal assents were obtained from families/caretakers. Moreover, all clinical samples of the children were also assessed for *Salmonella* and *Shigella* to give evidence-based treatment. Communications with physicians were made for positive cases to be managed and treated accordingly.

5. RESULTS

5.1. Socio-demographic characteristics of under-five children

A total of 214 under-five children with diarrhea were enrolled. About 109 (50.9%) were males. The age of study participants was ranged from 1-56 months with a mean age of 15.78 months (+/-11.8 SD). Seventy nine (36.9%) study participants were between 12-24 months of age [Figure-3].

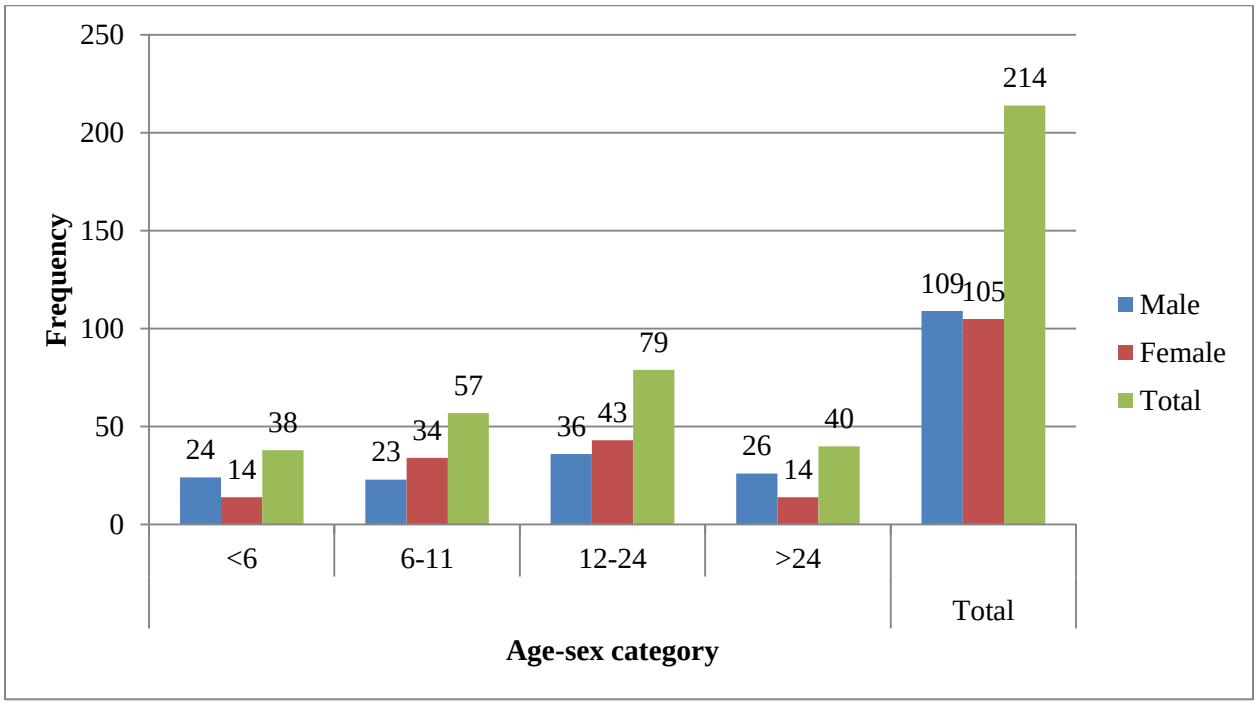

Figure 3: Age-sex distribution of under-five children investigated for *Campylobacter* infection at JMC from January to April 2020.

5.2. Socio-demographic characteristics of families/caretakers of under-five children

Regarding the families/caretakers of under-five children (respondents), most of the under-five children's mothers, 75 (35%) had no level of education and 145 (67.8%) were housewives. Sixty-six (30.8%) of the children's fathers had educational level of first-degree and above. The majority, 79 (36.9%) of under-five children's fathers were farmers [Table-2].

Table 2: Socio-demographic characteristics of mothers/caretakers of under-five children investigated for *Campylobacter* infection at JMC from January to April 2020

Variables		N (%)
Place of residence	Urban	114 (53.3)
	Rural	100 (46.7)
Maternal occupational status	Housewives	145 (67.8)
	Government employees	39 (18.2)
	Merchants	21 (9.8)
	Others*	9 (4.2)
Maternal/caretaker educational level	No level of education	75 (35.0)
	1-8	41 (19.2)
	9-12	26 (12.1)
	Diploma	47 (22.0)
	Frist degree & above	25 (11.7)
Paternal occupational status	Farmers	79 (38.6)
	Government employees	78 (38.0)
	Merchants	38 (18.5)
	Others*	10 (4.9)
Paternal educational level	No level of education	52 (25.4)
	1-8	36 (17.5)
	9-12	28 (13.7)
	Diploma	23 (11.2)
	Frist degree & above	66 (32.2)
Source of drinking water	Private tap	108 (50.5)
	Public tap	90 (42.0)
	Spring	16 (7.5)
Latrine availability	Yes	164 (76.6)
	No	50 (23.4)
Presence of domestic animals	Yes	76 (35.5)
	No	138 (64.5)

*N: Number, * Cafeteria workers, cleaners, office guards and daily laborers*

5.3. Behavioral characteristics of families/caretakers of under-five children

Of the 214 mothers/caretakers, 172 (80.4%) and 147 (68.7%) had handwashing behavior using soap and water after using the toilet and before preparing food for their children, respectively. More than half, 143 (66.8%) use treated drinking water for their children [Table-3].

Table 3: Behavioral characteristics of families/caretakers of under-five children investigated for *Campylobacter* infection at JMC from January to April 2020

Variables	Responses	N (%)
Handwashing before preparing food	Yes	147 (68.7)
	No	67 (31.3)
Handwashing after going to the toilet	Yes	172 (80.4)
	No	42 (19.6)
Handwashing before breast-feeding	Yes	19 (10.8)
	No	157 (89.2)
Water treatment before a child using	Yes	143 (66.8)
	No	71 (33.2)
Mothers'/caretakers' contact with domestic animals	Yes	60 (28)
	No	154 (72)

N: Number

5.4. Behavioral and health related characteristics of under-five children

Out of 214 under-five children, 52 (24.3%) had a habit of consuming raw dairy products. One hundred sixty-four (76.6%) and 10 (4.7%) children had vomiting and bloody diarrhea, respectively. The majority of the children, 127 (59.3%) had weight for age z-score value of less than -2 SD (underweight). Twenty-two, (10.3%) of the children were previously screened for HIV infection. About 1/4th of the children had a history of treatment with antibiotics in the last seven days before the visit [Table-4].

Table 4: Characteristics of under-five children investigated for *Campylobacter* infection at JMC

Variables		N (%)
Consumption raw/undercooked meat	Yes	9 (4.2)
	No	205 (95.8)
Consumption raw dairy products	Yes	52 (24.3)
	No	162 (75.7)
Consumption raw/Unpeeled fruits	Yes	24 (11.2)
	No	190 (88.8)
Consumption of poultry	Yes	8 (3.7)
	No	206 (96.3)
Vomiting	Yes	164 (76.6)
	No	50 (23.4)
Bloody Diarrhea	Yes	10 (4.7)
	No	204 (95.4)
Previous antibiotic treatment	Yes	47 (22)
	No	167 (78)
Known HIV status	Negative	22 (10.3)
	Positive	-
Nutritional status	Stunting	86 (40.2)
	Underweight	127 (59.3)
	Wasting	60 (28)

5.5. Prevalence of *Campylobacter* species among under-five children

Bacterial pathogens were isolated from 30 (14.02%) under-five children with diarrhea. The isolation rate of *Campylobacter* species was 19 (8.9%), while the isolation rate of *Shigella and Salmonella* species *were* 7 (3.27%) and 4 (1.9%), respectively. All *Campylobacter* species isolated were *C. jejuni/ C. coli*. The majority, 16 (84.2%) of the isolated *Campylobacter* species were among children aged 6-23 months. Most of *Campylobacter* species, 15 (78.9%) were isolated from children living in the rural area [Table-5].

Table 5: Distribution of *Campylobacter* infection among under-five children with diarrhea in age, sex and place of residence at JMC from January to April 2020

Variables		*Campylobacter* species		Total
		Positive N (%)	Negative N (%)	N (%)
Age in months	<6	-	38 (100)	38 (17.8)
	6-11	8 (14)	49 (86)	57 (26.6)
	12-24	8 (10.1)	71 (89.9)	79 (36.9)
	>24	3 (7.5)	37 (92.5)	40 (18.7)
Sex	Male	7 (6.4)	102 (93.6)	109 (50.9)
	Female	12 (11.4)	93 (88.6)	105 (49.1)
Place of residence	Urban	4 (3.5)	110 (96.5)	114 (53.3)
	Rural	15 (15)	85 (85)	100 (46.7)

5.6. Associated risk factors for *Campylobacter* infection among under-five children

Bivariate logistic regression analysis was conducted to identify the associated risk factors for *Campylobacter* infection among under-five children with diarrhea. All risk factor variables in the bivariate analysis with p-value ≤0.25 were a candidate for multiple logistic regression analysis.

Accordingly, place of residence, paternal occupational status, treatment of drinking water, hand washing before preparing child food, presence of domestic animals in the household, consumption of raw dairy products, and mothers/caretakers contact with domestic animals were candidate variables and selected for multiple logistic regression.

Multiple logistic regression analysis showed that the odds of *Campylobacter* infection was higher among under-five children whose mothers/caretakers do not wash their hands with soap before preparing child food [AOR: 3.7, 95% CI: (1.2, 10.8), p=0.019] and whose family had a domestic animal in the household [AOR: 3.6, 95% CI: (1.0, 12.7), p=0.044]. Similarly, the odds of *Campylobacter* infection were higher among under-five children who consumed raw dairy products [AOR: 4.5, 95% CI: (1.4, 13.9) p=0.009] [Table-6].

Table 6: Bivariate and multiple logistic regressions between candidate variables and *Campylobacter* infection among under-five children with diarrhea at JMC from January to April 2020

Variables	Positive N (%)	Negative N (%)	Total N (%)	COR (95%CI)	COR p-value	AOR (95%CI)	AOR p-value
Paternal occupation							
Gov't Employee	2 (2.6)	76 (97.4)	78 (38.0)	1		1	
Others	17 (13.4)	110 (86.6)	127 (62.0)	5.6 (1.3,25.1)	0.020	1.1 (0.1,9.1)	**0.948**
Place of residence							
Urban	4 (3.5)	110 (96.5)	114 (53.3)	1		1	
Rural	15 (15.0)	85 (85.0)	100 (46.7)	4.8 (1.6,15.2)	0.007	0.4 (0.05,2.8)	**0.333**
Handwashing before preparing food							
Yes	6 (4.1)	141 (95.9)	147 (68.7)	1		1	
No	13 (19.4)	54 (80.6)	67 (31.3)	5.6 (2.0,15.5)	0.000	3.7 (1.2,10.8)	**0.019***
Water treatment before child using							
Yes	5 (3.5)	138 (96.5)	143 (66.8)	1		1	
No	14 (19.4)	57 (80.3)	71 (33.2)	6.8 (2.3,19.7)	0.000	1.8 (0.5,6.7)	**0.394**
Presence of domestic animals							
Yes	15 (19.7)	61 (80.3)	76 (35.5)	8.2 (2.6,25.9)	0.000	3.6 (1.0,12.7)	**0.044***
No	4 (1.9)	134 (62.2)	138 (64.5)	1			
Mothers'/caretakers' contact with domestic animals							
Yes	12 (20)	48 (80)	60 (28)	5.3 (1.9,14.1)	0.001	1.1 (0.3,5.2)	**0.866**
No	7 (4.5)	147 (95.5)	154 (72)	1		1	
Consumption of raw dairy products							
Yes	13 (25)	39 (75)	52 (24.3)	8.6 (3.1,24.3)	0.001	4.5 (1.4,13.9)	**0.009***
No	6 (3.7)	156 (96.3)	162 (75.7)	1		1	

*N: Number, COR: Crude odds ratio, AOR: Adjusted odds ratio, CI: Confidence Interval, *p-value ≤ 0.05 (significant)*

5.7. Antimicrobial susceptibility patterns of the isolates

All isolated *Campylobacter* species were sensitive to gentamycin and azithromycin. Nearly 95% of the isolates were sensitive to ciprofloxacin and about 79% sensitive to erythromycin. From all *Campylobacter* species, 94.7% were resistant to ceftriaxone and trimethoprim-sulphamethoxazole, 78.5% to ampicillin, and 42.1 % to chloramphenicol. About 95% of the isolated strains were resistant to two or more classes of antibiotics [Table-7].

Table 7: Drug susceptibility pattern of *Campylobacter* species isolated from under-five children with diarrhea at JMC from January to April 2020

Selected Antibiotics		**Disk concentration (µg)**	**Susceptibility pattern**		
			S N (%)	**I N (%)**	**R N (%)**
Quinolones	Ciprofloxacin	5	18 (94.7)	-	1 (5.3)
	Nalidixic acid	30	19 (100)	-	-
Macrolides	Azithromycin	15	19 (100)	-	-
	Erythromycin	15	15 (78.9)	2 (10.5)	2 (10.5)
Phenicol	Chloramphenicol	30	8 (42.1)	3 (15.8)	8 (42.1)
Penicillin	Ampicillin	10	3 (15.8)	1 (5.3)	15 (78.9)
Beta-lactam/beta-lactamase inhibitor	Amoxicillin/ clavulanate	30	16 (84.2)	-	3 (15.8)
Cephalosporins	Cephalothin	30	-	-	19 (100)
	Ceftriaxone	30	1 (5.3)	-	18 (94.7)
Carbapenem	Meropenem	10	16 (84.2)	1 (5.3)	2 (10.5)
Aminoglycoside	Gentamycin	10	19 (100)	-	-
Folate pathway inhibitor	Trimethoprim-sulphamethoxazole	25	1 (5.3)	-	18 (94.7)

S: Sensitive, I: Intermediate, R: resistant, N: Number

Multi-drug resistant was detected among all isolated strains of *Campylobacter* species. Most of the isolates, 9 (47.4%), were resistant to four classes of antibiotics [Table-8].

Table 8 : Antibiogram of *Campylobacter* species isolated from under-five children with diarrhea at JMC from January to April 2020

Organism	**Antibiogram**					
	R_1 N (%)	**R_2 N (%)**	**R_3 N (%)**	**R_4 N (%)**	**R_5 N (%)**	**Total no. of isolates**
***Campylobacter* species**	-	2 (10.5)	7 (36.8)	9 (47.4)	1(5.3)	19

***R_1**: Resistance to one class of antibiotic, **R_2**: Resistance to two classes of antibiotics, **R_3**: Resistance to three classes of antibiotics, **R_4**: Resistance to four classes of antibiotics **R_5**: Resistance to five classes of antibiotics, N: Number*

6. DISCUSSION

Enteric pathogens are common causes of diarrhea among under-five children (52). *Campylobacter* species were the common cause of diarrhea among under-five children in Ethiopia (53). Patients with *C. jejuni/ C. coli* infection may experience acute watery or bloody diarrhea, fever, weight loss, and cramps. Most infections are self-limiting. However, antibiotics are used in immune-compromised patients, patients with severe symptoms, and extra-intestinal infections (8).

In this study, the prevalence of thermotolerant *Campylobacter* species among under-five children with diarrhea was 8.9%. All isolated strains of *Campylobacter* species were sensitive to gentamycin and azithromycin. About 95% of the strains and 79% of the strains were sensitive to ciprofloxacin and erythromycin respectively. The presences of domestic animals to the household level, consumption of raw dairy products, and mother/caretakers handwashing with soap and water before preparing food for child were the independent predictors of *Campylobacter* infection.

Our study showed that the prevalence of *Campylobacter* species among under-five children with diarrhea was 8.9%, which is consistent with studies conducted in Poland 9.3% (21), Egypt 9.37% (25), Tanzania 9.7% (28) and Uganda 9.3% (29). Lower prevalence of campylobacteriosis had been reported in the United Kingdom with a 6% isolation rate (22). On the other hand, a higher prevalence of *Campylobacter* species among under-five children was also reported in South Africa (26), India (23), and Kenya (27) with 25%, 17.35%, and 16% rates of isolation, respectively. The finding of this study was lower than other findings elsewhere in Ethiopia where isolation rates of *Campylobacter* species were 15.4%, 12.7%, and 16.7% in Gondar (18), Hawassa (17), Jimma (5), respectively. The variation in the isolation rates of *Campylobacter* species among different studies could be due to differences in socio-demographic characteristics geographical areas, study subjects, study periods, and methods employed. The major variation observed between this study and the previous studies conducted in the same study area could be due to variation in study periods, as seasonality is one of the factors which affect the prevalence of campylobacteriosis (20). Additionally the previous study was conducted on samples taken from health centers where acute diarrhea could be found common (5).

The odds of *Campylobacter* infection were higher among under-five children whose families had domestic animals. This finding is consistent with studies conducted in California (33), Sweden (30), Norway (31), and Jordan (34). A similar study conducted in Ethiopia also agrees with the finding of an association between *Campylobacter* infections and the presence of domestic animals in the household (37). This could be due to the fact that *Campylobacter* species are harbored by most domestic animals as normal gut microbiota. So, the presence of domestic animals increases the possibility of contamination of child food with animal feces and hence increases the rate of infection. Similarly, this study has also identified that under-five children who do not consume raw dairy products had low odds of infection as compared to those who consumed raw dairy products, which agrees with the finding of the study conducted in New Zealand (35), Sweden (30) and Norway (31). The possible reason for this finding is due to the probability of cross-contamination of animal feces with dairy products, mainly raw milk. However, the odds of *Campylobacter* infection among children who consumed poultry, raw meat, and raw/unpeeled fruits were not found statistically significant as these findings disagree with other findings in Sweden (30) and Norway (31). This could be due to the feeding habit of the community as poultry foods are not commercially available in the study area. Additionally, in this study, the majorities (3/4) of the study participants were children less than 24 months. So, these foods are not routinely consumed by children of less than 24 months. On the other hand, the odds of *Campylobacter* infection among under-five children whose mother/caretakers do not wash their hands with soap and water before preparing food was higher than those whose mother/caretakers wash their hands with soap and water. This result is consistent with a study conducted in 8 selected low-income countries (36). Although it is not statistically significant, the rate of infection with *Campylobacter* species was higher among under-five children whose mothers/caretakers had contact with domestic animals. So, the absence of routine handwashing practices could be the cause of *Campylobacter* infection in the presence of maternal contact with domestic animals.

Regarding the antibiotic susceptibility pattern of *Campylobacter* species, all isolated strains were sensitive to gentamycin and azithromycin, which agrees with the studies conducted in Bangladesh (42), Macedonia (44), and Korea (43). This finding is also in line with the previous study conducted in Ethiopia in which nearly 87% of the isolates were sensitive to gentamycin (5). Our study also revealed that about 94.7% of the isolates were sensitive to ciprofloxacin, which is consistent with another study conducted in Ethiopia (17) in which about 94.3% of the

isolates were sensitive to ciprofloxacin. Similarly, our study indicates about 78.9% of the isolates were sensitive to erythromycin. This finding also agrees with the studies conducted in South Africa (45) where the sensitivity rate of 73.3% and in Ethiopia where sensitivity rate of 77.3% (18) and 81.6% (5) were recorded. However, lower rates of resistance to erythromycin were recorded in Korea (43) and Macedonia (44). This could be due to the empirical use of antibiotics in our country. This study also indicates a high rate of resistance to ceftriaxone (94.7%), trimethoprim-sulphamethoxazole (94.7%), ampicillin (78.5%) and chloramphenicol (42.1%). This result is also in line with the study conducted in Pakistan (41). However, it was slightly higher as compared to the previous studies conducted in Ethiopia (5), Macedonia (44), Korea (43), and Bangladesh (42). This could be due to an increase in beta-lactamase-producing *Campylobacter* species against ampicillin and ceftriaxone. Additionally, the empirical use, self-medications, and usage of these antibiotics in animals (like ampicillin) could be the cause for the higher rate of resistance.

Limitations of the study

This study has a limitation as it was difficult to differentiate between *C. jejuni* and *C. coli* due to absence the hippurate hydrolysis test used for this purpose. The study is also not powered enough to determine additional risk factors of infection among under-five children due to the nature of the study design and limited sample size.

7. CONCLUSION AND RECOMMENDATIONS

7.1. Conclusion

The rate of isolation of thermotolerant *Campylobacter* species was lower at this study area. Azithromycin and gentamycin were the antibiotics to which all isolates were found sensitive. Although most of the isolated strains were susceptible to common antibiotics, significant resistance to a drug of choices and other antibiotic classes were also identified. Consumption of raw dairy products, absence of maternal/caretakers' routine handwashing with soap and water before preparing child food, and the presence of domestic animals in the household were the associated risk factors.

7.2. Recommendations

FMOH, Zonal and Woreda health office

- Should plan the surveillance program involving *Campylobacter* gastroenteritis

JUMC

- Should optimize and set the possible methods used for the diagnosis of *Campylobacter* species when required

Researchers

- Should also conduct further study on the burden and associated factors of *Campylobacter* gastroenteritis using longitudinal study

Families/caretakers of under-five children

- Should improve their hygiene and sanitation as much as possible noticing that their every sanitary activity has an impact to reduce infection with *Campylobacte*r species.
- Should pasteurize dairy products for child feeding before use.

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ANNEXES

Annex I - Questionnaire in an English version

Title: - *Campylobacter* species and antibiotic susceptibility pattern of isolates among under-five children with diarrhea at JMC, Jimma, Southwest Ethiopia

Dear respondent,

This study is conducted for the partial fulfillment of a Master of Science Degree in Medical Microbiology. Every response from you is valuable in producing fruitful findings related to the study, and your concise and clear responses would facilitate smooth data analysis. So please give your responses accordingly. All information provided will be treated confidentially.

Questionnaire ID number: _____ Patient Card no. _____

Site of collection: ward_____/OPD_____

Date of interview: _____

Part I – Socio-demographic characteristics of the respondent

No	**Questions**	**Response**
1.	Age of the child (months completed)	_____month/s
2.	Sex of the child	1. Male 2. Female
3.	Place of Residence	1. Rural 2. Urban
4.	Maternal Educational status	1. No level of Education 2. 1-8 3. 9-12 4. Certificate 5. Diploma

		6. Degree and Above
5.	Maternal Occupation	1. Housewife 2. Government Employee 3. Merchant 4. Other (specify)……
6.	Paternal Educational status	1. No level of Education 2. 1-8 3. 9-12 4. Certificate 5. Diploma 6. Degree and Above
7.	Paternal Occupation	1. Farmer 2. Government Employee 3. Merchant 4. Other (specify)……

Part II – Anthropometric Measurements

8. Anthropometric Measurements		
Weight(kg) _____	Height/length (in cm) _____	Edema status_____(yes/no)

Part III – Behavioral and Sanitation Characteristics

9.	What is the main source of drinking water for the household? 1. Private tap to a household level 2. Public tap or standpipe 3. Protected Spring 4. Unprotected spring 5. Dug well 6. River/dam/lake/irrigation channel

10.	When do you normally wash your hands using soap? (Circle all that applied!) 1. I don't wash my hands using soap 2. After going to the toilet 3. Before preparing food for the child 4. Before breastfeeding/feeding the child 5. After cleaning the child's feces 6. Before meal 7. After meal 8. When my hands are dirty	
11.	Is your water treated in any method to make it safe before drinking/usage? If 'Yes' specify the method	1. Yes 2. No 3. Don't know
12.	Is Latrine available in the household?	1. Yes 2. No
13.	If '**No'** to question no. 13, where do you use for defecation?	1. Open field 2. Communal latrine
14.	Does the household own any of the following domestic animals? Select all owned. If **'No'** any domestic animal, **Skip** to **Q. no**. **20**	1. Pets 4. Pig 2. Cattle 5. Chickens 3. Goats 6. Sheep
15.	Does the child's mother/guardian have any habit of contact with domestic animals?	1. Yes 2. No
16.	If **'Yes'** to question no. 16, please specify with which animal?	1. Pets 4. Pig 2. Cattle 5. Chickens 3. Goats 6. Sheep
17.	Does your child have any habit of contact with domestic animals?	1. Yes 2. No
18.	If **'Yes'** to question no. 18, please specify with which animal?	1. Pets 4. Pig 2. Cattle 5. Chickens 3. Goats 6. Sheep

19.	Does the household use indoor or outdoor animal manure or processed fertilizers in the garden?	1. Yes 2. No
20.	If **'Yes'** to Q. no. 20, Does the Child have any access to areas of fertilizer use?	1. Yes 2. No
21.	Has your child started feeding by him/herself?	1. Yes 2. No
22.	If '**Yes**' to Q. no. 21, does your child wash his/her hands with soap before meal?	1. Yes always 2. Not always 3. Never with soap
23.	Did your child consume any of the following sources of food in the last two weeks?	
	Raw meat/undercooked meat like kitfo	1. Yes 2. No
	Raw dairy product	1. Yes 2. No
	Poultry	1. Yes 2. No
	Raw Fruits and vegetables. E.g. Raw carrots, Raw cabbage, Unpeeled apples.	1. Yes 2. No
24.	For how many months was your child only on breast milk (Duration of exclusive breastfeeding)?	_____months
25.	Is your child still on breastfeeding?	1. Yes 2. No
26.	If '**No'** to question no. 26, how old was your child when she/he stopped breastfeeding (in months)?	_____months

Part IV - Questions about the current illness

27.	For how many days including today have this episode of DIARRHOEA lasted? (DIARRHOEA: ≥ 3 watery or loose stools (looser than normal stools) per 24 hours)	_____days
28.	Any bloody or blood-stained stools?	1. Yes 2. No
29.	Does your child have a symptom of Vomiting?	1. Yes 2. No

30.	If '**Yes'** to question no. 30, for how many days	_____days
31.	In the last week, has your child received any antibiotic treatments?	1. Yes 2. No
32.	If '**Yes'** to question no 32, please specify which antibiotic? (show the sample packet)	

Part V - Questions about past medical history

33.	Has your child ever been tested for HIV?	1. Yes 2. No
34.	If **'Yes'** to Q. no. 34, what was the result of the test?	1. HIV Positive 2. HIV Negative
35.	If the result was **'HIV positive'** to Q. no. 35, is your child on ART? If '**No**' why? _____	1. Yes 2. No

Interviewer signature ----------Date----------

Thank you for your participation!

Annex II - የቃለ-መጠይቅ ቅፅ በአማርኛ

በጂማ ዩኒቨርሲቲ የሕክምና ማዕከል እድሜያቸው ከ 5 ዓመት በታች በሆኑ ህፃናት ላይ ካምፒሎባክተር የተባለዉ ባክቴሪያ የተቅማት በሽታን የማምጣት ዓቅምና ተግዳሮቶቹ

የመጠየቂያ ቁጥር: _____ የታካሚዉ ካርድ ቁጥር_____

የቃለ መጠይቁ ቀን: _____ የመጠየቂያ ቦታ፡ ኦፒዲ_____ /ዋርድ_____

ክፍል 1 - ምላሽ ሰጭው ሶሺዮዲሞግራፊክ ባህሪያት

ቁጥር	ጥያቄዎች	መልሶች
1.	የሕፃኑ ዕድሜ (በወራት)	_____ / _____
2.	የሕፃኑ ፆታ	1. ወንድ 2. ሴት
3.	የመኖሪያ ቦታ	1. ገጠር 2. ከተማ
4.	የወላጅ እናት የትምህርት ደረጃ	1. የትምህርት ደረጃ የለም 2. 1-8 3. 9-12 4. ዲፕሎማ 5. ዲግሪና ከዛ በላይ
5.	የውላጅ እናት የስራ ድርሻ	1. የቤት እመቤት 2. የመንግስት ሰራተኛ 3. ነጋዴ 4. ሌላ (ይግለጹ) .....
6.	የወላጅ አባት የትምህርት ደረጃ	1. የትምህርት ደረጃ የለም 2. 1-8 3. 9-12 4. ዲፕሎማ 5. ዲግሪና ከዛ በላይ
7.	የውላጅ አባት የስራ ድርሻ	1. አርሶ አደር 2. የመንግስት ሰራተኛ 3. ነጋዴ

		4. ሌላ (ይግለጹ) .....

ክፍል 2 - አካላዊ ልኬቶች

8. **አካላዊ ልኬቶች**		
ክብደት (በኪ..ግ) _____	ቁመት / ርዝመት (በሴ.ሜ) _____	ኢዴማ አለ/የለም

ክፍል 3- ሌሎች ባህሪያት

9.	ለቤተሰቡ ዋነኛ የመጠጥ ውኃ ምንጭ ምንድነው? 1. የቤት ውስጥ ቧንቧ 2. የሕዝብ ቧንቧ 3. የዝናብ ውሃ 4. የተጠበቀ/የተከለለ ጉድጓድ 5. ያልተጠበቀ/ያልተከለለ ጉድጓድ 6. ወንዝ / ግድብ / ሐይቅ / የመስኖ ቦይ
10.	አብዛኛውን ጊዜ እጅዎትን ምን ጊዜ ነዉ በሳሙና የሚታጠቡት? (የሚመለከቶትን ሁሉ ያክብቡ!) 1. እጆቼን በፍጹም አልታጠብም 2. ወደ መጸዳጃ ቤት ከገቡ በኋላ 3. ለልጁ ምግብ ከማዘጋጀትዎ በፊት 4. ልጁን ከማጥባቱ በፊት 5. ከመመገብዎ በፊት 6. ከምግብ በኋላ 7. እጆቼ ሲቆሸሹ

11.	ከመጠጥዎ በፊት ውሃዎን በማንኛውም መንገድ ያክማሉ? 1. አዎ 2. አይ 3. አላውቀውም	
12.	በቤተሰብዎ መጸዳጃ ቤት አለ?	1. አዎን 2. አይ
13.	የጥያቄ 13 መልሶ አይ ከሆነ፣ቤተሰብዎ ለመጸዳጃነት የት ይጠቀማል?	1. ሜዳ ላይ 2. የህዝብ መጸዳጃ ቤት
14.	በቤተሰብዎ ማንኛውንም ህይወት ያላቸው እንስሳት (አንድ ወይም ከዚያ በላይ) ይኖራሉ? አዎ ከሆነ, ያሉትን እንስሳት ሁሉንም ይምረጡ: መልሶ አይ ከሆነ ወደ ጥያቄ ቁጥር 20 ይሂዱ	1. ውሻና ድመት 2. ከብቶች 3. በጎች 4. ፍየሎች 5. አሳማ 6. ዶሮዎች
15.	እርሶ/የልጅዎ እናት/ ኣሳዳጊ ከእንስሳት ጋር የመገናኘትና የመነካካት ነገር አላቸዉ ወይ?	1. አዎ 2. አይደለም
16.	የጥያቄ 16 መልሶ አዎ ከሆነ፣ ከየትኛው እንስሳ ጋር?	1. ውሻና ድመት 2. ከብቶች 3. በጎች 4. ፍየሎች 5. አሳማ 6. ዶሮዎች
17.	ልጅዎ ከእንስሳት ጋር የመገናኘትና የመነካካት ነገር አለው ወይ?	1. አዎ 2. አይደለም
18.	አዎ ከሆነ, ከየትኛው እንስሳ ጋር?	1. ውሻና ድመት 2. ከብቶች 3. በጎች

		4. ፍየሎች 5. አሳማ 6. ዶሮዎች
19.	በቤትዎ ውስጥ ወይም ከቤት ውጭ የእንስሳትን ፍግ ወይም ለማዳበሪያ ይጠቀማሉ?	1. አዎን 2. አይ
20.	ልጅዎ የእንስሳት ፍግ ወይም ማዳበሪያ ወዳለበት ደርሶ ያዉቃል?	1. አዎን 2. አይ
21.	ልጅዎ በራሱ / በራሷ እራሷን መመገብ ጀምሯል/ጀምራለች?	1. አዎን 2. አይ
22.	ልጅዎ ምግብ ከመብላቱ በፊት እጆቹን በሳሙና ይታጠባል?	1. አዎን ሁል ጊዜ 2. አልፎ አልፎ 3. በፍፁም
23.	ባለፉት ሁለት ሳምንታት ልጅዎ ከዚህ በታች ከተዘረዘሩት የምግቦች ውስጥ አንዱንም ቢሆን በልቶ ተገኝቷል ወይ?	
	ጥሬ ሥጋ	1. አዎን 2. አይ
	ጥሬ ወተትና የወተት ውጤቶች	1. አዎን 2. አይ
	ዶሮና የዶሮ ውጤቶች	1. አዎን 2. አይ
	ፍራፍሬዎችን	1. አዎን 2. አይ
24.	ልጅዎ ከመታመሙ በፊት ጡት እየጠባ ነበር?	1. አዎ 2. አይ
25.	ልጅዎ ከጡት ወተት በስተቀር ምንም ምግብ ወይም መጠጥ አይወስድም ነበር?	_____ወራት
26.	ስንት ወር በጡት ወተት ላይ ብቻ ቆየ?	1. አዎ 2. አይ

27.	አሁንም የጡት ወተት እየጠባ ነዉ?	_____ወራት
28.	ልጅዎ የጡት ወተት ሲያቆም እድሜዉ ስንት ይሆን ነበር?	

ክፍል 4- ስለ ወቅታዊ ህመም ጥያቄዎች

29.	ዛሬን ጨምሮ ተቅማጥ ከያዘዉ ስንት ቀን ይሆነዋል?	_____ቀናት
30.	ከተቅማቱ ጋር ደም ይወታዉ ነበር?	3. አዎ 4. አይ
31.	ያስመልስሰዉ ነበር?	5. አዎ 6. አይ
32.	አዎን ካሉ ለስንት ቀናት?	ለ _____ ቀናት
33.	በአለፈው ሳምንት ልጅዎ አንቲባዮቲክ መድሃኒትን ወስዶ ነበር	1. አዎ 2. አይ
34.	አዎ ከሆነ; እባክዎ የትኛውን አንቲባዮቲክ (ፓኬቱን ያሳዩን)	

ክፍል 5 - ያለፈው የህክምና ታሪክ ጥያቄዎች

35.	ልጅዎ በኤች አይ ቪ ምርመራ ተደርጎለታል?	7. አዎ 8. አይ 9. አላውቀውም
36.	አዎ ከሆነ, የምርመራው ውጤት ምን ነበር?	10. ኤች አይ ቪ + 11. ኤች አይ ቪ - 12. አላውቀውም

የምላሽ ሰጪዉ ፊርማ ---------- ቀን ----------

ለመሳተፍዎ እናመሰግናለን!

Annex III - Waraqaa gaaffii fi deebii Afaan Oromiffaatiin

Mata-duree: - Dhiibbaa baakteeriyaa 'kaampiloobacter' jedhamu ijoollee waggaa shanii gadii dhibee garaa kaasaan qabamanii Giddugala Meedikaalaa Jimmaatti yaalamaa jiran irratti qabu qorachuu ta'a!

Lakk Waraqaa Gaaffii: _____ Lakkoofsa kaardii _____

Iddoo funaaname: OPD___/kutaa ciisichaa___ Guyyaa gaaffiif deebii: _____

Kutaa I – Gaaffilee Hawaasummaa

Lakk	**Gaaffilee**	**Deebiiwwan Hirmaattotaa**
1.	Umrii	Ji'oota_____
2.	Saala	1. Dhiira 2. Durba
3.	Iddoo jireenyaa	1. Magaalaa 2. Baadiyyaa
4.	Sadarkaa Barnootaa Harmee	1. Sadarkaa barnootaa hin qabu 2. 1-8 3. 9-12 4. Dippiloomaa 5. Digirii fi isaa ol
5.	Gahee Hojii Harmee	1. Haadha manaa 2. Hojjettuu mootummaa 3. Daldaltuu 4. Kan biraa…….
6.	Sadarkaa Barnootaa Abbaa	1. Sadarkaa barnootaa hin qabu 2. 1-8 3. 9-12 4. Dippiloomaa 5. Digirii fi isaa ol
7.	Gahee Hojii Abbaa	1. Qotee bulaa 2. Hojjetaa mootummaa 3. Daldalaa 4. Kan biraa…….

Kutaa II – Safartoowwan Qaamaa

8. Safartoowwan qaamaa		
Ulfaatina (kg)_____	Dheerina (cm)_____	Ideemaa____Jira/hin jiru

Kutaa III – Gaaffilee barmaatilee Maatii tokko tokko

9.	Maddi bishaan dhugaatii maatii keessanii maali? 1. Kan dhuunfaa/mana keessaa 2. Kan hawaasa naannoo 3. Bishaan bokkaa 4. Burqituu daangeffame 5. Burqituu hin daangeffamin 6. Laga/Haroo/Bishaan jallisii	
10.	Harka keessan yeroo akkamii dhuqattu? 1. Gonkuma hin dhiqadhu 2. Mana fincaanii booddee 3. Nyaata mucaa qopheessuun dura 4. Harma hoosisuu dura 5. Ergan mucaa qulqulleessee booda 6. Nyaata booddee 7. Nyaata dura 8. Yeroo harki koo xuraawu	
11.	Madda bishaanii keessan karaa kamiinuu Qorichaan yaaltanii beektuu? 1. Eeyyee 2. Lakki 3. Hin Beeku	
12.	Maatiin keessan mana fincaanii ni qabaa?	1. Eeyyee 2. Lakki
13.	Yoo deebiin keessan ‘lakki” ta’e, maatiin keessan boobbaaf maal fayyadamu?	1. Dirree 2. Mana fincaanii hawaasaa
14.	Maatiin keessan beeylada manaa niqabaa? Yoo Eeyyee jettan maal fa’i? Yoo deebiin ‘lakki’ ta’e, lakkofsa gaaffii 20 bira dhaqaa.	1. Saree fi Adurree 2. Saawwa 3. Hoolaa 4. Re’ee 5. Booyyee Lukkuu
15.	Harmeen/ kunuunsituun mucaa keessanii beelada isin nuuf himtan waliin tuttuqinsa ni qabuu	1. Eeyyee 2. Lakki

16.	Yoo eeyyee ta'e, beetlada isa kam waaliin?	1. Saree fi Adurree 2. Saawwa 3. Hoolaa 4. Re'ee 5. Booyyee 6. Lukkuu
17.	Mucaan keessan barmaata beeylada manaan wal tuttuquu ni qabaa/qabdii?	1. Eeyyee 2. Lakki
18.	Yoo Eeyyee jettan, beeylada kam waliin?	1. Saree fi Adurree 2. Saawwa 3. Hoolaa 4. Re'ee 5. Booyyee 6. Lukkuu
19.	Maatiin keessan xuraawaa beeladaa waan garaagaraaf ni fayyadamuu?	1. Eeyyee 2. Lakki
20.	Yoo deebiin keessan 'eeyyee' ta'e, mucaan keessan xuraawaa beeladaa waliin wal tuttuqiinsa ni qabaa/qabdii?	1. Eeyyee 2. Lakki
21.	Mucaan keessaan ofii isaaf nyaachuu eegaleeraa/eegalteertii?	1. Eeyyee 2. Lakki
22.	Yoo deebiin keessan 'eeyyee' ta'e, mucaan keessan nyaata dura harka isaa saamunaan ni dhiqataa/dhiqattii?	1. Eeyyee yeroo hunda 2. Yeroo tokko tokkko 3. Gonkumaa
23.	Mucaan keessan torbee lamaan darban keessattibarmaatilee gosa nyaataa armaan gadii nyaatee turee?	
	Foon dheedhii	1. Eeyyee 2. Lakki
	Aannanii fi bu'aalee aannanii	1. Eeyyee 2. Lakki
	Lukkuu fi bu'aalee lukkuu	1. Eeyyee 2. Lakki
	Kuduraaf muduraa akka kaarotii dheedhii	3.
24.	Mucaan keessan utuu nyaata biraa hin fudhatin kan harma duwwaa hodhaa ture ji'oota meeqaaf?	Ji'oota_____
25.	Ammayyuu harma hodhaa jiraa?	1. Eeyyee 2. Lakki

26.	Yoo deebiin keessan Lakki ta,e, yeroo ji,a meeqaffaasaa harma hodhuu dhaabe?	Ji'a_____

Kutaa IV – Gaaffilee Waa'ee dhibee garaa kaasaan walqabatee

27.	Guyyaa har'aa dabalatee guyyoota meeqaaf dhibeen garaa kaasaa irra ture? (GARAA KAASAA: sa'aa 24 keessatti yeroo 3 ol gad-teessisuu	Guyyoota_____
28.	Gad teessumni mucaa dhiigan walmakaa turee?	1. Eeyyee 2. Lakki
29.	Mucaa keessan ni ballaqqamsiisaa?	1. Eeyyee 2. Lakki
30.	Yoo deebiin keessan Eeyyee ta'e guyyoota meeqaaaf?	Guyyoota_____
31.	Torbee darbe keessa mucaan keessan gosa qoricha farra baacteeriyaa fudhatee jiraa?	1. Eeyyee 2. Lakki
32.	Yoo eeyyee jettan, mee qodaa isaa nutti argisiisuu dandeessuu?	

Kutaa V – Gaaffilee waa'ee fayyuummaa mucaa ji'oota muraasa dura.

33.	Mucaa keesaniif amma dura qorannoon HIV taasifamee beekaa?	1. Eeyyee 2. Lakki
34.	Yoo Eeyyee jettan bu'aan qarfannichaa maal ture?	1. HIV Poosatiivii 2. HIV Neegatiivii 3. Himuu hin barbaadu
35.	Yoo bu'aan qorannoo mucaa keessanii 'hiv poosatiivii' ta'e, mucaan keessaan hordoffii fi qorichaa dhibee kanaa fudhataa jiraa? Yoo lakki ta'e maaliif?	1. Eeyyee Lakki

Mallattoo Gaafataa _____Guyyaa_____

Hirmaannaa keessaniif Galatoomaa!

Annex IV- Informed Consent/Assent Form English version

This informed consent/assent form is for the parents of under-five attending Jimma Medical Center.

Title of Study:	***Campylobacter* species and antibiotic susceptibility pattern of isolates among under-five children with diarrhea at Jimma Medical Center**			
Investigator:	**Yared Nigusu**	**Dep't:**	Medical Microbiology	**Phone: 09 21 20 46 63**

This Informed Consent Form has two parts:

- Information Sheet (to share information about the study with you)
- Certificate of Consent (for signatures if you agree that your child may participate)

PART I: Information Sheet

Introduction

I am a data collector of research going to be conducted on *Campylobacter* species and drug susceptibility pattern among under-five children with diarrhea in Jimma Medical Center, which is very common in developing countries. I am going to give you information and invite you to have your child participate in this research. Before you decide, you can talk to anyone you feel comfortable with. If you do not understand some of the words or concepts, I will take time to explain it as you go along, and you can ask questions anytime you want. If you have questions about me, you can ask the staff.

Purpose of the Study

The purpose of the study is to know the figure indicating disease causation magnitude and burden of *Campylobacter* species among under-five children. In developing countries like Ethiopia, under-five children are known to be affected by diarrhea. But the major causes of diarrhea among under-five children are inconsistent which could be due to variation in the causative agents, of which *Campylobacter* species is one of the common causes.

This study is aimed at revealing the magnitude of *Campylobacter* species in causing diarrhea. Besides this aim, the study will also deal with the major factors associated with *Campylobacter* infection.

Furthermore, the result of this study will help the concerned body/stakeholders to intervene in the problem identified as a major factor associated with *Campylobacter* species.

Participant selection

We are inviting you to take part in this research because your child has already been with diarrhea, and we want to identify the bacteriological causes of diarrhea, particularly *Campylobacter* species. You are selected because you and your child live around this area and attend Jimma Medical Center. I need to notify you that it is not only your child who is selected but all under-five children with diarrhea attending Jimma Medical Center during the study period will be selected.

Voluntary Participation

Your decision to have your child participate in this study is entirely voluntary. It is your choice whether to have your child participate or not. If you choose not to consent, all the services you and your child receive at this clinic will continue and nothing will change. You may also choose to change your mind later and stop participating even if you agreed earlier, and the services you and/or your child receive at the clinic will continue.

Study Procedures and protocols

If you agree to be in this study, you will be asked for direct questions of socio-demographic and behavioral characteristics, and you will help as in collecting a stool sample for your child.

Risks/Discomforts of Being in this Study

By participating in this research, there is no possible risk or discomfort that may happen as a result of the participation of your child.

Benefits of Being in the Study

There is no direct benefit to you from participating in this study. However, you and your community will be benefited from the majors to be taken upon the finding of this study.

Confidentiality

The information that we collect from this research project will keep confidential. Information about your child that will be collected from the research will be put away and no-one but the researchers will be able to see it. Any information about your child will have a number on it instead of his/her name. Only the researchers will know what his/her number is, and we will lock that information up with a lock and key. It will not be shared with or given to anyone.

Right to Refuse or Withdraw

You do not have to agree to your child to take part in this research if you do not wish to do so, and refusing to allow your child to participate will not affect your treatment or your child's treatment at this center in any way. You and your child will still have all the benefits that you would. You may stop your child from participating in the research at any time that you wish without either you or your child losing any of your rights as a patient here. Neither your treatment nor your child's treatment will be affected.

Who to Contact

You have the right to ask questions about this research study and to have those questions answered by me before, during, or after the research. If you have any further questions about the study, at any time feel free to contact using the following address.

Name: - Yared Nigusu

Phone no.: - 09 21 20 46 63

Email: - nyared@yahoo.com

PART II: Certificate of Consent

I have been invited to have my child participate in the research of *Campylobacter* species and antibiotic susceptibility pattern among under-five children with diarrhea at Jimma Medical Center. The information has been read to me. I have had the opportunity to ask questions about it, and any questions that I have asked have been answered to my satisfaction. I am a volunteer for my child to participate in this study.

Name of Participant_____

Name of Parent or Guardian_____

Signature of Parent or Guardian _____

Date _____

Thank you for your participation!

Annex V - የፈቃድ መጠየቂያ ቅፅ በአማርኛ

ይህ የስምምነት ቅጽ የሚሞላዉ በጅማ ዩኒቨርሲቲ የሕክምና ማዕከል የሚከታተሉ እድሜያቸው ከ 5 ዓመት በታች በሆኑት ህፃናት ወላጆች ነው::

የጥናቱ ርዕስ:	በጂማ ዩኒቨርሲቲ የሕክምና ማዕከል እድሜያቸው ከ 5 ዓመት በታች በሆኑ ህፃናት ላይ ካምፒሎባክተር የተባለዉ ባክቴሪያ የተቅማት በሽታን የማምጣት ዓቅምና ተግዳሮቶቹ

ጥናቱን የሚያካሄደዉ	የት/ት ዐይነት:	ስልክ ቁጥር:
ያሬድ ንጉሡ	ማይክሮ ባዮሎጊ	09 21 20 46 63

ይህ መረጃ የሚሰጥበት ቅጽ ሁለት ክፍሎች አሉት

- የመረጃ ሰነድ (ለእርስዎ የጥናቱን መረጃ ለማጋራት)
- የስምምነት ምስክር ወረቀት (ልጅዎ ተሳታፊ ሊሆን ይችላል ብለው ከተስማሙ ሊፈርሙበት)

ክፍል 1: የመረጃ ዝርዝር

መግቢያ

በጂማ ዩኒቨርሲቲ የሕክምና ማዕከል ውስጥ በተቅማጥ በሽታ በሚያዙ እድሜያቸው ከ 5 ዓመት በታች በሆኑ ሕፃናት መካከል ካምፒሎባክተር የተባለዉ ባክቴሪያ ላይ የሚደረገውን የምርምር ጥናት ሰብሳቢ ነኝ፡፡ መረጃን እሰጥዎታለሁ እናም ልጅዎ በዚህ ጥናት እንዲሳተፍ እንድትጋብዙ ተጋብዘዋል፡፡ ልጅዎ በጥናቱ ውስጥ መሳተፍ ይችል እንደሆነ ወይም እንዳልሆነ ዛሬ ለመወሰን ላይፈልጉ ይችላሉ፡፡ ከመወሰንዎ በፊት ከሚፈልጉት ማንኛውም ሰው ጋር መነጋገር ይችላሉ.፡፡ አንዳንድ ቃላትን ወይም ጽንሰ-ሐሳቦችን እርስዎ የማይገባዎት ከሆነ፤ እነሱን ለማብራራት እችላለዉ፡፡ ጥያቄ ካለዎት በፈለጉት ጊዜ መጠየቅ ይችላሉ፡፡

የጥናት ዓላማ

እድሜያቸው ከ 5 ዓመት በታች በሆኑ ሕፃናት ላይ ካምፒሎባክተር የተባለዉ ባክቴሪያ የተቅማት በሽታን የማምጣት ዓቅምና ተግዳሮቶቹን ማጥናት ነዉ። ይህ ጥናት ተቅማጥ የሚያስከትል ካምፒሎባክተር የተባለዉ ባክቴሪያ ዝርያዎችን መጠን ለማጥናት ዕና ከዚህ ዓላማ በተጨማሪ ጥናቱ ካምፒሎባክተር ኢንፌክሽን ጋር የተያያዙ ዋናዋና ጉዳዮችን ማጥናት ይሆናል።

የተሳታፊዎች ምርጫ

ልጅዎ ከዚህ ቀደም የተቅማጥ በሽታ ይዞት ስለነበረ በዚህ ጥናት ላይ እንዲሳተፉ እየጋበዝንዎ ነው። በተጨማሪም እርስዎ እና ልጅዎ በዚህ አካባቢ ነዋሪ ስለሆኑና እና እርስዎ በጅማ ዩኒቨርሲቲ የሕክምና ማዕከል ውስጥ በመገኘቶ ልጅዎ ተመርጧል። የተመረጠው ልጅዎ ብቻ ሳይሆን በጥናቱ ወቅት በጅማ ዩኒቨርሲቲ የሕክምና ማዕከል የሚመጡ እድሜያቸው ከ 5 ዓመት በታች የሆኑና በተቅማት በሽታ የተጎዱ ሕፃናት ሁሉ የሚመረጡ ይሆናል።

በፈቃደኝነት ተሳትፎ

ልጅዎ በዚህ ጥናት እንዲሳተፍ የሚወስኑት ውሳኔ በፈቃደኝነት ነው። ልጅዎ እንዲሳተፍ ወይም እንዳይሳተፍ ምርጫዎ ነው። መስማማት ካልፈለጉ በዚህ የሕክምና ማዕከል ውስጥ እርስዎ እና ልጅዎ የሚያገኟቸው ሁሉም አገልግሎቶች ሳይቋረጡ ማግኘት ይችላሉ።

የጥናቱ ደንቦችና ፕሮቶኮሎች

በዚህ ጥናት ውስጥ ለመሳተፍ ከተስማሙ የተወሰኑ ቀጥተኛ ጥያቄዎችን እንዲመልሱ ይጠየቃሉ። ከልጅዎም ናሙና በመሰብሰብ ይረዱናል።

በዚህ ጥናት ውስጥ የሚያጋጥሙ አደጋዎች / አለመመቻቸቶች

በዚህ ጥናት ውስጥ በልጅዎ ተሳትፎም ምክንያት ሊከሰት የሚችል ምንም ዓይነት አደጋ አይኖርም።

በጥናቱ ውስጥ የመሆን ጥቅሞች

በዚህ ጥናት ውስጥ በመሳተፍ ምንም ቀጥተኛ ጥቅሞች የሉም:: ሆኖምግን,፤ እርስዎ እና ማህበረሰብዎ በዚህ ጥናት ግኝት ብዙ ጥቅም ያገኛሉ፡፡

ሚስጢራዊነት

ከዚህ የምርምር ፕሮጀክት የምንሰበስበው መረጃዎች በሚስጢር ይያዛሉ፡፡

የማቋረጥ ወይም የመተው መብት

ይህን ማድረግ ካልፈለጉ ልጅዎ በዚህ ጥናት ውስጥ መሳተፍ እና ልጅዎን እንዲሳተፍ አለመፍቀድ በየትኛውም መንገድ በዚህ ህክምናዎ ወይም በልጅዎ ህክምና ላይ ተጽዕኖ አይኖረውም፡፡

ከፈለጉ ማንን ዕንደሚያገኙ

ስም: - **ያሬድ ንጉሡ**

ስልክ ቁጥር: - 09 21 20 46 63

ኢሜይል: - nyared@yahoo.com

ክፍል ሁለት- የማረጋገጫ የምስክር ወረቀት

በጂማ ዩኒቨርሲቲ የሕክምና ማዕከል እድሜያቸው ከ 5 ዓመት በታች በሆኑ ህፃናት ላይ ካምፒሎባክተር የተባለዉ ባክቴሪያ የተቅማት በሽታን የማምጣት ዓቅም፡ ተግዳሮቶቹና ተጋላጭነት ጥናት ላይ ልጄ እንዲሳተፍ ወስኛለሁ፡፡

የወላጅ ወይም የአሳዳጊ ስም _____

የወላጅ ወይም አሳዳጊ ፊርማ _____

ቀን _____

ለተሳትፎዎ እናመሰግናለን

Annex VI - Guca gaaffii fedhii hirmaannaa Afaan Oromiffaatiin

Gucni kun kan maatii ijoollee waggaa shanii gadii ta'an Giddugala meedikaalaa Yuunivarsiitii Jimmaattii yaalsisaa jiraniif qophaa'edha.

Mata-duree Qorannoo:	**Dhiibbaa Baakteeriyaa 'kaampiloobacter' jedhamu ijoollee waggaa shanii gadii dhibee garaa kaasaan qabamanii irratti qabu qorachuu ta'a.**	
Qorataa:	**Gosa barnoota:**	**Lakk. bilbilaa**
Yaareed Nugusuu	Medical Microbiology	09 21 20 46 63

Gucni Gaaffii Fedhii Hirmaannaa kun Kutaalee lama qaba: Isaanis

- Kutaa waa'ee qorannichaa gadi-fageenyaan ibsu fi
- Kutaa ragaa fedhii hirmaannaa mallattoodhaan ibsu

Kutaa 1ffaa: - Waa'ee qorannichaa

Seensa

Ani ragaa funaanaa qorannoo mata duree armaan olitti geggeeffamu kanaa yoon ta'u, dhibeen garaa kaasaa baakteeriyaa 'kaampiloobaakter' jedhamuun dhufu daa'imman wagga shanii keessatti baay'inaa mul'ata. Kanaafuu mucaan keessan qorannoo kana irratti akka hirmaatuuf fedhii keessan ibsuuf waa'ee qorannoo kanaa gadi fageenyaanan isiniif ibsuu barbaada. Murtoo hirmaanaa mucaa keessanii amma kana murteessuun dirqama miti, yeroo itti amantanitti murtessuu dandeessu. Nama isin barbaaddan waliinis waa'ee qorannoo kanaa mar'achuu dandeessu. Gaaffiilee isin hubachuu dhabdaniifis an isiniifin ibsa.

Kaayyoo qorannichaa

Biyyoota guddataa jiran kan akka Itoophiyaa keessatti ijoolleen waggaa shanii gadii yeroo baay'ee dhukkuba garaa kaasaatiin ni miidhamu. Haa ta'u malee hanqina qorannoo fi sababoota adda addaatiin kan ka'e ka'umsi dhibee garaa kaasaa isaanii maal akka ta'e ifa miti. Kaayyoon qorannoo kanaas gaheen baakteeriyaa 'kaampiloobacter' jedhamu dhibee garaa kaasaa fiduu keessatti hagam akka ta'eefi sababoota ijoolleen waggaa shanii gadii baacteriyaa kanaaf akka

saaxilam taasisan qorachuu ta’a. Bu’aaleen qorannoo kanaas qaamoleen dhimmi kun ilaallatu fi maatiin ijoollee sababoota adda bahan kanaf ijoolleen isaanii akka hin saaxilamneef xiyyeeffaannoo akka kennan taasisa.

Akkataa Filannoo Hirmaattota Qorannoo kanaa

Mucaan keessan qorannoo kana irratti akka hirmaatuuf kan filatame, yeroo kana dhibee garaa kaasaa waan qabuufii jiraataa naannoo kanaa fi maatiin keessan tajaajilamaa giddugala medikaaalaa yuunivarsiitii Jimmaa waan taataniif qofadha. Haaluma kanaan ijoolleen waggaa shanii gadii dhibee garaa kaasaan qabaman hundi, yeroo qorannoo kana keessatti carraa qorannoo kana irratti hirmaachuu ni qabu.

Duraa duuba qorannichaa

Mucaan keessan qorannoo kana irratti akka hirmaatuuf yoo eyyamtan, gaaffillee afaanii tokko tokko kan isin gaafannuu fi qorannoon laaboraatorii mucaa keessanii akka taasifamu kan nu gargaartan ta’a.

Dhiibbaa Qorannoo Keessatti Hirmaachuun Ykn Hirmaachuu Dhabuun Qabu

Sababa mucaan keessan qorannoo kana irratti hirmaateef/tteef rakkoon isinirra gahu tokkoyyuu hin jiru.

Faayidaa Qorannoo Keessatti Hirmaachuun Qabu

Qorannoo kana keessatti hirmaachuu keessaniif faayidaan kallattiin isin argattan hin jiru. Haa ta’u malee bu’aan qorannoo kana irraa argamu isiniifis ta’ee hawaasa naannoo keessaniitiif dhukkuba garaa kaasaa daa’imman waggaa shanii hir’isuuf gahee guddaa taphata.

Iccitii Ragaalee

Ragaaleen qorannoon kanaaf jecha isin nuuf laattanis ta’ee kan nuti qoranne arganne hundi iccitiidhaan kan eegaman yoo ta’u, waraqaa gaaffii kana irratti maqaan keessanis ta’ee kan mucaa keessanii kan hin caqasamnee fi koodii namni biraa beekuu hin dandeenye kan qabuudha.

Mirga Hirmaachuu Dhabuu

Yoo hirmaannaan mucaa keessaniitti hin amanne ta’e, fedhii hirmaannaa agarsiisuun dirqama miti. Qorannoo kana irrattti hirmaachuuf fedhii dhabuu kessaniin kan walqabee tajaajila yaala gahaa argachuu keessan irratti dhiibbaa tokkollee kan hin qabne ta’uu hubadhaa. Isiniif mucaan keessan tajaajila barbaachisu hunda argachuuf mirga guutuu qabdu.

Namni Quunnamuu barbaaddan yoo jiraate

Maqaa**: Yaareed Nugusuu**

Lakk bilb**: 09 21 20 46 63**

Email: **nyared@yahoo.com**

Kutaa 2ffaa: Waraqaa Ragaa Fedhii Hirmaannaa

Akkaataa odeeffannoo armaan olitti naaf dubbifame kanaan qorannoo mata duree “Dhiibbaa Baakteeriyaa ‘kaampiloobacter’ jedhamu ijoollee waggaa shanii gadii dhibee garaa kaasaan qabamanii irratti qabu” jedhu irratti aniif mucaan koo akka hirmaannuf fedhiin qabu mallattoo kootin nan mirkanneessa.

Maqaa Maatii Mucaa_____

Mallattoo_____

Guyyaa_____

Annex VII - Laboratory Procedures

Stool sample collection, Transportation and Processing

- Stool samples were taken using clean disposable swabs, transferred, and mixed into 6ml Cary-Blair transport media. The procedure was repeated until the mixture becomes heavily turbid; the swab was immersed within the media and the tube was recapped.

  NB: Fecal samples should be liquid or semi-formed (i.e. take the shape of the container).

- Approximately 3g of stool samples were collected.
- Specimens were transported and processed as soon as possible within 2 hours of collection.
- Samples were placed in the refrigerator in case delay was anticipated

Inoculation

- The samples were mixed within the tube by a vortex
- Campylobacter agar plate was inoculated using swab placed in the Cary-Blair medium at the edge of the medium and then distributed/streaked by a sterile wire loop.

Figure 4: Inoculation of stool sample of under-five children with diarrhea on *Campylobacter* agar media

Incubation

- The plates were then incubated micro-aerobically at 42^{o}C for 48 hours

Figure 5: Incubation of an inoculated medium at microaerobic environment using anaerobic jar and 3.5L sachet

Identification

Preliminary identification of *Campylobacter* species from primary culture was by the colonial appearance, gram stain, catalase test, and oxidase test.

Gram Staining

Campylobacter species are not easily visualized with the Safranin counterstain normally used in the Gram stain procedure; therefore, carbon fuchsin or 0.1% aqueous basic fuchsin can be used as the counterstain, or extending the staining time of the Safranin to at least 10 minutes can improve the intensity of the stain.

- Smears were prepared and heat-fixed by passing three times over the flame
- Slides were flooded with 0.5% crystal violet
- After a minute, the slides were tilted and rinsed gently with distilled water
- Then, the slides were covered with grams iodine for 1 minute
- The slides were again tilted and rinsed gently with distilled water

- Using ethanol or acetone, the slides were decolorized until the color ceases to run out of the smear and rinsed with water
- The slides were flooded with 0.1% counterstain carbol fuchsin for 1 minute
- The slides were thoroughly washed with water and allowed to dry
- Slides were examined using an oil immersion objective, morphology and gram reaction were observed
- **Interpretation:** Gram-negative organisms staining pink/red and having a gullwing shaped/ curved/s-shaped appearance under a microscope.

Results

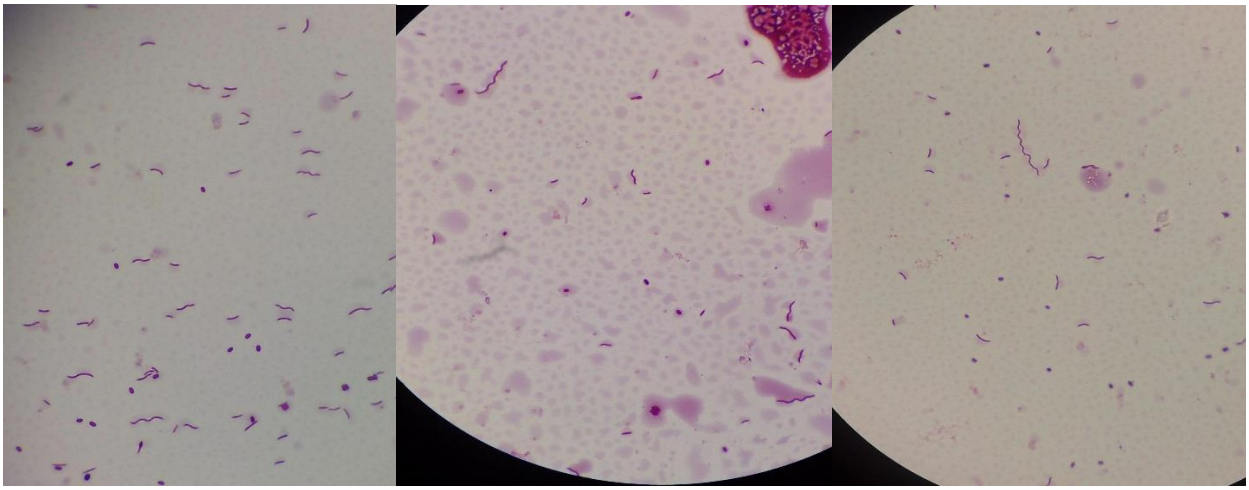

Figure 6: Gram staining of *Campylobacter* species isolated from stool samples of under-five children with diarrhea with a sea-gull wing, spiral, S-shaped and curved appearance

Catalase test

- Suspected colonies were picked carefully with wire/loop
- Rubbed on clean slides
- Two drops of hydrogen peroxide solution were placed on a colony previously smeared on a slide
- Bubble formation was observed
- **Interpretation**: Vigorous bubbling indicates the presence of catalase.

Result

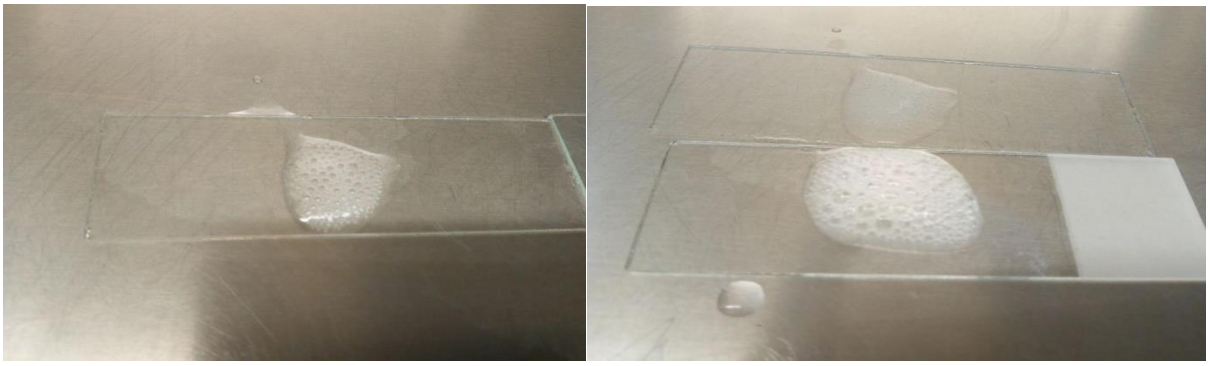

Figure 7: Catalase-positive reaction of *Campylobacter* species isolated from under-five children with diarrhea at JMC

Oxidase test

- A filter paper was soaked with a few drops of oxidase reagent.
- Colonies of the test organisms were smeared on the filter paper.

NB: When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple color.

- The color change was observed within 10s
- **Interpretation:** The formation of a deep purple-blue/blue color indicates oxidase production (oxidase positive).

Result

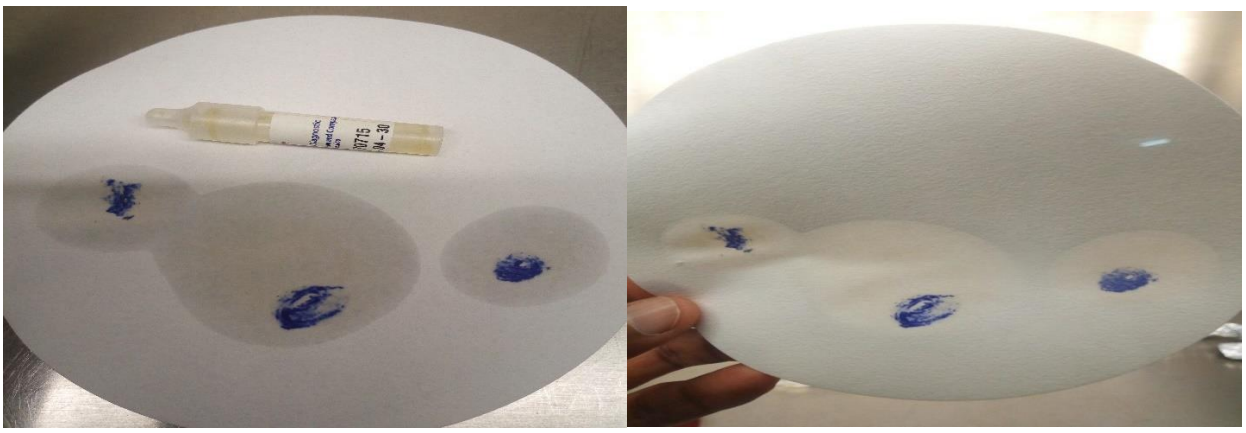

Figure 8: Oxidase-positive reactions of *Campylobacter* species isolated from a stool sample of under-five children with diarrhea at JMC

Inoculation of suspected colonies of *Campylobacter* species on Blood agar

- Blood agar plates were prepared from blood agar base and 5% sheep blood
- Suspected colonies were inoculated using a sterile wire loop
- The media were incubated at the micro-aerobic environment for 48 hours
- **Interpretation:** Presence of non-hemolytic grayish colonies

Results

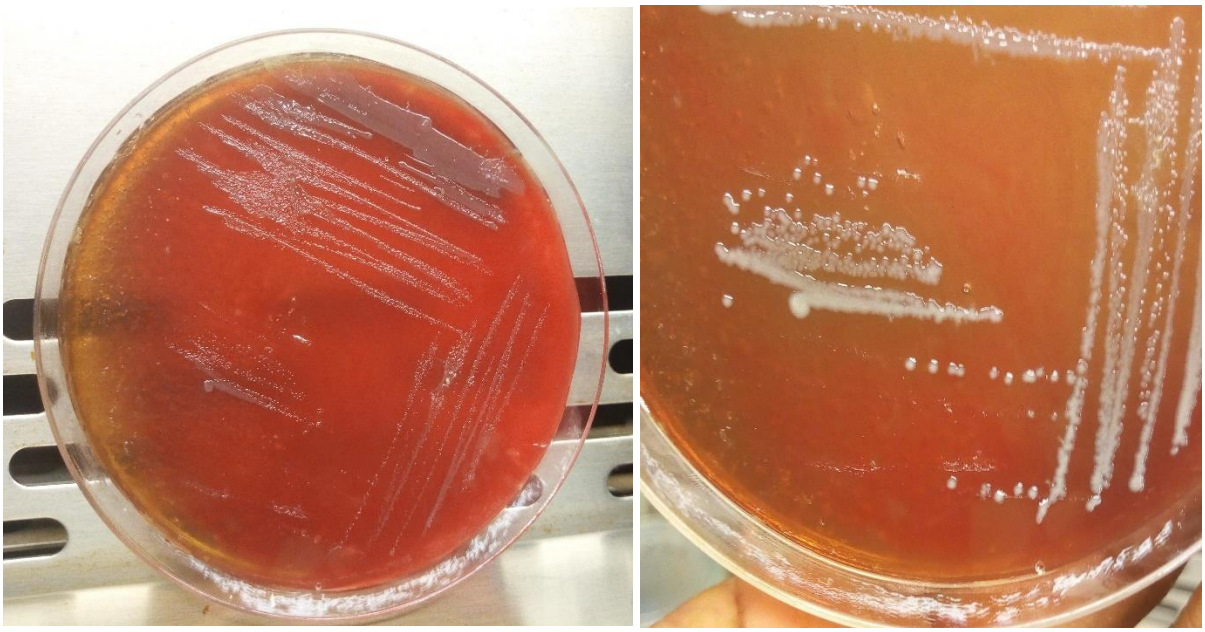

Figure 9: Non-hemolytic colonies of *Campylobacter* species isolated from under-five children with diarrhea on blood agar media

Antibiotic Susceptibility Testing

Disk diffusion Technique

Medium: Mueller-Hinton agar supplemented with 5% defibrinated sheep blood was used. The Mueller-Hinton plates were dried before inoculation to reduce swarming

Inoculum: Bacterial suspension of turbidity equivalent with 0.5McFarland standard was prepared. This was prepared by mixing 1 or 2 colonies in sterile normal saline

Inoculation: A sterile cotton swabs were immersed within the bacterial suspension. Then, swabs were rubbed on the wall of the tube to remove excess fluid, and the whole surface of Mueller-Hinton plates was streaked homogenously with the swab.

Application of disks: Disks of selected antibiotics were placed over inoculated plates using forceps.

NB: - The number of disks to be placed on a single plate should not be more than six to be evenly dispersed.

Incubation: Plates with antibiotic disks were incubated at a micro-aerobic environment, at 42ºC for 24 hours and the zones of diameter were measured using a caliper.

Interpretation

Table 9: CLSI breakpoints for antimicrobial susceptibility pattern of *Campylobacter* species isolated from under-five children with diarrhea at JMC from January to April 2020.

Selected Antibiotics		**Code**	**Disk concentration (µg)**	**Zone diameter breakpoint(mm)**		
				S ≥	**I**	**R ≤**
Quinolone and Flouroquinolone	Ciprofloxacin	CIP	5	21	16-20	15
	Nalidixic acid	NA	30	19	14-18	13
Macrolide	Erythromycin	E	15	23	14-22	13
	Azithromycin	AZM	15	13	-	12
Phenicol	Chloramphenicol	C	30	18	13-17	12
Penicillin	Ampicillin	AMP	10	17	14-16	13
Beta-lactam/beta-lactamase inhibitor	Amoxicillin + clavulanate	AUG	30	18	14-17	13
Cephalosporins	Cephalothin	CEF	30	18	15-17	14
	Ceftriaxone	CTX	30	23	20-22	19
Carbapenem	Meropenem	MEM	10	23	20-22	19
Aminoglycoside	Gentamycin	GEN	10	15	13-14	12