ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF ENTEROPATHOGEN AMONG UNDER-FIVE CHILDREN WITH DIARRHEA IN YEKATIT 12 HOSPITAL MEDICAL COLLEGE, ADDIS ABABA, ETHIOPIA

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Research Project Submission Form

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Contents

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Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
AST	Antimicrobial Susceptibility Test
CDC	Centers for Disease Control and Prevention
DCA	Deoxychocolate Citrate Agar
MAC	MacConkey
MUAC	Middle Upper Arm Circumference
NICU	Neonatal Intensive Care Unit
ORS	Oral Rehydration Solutions
RBCs	Red Blood Cells
SOPs	Standard Operating Procedures
SPSS	Statistical Package for the Social Sciences
TCBS	Thiosulfate Citrate Bile Salts Sucrose Agar
UNICEF	United Nations International Children's Emergency Fund
WHO	World Health Organization
WBCs	White Blood Cells
XLD	Xylose Lysine Deoxycholate Medium
VIP	Ventilated Improved Pit Latrine
TPL	Traditional Pit Latrine

Abstract

Background: Globally, diarrheal disease is a common condition associated with high morbidity and mortality in under 5 children. Every day, it is estimated that more than 4000 children lose their lives due to diarrhea. Despite its significant public health concern, little is known about prevalence and Antibiotic Susceptibility Patterns of enteropathogen in many countries, including Ethiopia.

Objectives: To determine Prevalence and Antibiotic Susceptibility Patterns of Enteropathogen among under-five children with diarrhea in Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia

Methods: A hospital-based cross-sectional study design was employed from October to march 2021 among 411 conveniently selected diarrheic patient in Yekatit 12 hospital Addis Ababa, Ethiopia. Data were collected by using Structured questionary. Stool samples was examined for parasites and enteric bacteria. Each bacteria then identified using colony morphology and standard biochemical tests. Anti-microbial susceptibility testing was performed by using disc diffusion techniques on Muller Hinton Agar. The collected data were analyzed using SPSS version 25 software package. To identify factors associated with enteropathogen, Chi-square ($\chi 2$) test was applied statistical significance was considered at level of significance of 5%.

Result: Among 411 examined stool samples, in more than a quarter (106(25.8%)) of the samples enteropathogen was detected. Of those majority (90(84.9%)) were intestinal parasites the rest (16(15.1%)) were positive for bacteria. Entamoeba histolytica and salmonella were the predominant enteropathogen which accounts for (51(12.4.7%)) and (10(2.4%)) respectively. unprotected drinking water source(P=0.002), poor hand washing practice of care giver (P=0.020) and poor Cleaning of utensil for child feeding(P=0.025) were statistically significant for the presence of enteropathogen in stool. Among patients who had Salmonella and shigella infections almost all were resistant for ampicillin and amoxicillin. But all the identified Salmonella and shigella isolates were sensitive to Ciprofloxacin and Ceftriaxone.

Conclusion and Recommendation: The finding of this study indicates that Entamoeba histolytica and salmonella species were the predominant etiologies and the presence of

bacterial isolates resistant to the commonly prescribed drugs for treating diarrhea in children Therefore, carry on identified associated factors and regular drug susceptibility test is essential to reduce the problem.
Key words: Prevalence, Enteropathogen, Diarrhea in under-Five Children

1. Background

1.1 Introduction

According to world health organization (WHO), Diarrhea is characterized by three or more loose or liquid stools per day due to abnormally high fluid content of stool or an abnormal increase in daily stool fluidity, frequency, and volume from what is considered normal for an individual [1]. Diarrheal diseases have been a major public health concern of low-income countries leading to high morbidity and mortality among under-five children [2]. In developing countries, diarrhea is constantly a symptom of gastrointestinal infection caused by parasites, bacteria or viruses. Regularly, these pathogens are transmitted through the feco-oral route, where the pathogens are excreted from the intestinal tract of a person or animal carrying the pathogens and are ingested by another [12]

It is estimated that 3.5 billion people around the world are infected in diarrhea from this number the majority of which are children. These infections cause diarrhea in 30-80% of patients, and the most common pathogenic agents include Giardia lamblia, Entamoeba histolytica, Cyclospora cayetanensis, Cryptosporidium, Ascaris lumbricoides, Trichuris trichiura, and Hymnolepis nana. Bacterial pathogens, including Escherichia coli, Shigella species, Salmonella species, Proteus species, Yersinia species, Vibrio cholera and Campylobacter species as well as virus such as Adenovirus, Norwalk virus and Rota virus [13] are some of the common infectious agents which cause enteric disease in under-five children.

Diarrheal illness remains the leading reason for morbidity and mortality among under-five children worldwide. Every day, above 4000 children lose their lives due to diarrhea [3]. Every year, as predicted 2.5 billion cases of diarrhea occur among under five children, and estimates suggest that all incidence has remained comparably stable over the past two decades, likewise half of these cases are in Africa and South Asia [4].

The highest mortality rate was in sub-Saharan Africa and Asia, and if this trend continues, it is estimated that 4.4 million children under the age of five will die from infectious diseases by 2030 [5,6]. The World Health Organization (WHO) reported that Africa and South-East Asia account for 78% of all diarrheal deaths among

children in the developing world. Sub-Saharan Africa, which incorporates Ethiopia, has the very best rates of child mortality because of diarrhea. Poor environmental conditions, socioeconomic status, behavioral factors, overcrowding and low maternal education, poor sanitation, contaminated water, failure to continue breast feeding until one year of age, using infant bottles that are tough to clean, failure to dispose of feces hygienically, storing food at room temperature, inadequate food hygiene, and failure to clean hands were related with a high incidence of diarrheic diseases [7].

Although there is global recession in the rates of children younger than 5 years old, the risk of a child dying before becoming 5 years of age remains highest in the WHO African Region 90 per 1000 live births, which is approximately seven times higher than in the WHO European Region which is 12 per 1000 live births [9].

In Ethiopia, the morbidity rate reports and community-based studies indicate that diarrheal diseases are a major public health problem that causes high morbidity and mortality among children and it is the second killer of under-five children next to pneumonia [8, 10].

1.2 Statement of the problem

The main risk factors for the mortality-morbidity of diarrhea in developing countries are known and related to lack of sanitation, poor quality of life and clean water supply [14]. These factors may lead to a significant disease burden and negative economic effects, resulting from medical costs, loss of work, lower quality of life and high mortality [14].

Infectious diarrhea, a typical disease of children, deserves permanent observance in all social teams. Infectious diarrhea continues to be a health burden worldwide, particularly in children living in developing countries [15].

In low-income countries, under five years old children's experience on average three episodes of diarrhea every year. Each incident deprives the child of the nutrition necessary for growth. As a result, diarrhea is a major reason of malnutrition, and undernourished children likely to fall ill from diarrhea (16).

Diarrhea because of infection is widespread throughout the developing world. In step with WHO, in Southeast Asia and Africa, diarrhea is responsible for as much as 8.5% and 7.7% of all deaths respectively. In Africa, it has been estimated that every child has five incident of diarrhea per year and that 800,000 children die each year from diarrhea and dehydration [16].

In Ethiopia, diarrhea is one of the most common reasons for children to visit health care clinics [6]. Surveys conducted among under-five children in Ethiopia revealed five diarrheal incident per child per year. Released studies conducted between 1994 and 2000 in Ethiopia on the prevalence of under-five diarrhea showed that the variability of the disease across the country are 11.4% to 37% [6] respectively. Despite the emphasis given by the Ethiopian ministry of health and the respective regional health offices to improve child health still there are many children are dying due to easily preventable and treatable diarrheal disease in Ethiopia.

The aim of this study is to determine Prevalence of Enteropathogen and Antibiotic Susceptibility Patterns among under-five children with diarrhea in Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia

1.3 Significance of the study

Diarrheal disease is a common condition seen in the emergency room. And also, it is a public health problem that affects many people all over the world. Identifying the Prevalence of enteropathogen, associated risk and antibiotic Susceptibility patterns may contribute in reducing morbidity and mortality.

The findings from this study would serve as an input data for policymakers in planning and implementing interventions associated with prevention and management of diarrheal disease. The finding of AST would also help clinicians take in to account and be curious about prescribing antibiotic. Moreover, the findings of this study could serve as baseline data for further research in this area.

2. Literature review

2.1 Epidemiology of diarrheal infection

Diarrhea is a leading killer of children, accounting for 9% of all deaths among children under age 5 worldwide in 2015. This translates to over 1,400 young children dying each day, or about 526,000 children a year, despite the availability of simple effective treatment [15,17].

In Africa, it's been estimated that every child has five episodes of diarrheal symptom each year and that 800,000 children die each year from diarrhea and dehydration. Each year, associate degree calculable 2.5 billion cases of diarrhea occur among children under five years of age, and estimates recommend that overall incidence has remained comparatively stable over the past twenty years, over half these cases are in Africa and South Asia, where about of diarrhea are more likely to result in death or other severe outcomes [16]. Diarrheal infection is widespread throughout the developing world. According to WHO, in Southeast Asia and Africa, diarrhea is responsible for as much as 8.5% and 7.7% of all deaths respectively [18].

Diarrhea is also the second most cause of mortality and morbidity of under-five childhood illnesses in sub-Saharan Africa countries.[16] Despite the substantial reduction in child mortality between 2000 and 2010 it is estimated that globally 800,000 children under the age of 5 years die annually, mostly in sub-Saharan Africa and South Asia [5].

The United Nations Children's Fund (UNICEF) has disclosed that Diarrhea kills about 2195 children every day which is more than AIDS, malaria, and measles combined [16]. It records 7.6 million deaths in children younger than 5 years in 2010, 64% which 4.879 million were attributable to infectious causes. Between 2000 and 2010, the global burden of deaths in children younger than five years decreased by 2 million, of which pneumonia, measles, and diarrhea contributed the most to the overall reduction 0.451 million, 0.363 million, and 0.359 million respectively [7]. It is estimated that diarrhea accounted for 9.9% of the 6.9 million deaths among children under 5 in 2011 [19,21].

Diarrhea is a highly prevalent disease in Ethiopia. Published studies conducted between 1994 and 2000 in Ethiopia on the prevalence of under-five diarrhea showed the variability of the diseases across the country which is 11.4% to 37% respectively

[8]. In these countries, only 40% of people living in urban setting were accessing improved sanitation and 72% of the people in Ethiopia were living without improved sanitation facilities [16]

2.2 Etiology agents involved in diarrheal infection

A few older studies provide relevant etiological data. In the early 1980s, microbiologists could isolate the causative agent of diarrhea in only 20% of cases, but now they can also isolate an agent in about 80% of cases [18]. Since then, they identified more enteropathogens. Some enteropathogen causing bacteria are Shigella, Salmonella, Vibrio cholere, Escherichia coli, Yersinia and Aeromonas. Rotavirus is also a common virus causing diarrhea. Protozoans also cause significant diarrhea including Entamoeba histolytica, Giardia lamblia, and Cryptosporidium species [21]. In developing countries 50-60% cases are of bacterial like Escherichia coli 25%, Campylobacter jejuni 10-18%, Salmonella spp. and Shigella spp 5% each, 35% of viral 15-25% rotavirus origin [22]. Intestinal parasitic infections are the most common infections among children in developing countries. Giardia, Cryptosporidium parvum and Entamoeba histolytica are the most common protozoan parasites that cause acute diarrheal illnesses in children. The main clinical manifestation of infections with Intestinal Parasites is diarrhea, with abdominal cramping, vomiting, flatulence and weight loss also being common symptoms [23]

2.3 Predisposing factors for diarrheal infection

As CDC stated in 2014, About 88% of diarrhea -associated deaths are attributable to unsafe water, inadequate sanitation, and insufficient hygiene, limited knowledge about diarrhea prevention and treatment, limited access of vaccines.

Inadequate and unsafe water, lack of sanitation, poor hygiene practice has a grim impact on child mortality in particular and public health [24]and it’s a complex issue for different disease and accountability for the occurrence of 90% of diarrheal diseases. Approximately 1.5 to 2.2 million people die each year from diarrhea and related disease due to poor sanitation, open defecation, lack of awareness of hygienic practices, and using contaminated water [25].

Ethiopia is one among the developing sub–Saharan African countries sharing the high burden of diarrhetic morbidity and mortality. The incidence of sicknesses contributory to avoidable deaths diarrhea is higher in Ethiopia compared to other Sub Saharan

African nations partially because of living conditions, high incidence of unwellness, lack of safe drinking water, sanitation and hygiene, similarly as poorer overall health and nutritional status [26]

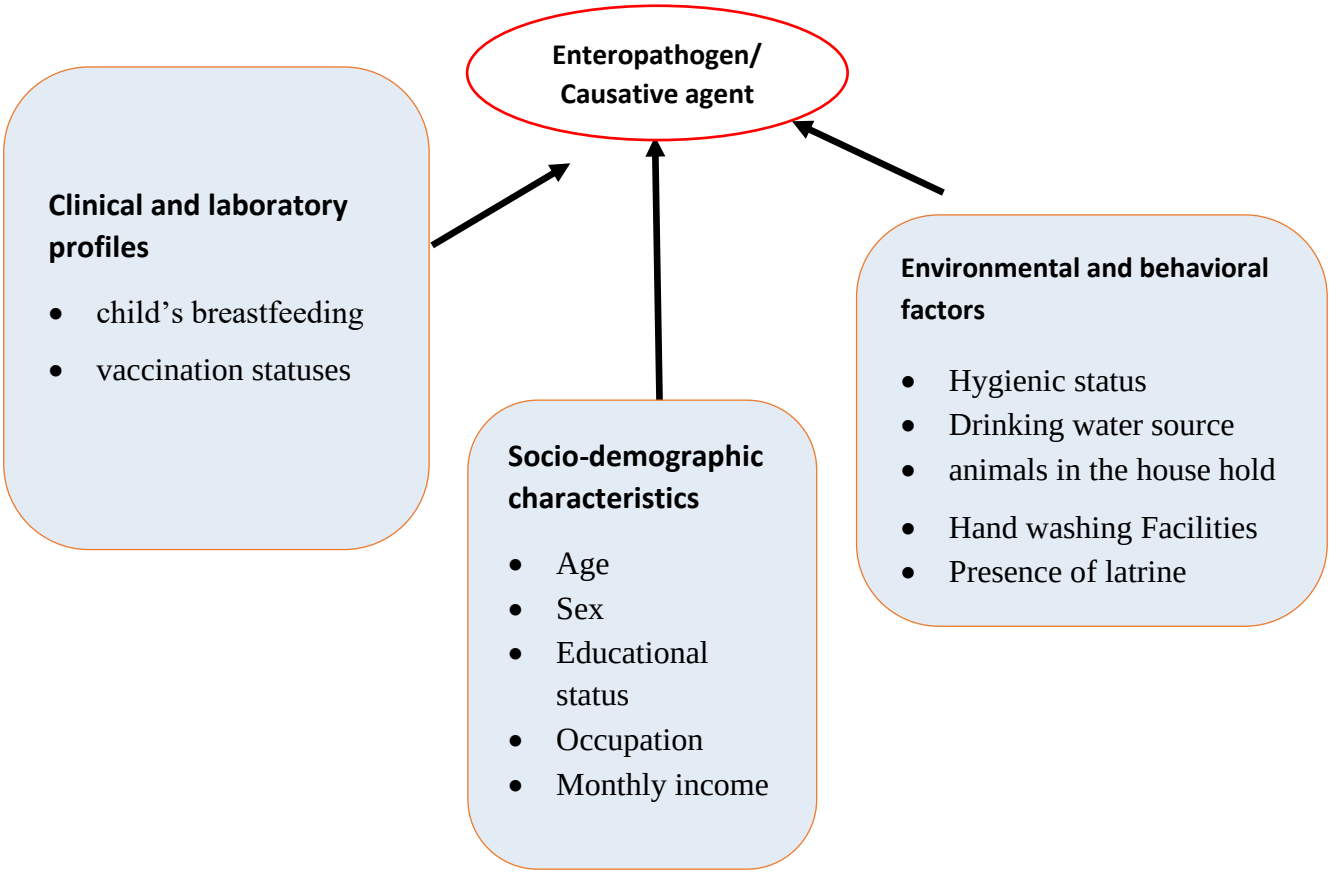
Even though poor environmental conditions typically believed to increase the danger of diarrhetic morbidity, there was a discrepancy of findings in studies conducted in several parts of Ethiopia on the results of majority of the environmental variables on the incidence of childhood diarrhea [27]. This discrepancy is due to the variation in socio-cultural and economic environments of households from place to place. This directly affects the behavioral patterns of mother and ability of getting access to water and sanitation service [28]

Different studies in Ethiopia showed that, socioeconomic status, monthly financial income, range of under-five children, strategies of complementary feeding, varieties of water storage equipment, mother's poor hand washing practices, lack of hand washing facilities, duration of breast feeding and improper waste disposal practices were significant factors for diarrhea occurrences [29]

The Ethiopian ministry of health has been troubled to manage the morbidity and mortality of children because of poor sanitation, inadequate safe water and poor hygiene practices by formulating and implementing totally different policies and methods [29].

Conceptual frame work

Relationship between dependent and independent variable



3. Objectives

3.1 General objective

To determine the Prevalence and Antibiotic Susceptibility Patterns of Enteropathogen among under-five children with diarrhea in Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia from October to March 2021.

3.2 Specific Objective

- To assess the prevalence of intestinal parasites and bacterias in under 5 children with compliant of diarrhea.
- To determine associated factors with enteropathogen.
- To determine Antibiotic Sensitivities profile for enteropathogen bacteria.

4. Material and Methods

4.1 Study Area and period

The study was conducted in Yekatit 12 hospital medical college, Addis Ababa, Ethiopia from October to March 2021. The hospital is a teaching hospital managed by Addis Ababa administrative health bureau. It is located in Arada sub city of city government of Addis Ababa. The hospital is established in 1915 with a total of 25 beds and 27 health professionals. It is the only hospital under the city administrative health bureau of Addis Ababa with high number of neonatal and pediatric beds and providing services for high number neonates and pediatric patients. It is also the only hospital under the city administrative health bureau of Addis Ababa, where there are culture and drug susceptibility test services available. According to the data obtained from the hospital, currently the hospital has 725 health professionals and 375 administrative staff with around 272 beds and provides different medical services for around 4 million people. According to current profiles, Pediatrics and NICU have totally 86% beds and have around 165 admissions per month.

4.2 Study Design

A hospital based cross-sectional study was carried out

4.3 Population

4.3.1 Source population

All under five children attended Yekatit 12 hospital medical college

4.3.2 Study population

Under 5 Children attended Yekatit 12 Hospital from October to March 2021 with complaints of diarrhea.

4.4 Inclusion and exclusion criteria

4.4.1 Inclusion Criteria

- All the children less than five years of age voluntarily accepted to participate in the study.
- The stool samples were collected from those patients who did not take antibiotics before attending the hospital.

4.4.2 Exclusion Criteria

- Patients who take antibiotics before 2 weeks of visiting the hospital.
- Any patient did not accept to participate in the interview and collection of stool sample from his or her child.

4.5 Study variables

4.5.1 Dependent variables

- The causative agent diarrhea in under 5children

4.5.2 Independent variables

- Socio-demographic variables
  - Socio-demographic variables of the guardian (Marital status, educational status, Occupation, average monthly income)
  - Socio-demographic variables of the child (age, Sex)
- Environmental and behavioral factors:
  - sanitation, hygiene, and water-related factors, and
- Clinical and laboratory profiles:
  - Fever, Dehydration, Duration of diarrhea
  - stool appearance

4.6 Measurement and Data collection

4.6.1 Sample size determination

The sample size was calculated using the formula for single proportion sample size calculation by taking a 95% confidence interval level and a 5% margin of error .and proportion or prevalence from previous study is 55.8% [6] and it becomes 374 sample size.

$$N=\frac{Z^2P(1-P)}{D2}$$

Where Z=95% Confidence interval (1.96)

P=Prevalence from previous study (55.8%)

D=Marginal of sampling error

N=Minimum sample size

$$=\frac{(1.96)^2\ 0.558(1-0.558)}{0.05^2}=\frac{3.8\times0.558(0.442)}{0.0025}=374$$

Therefor by adding 10% non-response rates, a total of 411 study subjects was participated in the study.

4.6.2 Sampling method

Convenience sampling methodology was applied within the study as a result of its comparatively simple and inexpensive to conduct. By this way, all children less than five visiting Yekatit 12 hospital was selected into the study.

4.6.3 Data collection procedure

A minimum sample size of 411 study participants was calculated and enrolled in the study.

To avoid ambiguous answers, a questionnaire with clear and straightforward questions was designed. The questionnaire has different sections: a section on demographic and socioeconomic characteristics; a section on knowledge of diarrhea by the mothers; a section on sanitation and rubbish disposal; a section on hygiene related practices; and a section on breastfeeding and vaccination status of the children. The questionnaire was developed in English language and translated into Amharic, the main language for communication in the country.

Face-to-face interviews based on the questionnaire was conducted on mothers of the children. Written informed consent was obtained from interviewees/participants parents / guardian after complete explanation of the study content and purpose. Interviews was conducted on the day of admission. Interviewers informed interviewees that participation in the study are voluntary. Interviewers explained the purpose of the study and asked interviewees for their permission to interview and collect stool samples from their children. Interviewees also informed that the information they provided has handled as confidential and their individual answers would not be known, except by the interviewer and the coordinator of this study.

4.6.4 Sample collection and processing

Stool samples were collected from children with acute diarrhea. These samples were collected in stool cap in the toilet.

Isolation and characterization of parasites were examined by preparing a slide using normal saline to observe the ova, cysts and trophozoite stage of the parasites presents in the stool. Samples has examined under a magnification of 40x to detect ova and cysts of parasites. Each Stool samples was concentrated using a formyl-ether technique for identification of some parasites. For bacteriological examination each stool sample for bacterial isolation were plated on to culture media in the Microbiology. For isolation of Salmonella and Shigella cultured on MacConkey agar (Mac), Xylose Lysine Deoxycholate medium (XLD), and Deoxycholate citrate agar (DCA) [6]. All plate then incubated overnight at 37c.Confirmatory identification was done by the pattern of biochemical reactions using a standard bacterial identification system. All the suspected isolates were examined biochemically using indole test, urease test, glucose test, maltose, lactose fermentation test, H2S, and motility test to identify the significant characteristic of bacteria according to the standard methods [6]. Based on culture results and biochemical characteristics, bacterial isolates were identified to the genus level.

Antimicrobial susceptibility patterns were determined by disk diffusion which is also known Kirby Bauer disk method. The bacterial inoculum has uniformly spread using sterile cotton swab on sterile petri dish Mueller Hinton Agar. Each disc must press down to ensure complete contact with the agar surface. The plates were incubated for 18-24 h at 37∘C which was done according to the guideline of Clinical and

Laboratory Standards Institute (CLSI). The antibiotics tested against bacteria were the commonly prescribed antibiotics. These were Ampicillin (AMP, 10µg), Amoxicillin (AMX, 10µg), Chloramphenicol (C, 30µg), Ciprofloxacin (CIP, 5µg), Gentamycin (GEN, 10µg), Ceftriaxone (CRO,30µg), Tetracycline (TET,30µg), Norfloxacin (NOR,10µg) and According to the size of the zone of inhibition, the organisms were classified as sensitive, intermediately, or resistant to each antibiotic using CLSI interpretation guideline [30].

4.7 Data Quality Assurance

The reliability of study is guaranteed by implementing quality control measures throughout the whole process of data collection and laboratory works.
Data was collected by pretested questioner and clean sample collection materials. Data collectors are identified, trained and informed to collect the data as per the pre structured questionnaire, and interviews are conducted by some selected assistants. Also, they were trained in how to use the instruments and how they should introduce themselves and the research objectives modesty to the guardians /parents during the interview. The purpose of the study as well as any related harm and benefits was explained to the study participants accordingly.
The reliability of study is guaranteed by implementing quality control measures throughout the whole process of data collection and laboratory works.
For laboratory analysis, Pre-analytical, Analytical and Post-analytical stages of quality assurance that is incorporated in standard operating procedures (SOPs)was strictly followed.

4.7.1 Pre-analytical phase

First of all, we are asked the parents/guardians verbally and by written consent/assent for their willingness and then we filled all the information on the performed questionnaire. Finally, by labeling the stool cup/container with participant identification number and information we informed them to bring the sample. The specimen quality assured by stool specimen rejection criteria of the hospital laboratory which is indicated in SOPs, following collection, specimen was transported to microbiology laboratory immediately

4.7.2 Analytical phase

The sample was analyzed at Yekatit 12 hospital which is located in 6kilo.The test was performed by the well experienced laboratory technicians/technologist. The collected sample are tasted once for bacterial culture and for intestinal parasite. All materials, equipment and procedures were adequately controlled.

4.7.3 Post-analytical

The results are recorded with identification number in order to avoid the errors in the results of the test, the reporting was repeatedly checked and evaluated by the head of the department. The laboratory results are given freely of charge for parents/guardians at the end of the test. For children with positive results, we are linked to the hospital for medical treatments.

4.8 Data analysis and Interpretation

After data collection each completed forms were checked for completeness and consistency then exported to SPSS version 25 for analysis. Mean, and standard deviation were used to describe Continuous data; and frequency and percentage were used to describe categorical data. Chi-square ($\chi 2$) test was applied to see if there was any association between the different categorical variables related to enteropathogen. Statically significant association was declared at p-values <0.05. Finally, the results were presented using text, table, and charts.

4.9 Ethical Considerations

Ethical clearance was obtained from departmental research and ethics review committee (DRERC) of the medical laboratory sciences of Addis Ababa University and from Addis Ababa public health researcher.

Ethical approval was obtained from the concerned authorities of the Yekatit 12 hospital for sample collection.

All the participants are asked for permission in the interview and they are not forced to participate. We are deal only to those accepted to participate and those did not accept are left freely without any objection. They are guaranteed that the provided information was confidential and disclosed to anyone.

4.10 Dissemination of result

The findings of this result are forwarded to department of Medical Laboratory Sciences, school of allied health science, and college of health sciences Addis Ababa University. The findings are also presented and manuscript was sent to peer reviled journals for publication.

The test results are recorded on the logbook and disseminated to clinicians for patient's treatment after the completion of the study, the research is disseminated to ministry of health, Addis Ababa university, Addis Ababa health bureau. It is also submitted to scientific publication.

4.11 Operational Definition

Acute diarrhea: Is an abnormally frequent discharge of semisolid or fluid fecal matter from the bowel, lasting fewer than 14days.

Diarrheic children: Refer to participating children who had acute diarrhea during the two weeks prior to the survey.

Enteric pathogens: Are microbes that are able to cause enteric disease of which more than 3 or more unformed stools per day and any documented intestinal infection associated with disrupted intestinal absorptive and barrier function.

Caregiver hygiene: Is personal hygiene of the caregivers like short finger nail cut or long finger nail which could be factors for the occurrence of diarrheal disease among children.

Hand washing during critical time: Refers to caregivers' hand washing practice after utilization of latrine, before food preparation and child-feeding as identified by caregivers' oral report of their practice to identify its relationship with diarrheal occurrence

Protected water: A type of water source that, by nature of its construction or through active intervention, is likely to be protected from outside contamination, in particular from contamination with fecal matter

Unprotected water: A type of water source that, by nature of its construction or through active intervention, is likely to be unprotected from outside contamination, in particular from contamination with fecal matter

5. RESULTS:

5.1 Demographic and Socio-economic characteristics

In this study, a total of 411 under-five children with acute diarrhea were included making a response rate of 100 %. Regarding the guardian's information majority 342(83.2%) of the guardians were married, more than half of them 213(51.8%) completed primary school, the Majority 294(71.5%) of them were unemployed, almost three-forth 297(72.3%) of the guardian's had monthly income less than 500. Among the 411 under-five children, 199 48.4%) were male and 212 (51.6%) were female resulting in a male-to-female ratio of 1:1.

Table 1 Socio-demographic data of the guardians in Yekatit 12 Hospital, Addis Ababa, Ethiopia from October to March 2021

Variable		Frequency (n=411)	Percent %
marital status	Single	28	6.8%
	Married	342	83.2%
	Divorced	38	9.2%
	Widowed	3	0.7%
educational status	Illiterate	30	7.3%
	Primary school	213	51.8%
	Secondary school	154	37.5%
	College	14	3.4%
Occupation	Unemployed	294	71.5%
	Employed	60	14.6%
	Self-employed	57	13.9%
Income	<500	297	72.3%
	500-1000	17	4.1%
	>1000	97	23.6%

The mean age of the study participants was 2.76 years (SD±1.34). More than half 259 (63.0%) of the patients were between the age group of 25 to 60 month. The detection rate of enteropathogen in the age group 25 to 60 months was 30.5 %, significantly higher than that in children in the other groups (P=0.006).

Variable		Frequency	Percent %
Age in month	0-12	48	11.7%
	13-24	104	25.3%
	25-60	259	63.0%
Sex	Male	199	48.4%
	Female	212	51.6%
Fever	Yes	114	27.7%
	No	249	60.6%
Vomiting	Yes	2	.5%
	No	409	99.5%
Dehydration	Yes	6	1.5%
	No	405	98.5%
Stool appearance	Watery	253	61.6%
	Mucoid	127	30.9%
	Bloody	31	7.5%

Table 2 Sociodemographic data of under-five children with diarrhea

5.2 Prevalence of enteropathogen

Among 411 examined stool samples, in more than a quarter 106(25.8%) of the samples enteropathogen was detected. Most 90(21.9%) of them were intestinal parasites the rest 16(3.9%) were positive for bacteria.

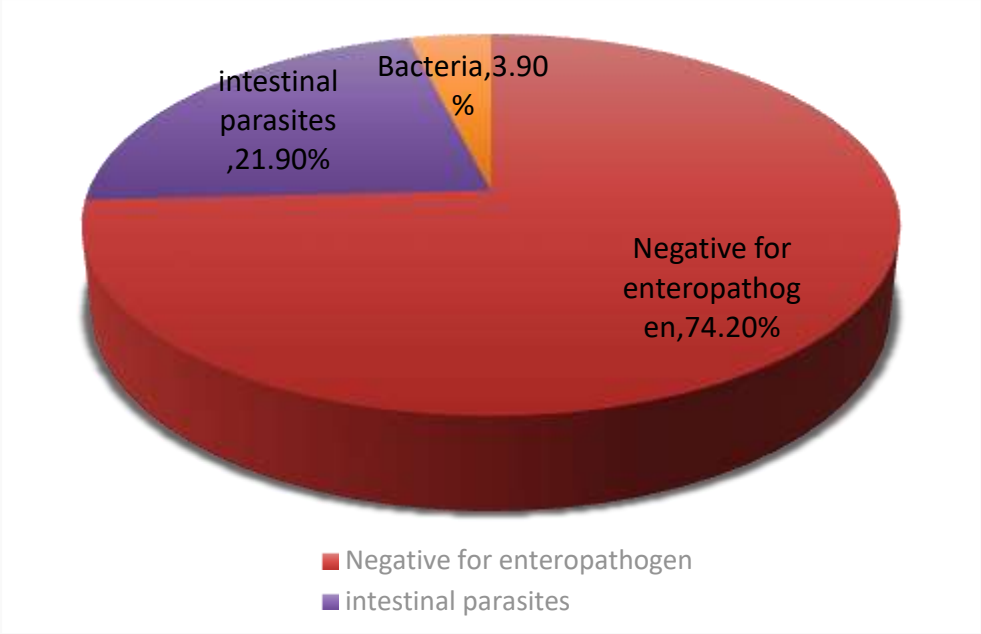
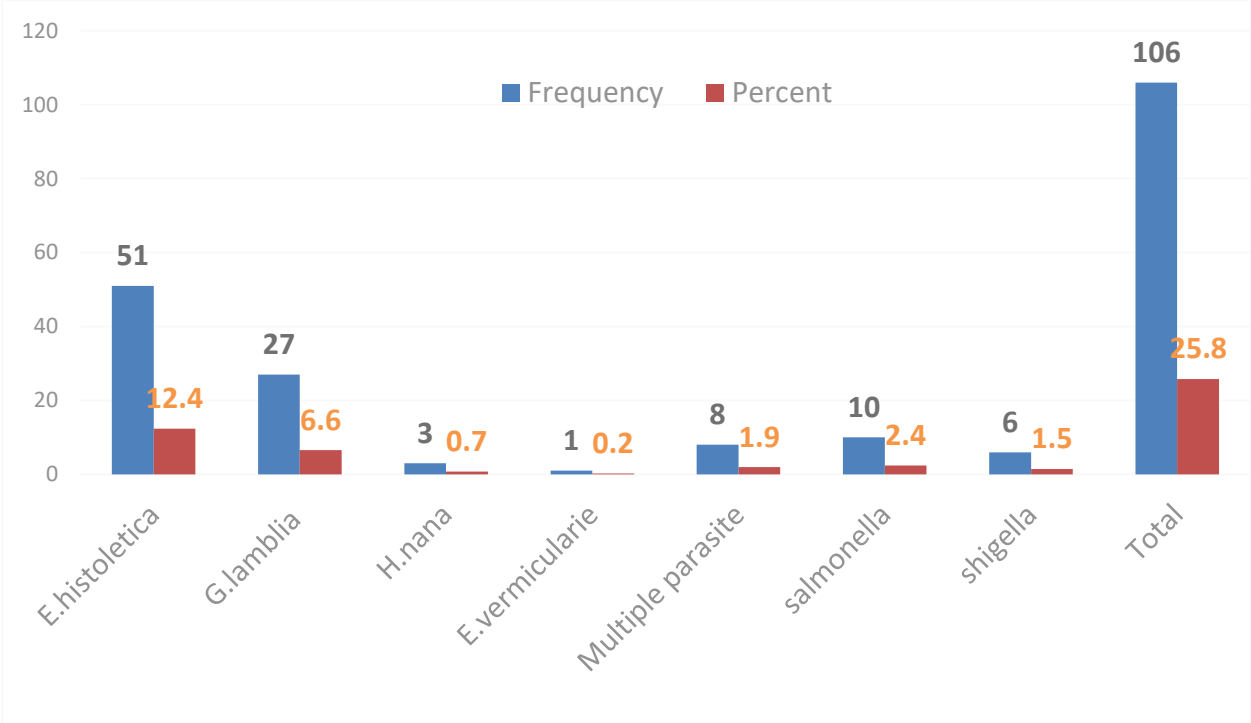


Figure 1. Prevalence of enteropathogen in under-five children with a compliant of diarrhea in yekatit 12 Hospital, Addis Ababa, Ethiopia from October to March 2021

Among parasite, Entamoeba histolytica was predominant with detection rate of 51(12.4.7%), followed by Giardia lamblia 27(6.6%), Hymnoleps nana 3(0.7%), multiple parasites 8(1.9%) and Enterobius vermicularis 1(0.2%) (Fig 1). In the case of bacterial isolate, salmonella was most predominant which accounts for more than half 10(2.4%) of the cases whereas Shigella accounts for 6(1.5%) (Fig 1).



Vertical line: -shows random number

Horizontal line: -shows types of entropathogen found on the study

Figure 1. Magnitude of identified Enteropathogen in under-five children with a compliant of diarrhea in Yekatit 12 Hospital, Addis Ababa, Ethiopia from October to March 2021

5.3 Associated factors for enteropathogen

In this study, the majority 396 (96.4%) of participants were uses unprotected water for drinking and 324(78.8%) of the care givers has poor hand washing practices. Around 231(56.2%) care giver had a good hygienic status and 321 (78.1%%) of them did not cleaning utensils for their child's. Only 17.2% of mothers/caregivers uses ventilated improved pit latrine the rest 82.7% are uses traditional pit latrine.

Associated factors related to child diarrhea were assessed, and it was found that unprotected drinking water source(P=0.002), poor handwashing practice of caregiver(P=0.020) and poor Cleaning of utensil for child feeding(P=0.025) were statistically significant for the presence of enteropathogen in stool samples (Table 3). There was no statistically significant difference between Socio-demographic data of the guardians and the presence of enteropathogen in the stool of under 5 children. The details of the sociodemographic data of the guardians are presented below.

There was no statistically any significant difference between gender and Presence of enteropathogen in the stool (P=0.879).

Table 3 Environmental and behavioral factors in under-five children with a compliant of diarrhea in Yekatit 12 Hospital, Addis Ababa, Ethiopia from October to March 2021

Variable		Presence of enteropathogen in stool		P-value
		Yes	No	
marital status	Single	6(21.4%)	22(78.6%)	0.432
	Married	87(25.4%)	255(74.6%)	
	Divorced	13(34.2%)	25(65.8%)	
	Widowed	0(0.0%)	3(100.0%)	
educational status	Illiterate	7(23.7%)	23(76.7%)	0.620
	Primary school	53(24.9%)	160(75.1%)	
	Secondary school	44(28.6%)	110(71.4%)	
	College	2(14.3%)	12(85.7%)	
Occupation	Unemployed	75(25.5%)	219(74.5%)	0.659
	Employed	18(30%)	42(70%)	
	Self-employed	13(22.8%)	44(77.2%)	
Income	<500	75(25.3%)	222(74.7%)	0.655
	500-1000	6(35.3%)	11(64.7%)	
	>1000	25(25.8%)	72(74.2%)	

Variable		Frequency (n=411)	Percent %	Presence of enteropathogen in stool		P-value
				Yes	No	
HH drinking water	Protected	15	3.6%	9(60.0%)	6(40.0%)	**0.002**
	Un protected	396	96.4%	97(24.5%)	299(75.5%)	
Hand washing Facilities presence	Yes	349	84.9%	89(25.5%)	260(74.5%)	0.750
	No	62	15.1%	17(27.4%)	45(72.6%)	
Presence of latrine	Yes	387	94.2%	100(99.8)	287(74.2)	0.927
	No	24	5.8%	6(25.0%)	18(75.0%)	
Type of latrine	TPL	321	82.7%	79(24.6%)	242(75.4%)	0.518
	VIP	67	17.2%	21(31.3%)	46(68.7%)	
Latrine cleaning frequency	Every day	32	8.9%	13(21.0%)	49(79.0%)	0.661
	1-2/week	151	42.2%	37(24.5%)	114(75.5%)	
	Never	175	48.9%	50(28.6%)	125(71.4%)	
HH environmental cleaning frequency	Every day	69	16.8%	14(20.3%)	55(79.7%)	0.519
	1-2/week	205	49.9%	55(26.8%)	150(73.2%)	
	Never	137	33.3%	37(27.0%)	100(73.0%)	
Hygienic status of care giver	Poor	180	43.8%	41(22.8%)	139(77.2%)	0.218
	good	231	56.2%	65(28.1%)	166(71.9%)	
Care giver hand washing practice during critical time	Yes	87	21.2%	14(16.1%)	73(83.9%)	**0.020**
	No	324	78.8%	92(28.4%)	232(71.6%)	
Cleaning of utensil for child feeding	Yes	90	21.9%	15(16.7%)	75(83.3%)	**0.025**
	No	321	78.1%	91(28.3%)	230(71.7%)	
animals in the house hold	Yes	194	47.2%	54(27.8%)	140((72.2%)	0.370
	No	217	52.8%	52(24.0%)	165(76.0%)	

5.4 Antibiotic Sensitivity Patterns

Regarding antimicrobial susceptibility, among 411 stool samples 16 samples were culture positive for bacteria. Of those 10 of them were positive for Salmonella species the rest 6 were positive for Shigella species. From six isolates of shigella species all of them has shown resistance for ampicillin and amoxicillin and all of the strains were sensitive to ciprofloxacin, and ceftriaxone. Among patients who had Salmonella infections almost all were resistant for ampicillin and amoxicillin and 7 ,5,3 and 2 of the isolates were resistant to Tetracycline, Chloramphenicol, and Gentamycin. But all of the Salmonella isolates were sensitive to Ciprofloxacin and Ceftriaxone. Norfloxacin and Cotrimoxazole shows slightly sensitive to salmonella.

Table 4, Antimicrobial susceptibility patterns of Shigella, and Salmonella isolates among under five years of children in Yekatit 12 Hospital,Addis Ababa, Ethiopia

	Salmonella spp(10)			**Shigella spp(6)**			**Total (16)**		
Antibiotics	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
AMP	0	0	10	0	0	6	0	0	16
CIP	10	0	0	6	0	0	16	0	0
CRO	10	0	0	6	0	0	16	0	0
AMX	0	0	10	0	0	6	0	0	16
TET	3	0	7	1	1	4	4	1	11
C	2	3	5	2	1	3	4	4	8
GENT	5	2	3	4	2	0	9	4	3
NOR	7	2	1	3	2	1	10	4	2

S sensitive, R resistant, I intermediate TET(Tetracycline), AMX(Amoxicillin), CIP(Ciprofloxacin), GEN(Gentamicin), AMP (Ampicillin, C(Chloramphenicol), NOR(Norfloxacin), CRO(Ceftriaxone)

6. Discussion

Diarrhea remains one of the major illnesses in under-five children, and enteropathogens play significant roles as etiologic agents. In this study, the burden of bacterial and parasitic agents in children suffering from diarrhea and drug susceptibility pattern of bacterial isolates were investigated. The isolation of enteropathogens was statistically associated with poor hand washing practices, drinking of unprotected water and poor cleaning of utensils used to feed the children. Enteropathogens were detected in 25.8% of the patients in this study. Specifically, regarding the prevalence of bacteria, studies in other parts of the world show more variation, some reporting lower rates and some reporting higher rates. The higher rates were from, Mozambique, 42.2% (44), Burkina Faso [48] and Palestine, 57.9% [47]. However, the similar rate is in another report from Trinidad [49], which found a prevalence of 17.4%.

Despite the relatively high prevalence of parasitic infection observed in our study (21.9%), low prevalence (3.9%) of bacterial infections was seen among children with diarrhea. The low prevalence of bacterial infection in the present study could be due to differences in the types of enteropathogen assayed in the study, true difference of the bacterial infection in the area and also seasonal variations for example, our study was conducted in the time of corona virus pandemic in the world and there were a few numbers of patients are visited the hospital some of them are frightened the contamination and transferring of the pandemic while coming in hospital and the other was their parents are started taking care of more for themselves and also for their children's from exposing to the virus that might be a reason for getting a low prevalence of entropathogens

Among parasitic infections, Entamoeba histolytica and Giardia lambilia respectively were relatively higher than other parasites 12.4% and 6.6% respectively. This may be because of their nature for easily transmitting in the community. The other parasites will follow by 3% and 1% Hymenelopis nana and E.vermicularis respectively .

But the current study showed a lower prevalence of intestinal parasite infection as compared to those studies conducted in Cuba 45.2% [42], Egypt 47.3% [43]and Addis Ababa 27.5%, Wondogenet 85.1% [44].

Among the overall prevalence of bacteria which counts 16(3.9%) of them positive cultures 10(2.4%) children were found to be infected with

Salmonella species and 6(1.5%) with shigella species. On prevalence of salmonella our finding was comparable with previous reports in Korem,ethiopia[33], Addis Ababa[34]Hawassa[23],kenya[35] with 2.01%,3.95%,1.5% and 2.5% respectively and lower than Bahir Dar, North Ethiopia [36] and Arba Minch, South Ethiopia [37] with 7.8% and 12.6% respectively. The prevalence of Shigella in stool samples was found to be 1.5%. Our study has lower prevalence of Shigella compared to the study conducted in Bahir Dar [34], Mekelle [36], south Ethiopia [39] and Gaborone [40] with prevalence of 7.85, 13.3, 8.3 and 21% respectively. In contrast, the study conducted in Nigeria–Benin Teaching Hospital, is similar to our finding (1.4%) [41]. The isolation of enteropathogens was statistically associated with poor hand washing practices ,drinking of inadequate and unsafe water ,lack of sanitation, poor hygiene practice have a grim impact on child mortality in particular and public health [24]and it's a complex issue for different disease and accountability for the occurrence of 90% of diarrheal diseases(25).On this study we can only get significant effect on hand washing practices, drinking of unsafe water and using of unclean utensils for feeding of Childs. The incidence of sicknesses contributory to diarrheal disease is higher in Ethiopia compared to other Sub-Saharan African nations partially because of living conditions, lack of safe drinking water, sanitation and hygiene, similarly as poorer overall health and nutritional status [26]. That might be due to the variation in socio-cultural and economic environments of households from place to place. This directly affects the behavioral patterns of mother and ability of getting access to water and sanitation service [28]. However, a more extensive community-based study is needed in order to explore the etiologic importance of this pathogen in diarrheic children. providing education and reach out the critical components are valuable to reducing the burden of diarrhea observed in this study.

Antimicrobial resistance by enteric pathogens is of major concern because of indiscriminate use of drugs. In most areas of developing countries, knowledge of antibiotic resistance profiles among various bacterial isolates would be highly valuable, as it can help inform antibiotic choices by healthcare professionals. Our findings showed that 100% of the Shigella and salmonella isolates were resistant to ampicillin and amoxicillin and the susceptibility of Salmonella and shigella isolates shows ciprofloxacin and ceftriaxone (100%) was higher and consistent with some studies in Ethiopia and elsewhere. Salmonella isolates revealed reasonably high rate of resistance to a number of commonly used antibiotics in Ethiopia such as,

Tetracycline (70%), chloramphenicol (50%) and Gentamycin (30%). and in shigella, Tetracycline (66.6%) and chloramphenicol (50%). The high resistance of bacterial isolates in this study may be due to misuse of therapeutic doses of commonly available antibiotics. The absence of Salmonella And shigella isolates resistance for ciprofloxacin and ceftriaxone in the present study suggests that ciprofloxacin and ceftriaxone are commonly used to treat children with Salmonella infections, particularly invasive infections, because of its favorable pharmacokinetic properties and the low prevalence of resistance. Healthcare providers, together to reduce unnecessary use of antibiotics in order to reduce the risk of resistance. It is important to continue surveillance on these microorganisms.

7. Limitation of the study

The study was limited for further microbial tastes, intestinal parasites and viruses because of lack of the availability of resources

8. Conclusion And Recommendation

The Salmonella and Shigella isolates displayed high rate of resistance to commonly used drugs such as ampicillin, and amoxicillin and they were highly susceptible to ciprofloxacin and ceftriaxone drugs which is the choice recommended for diarrheal diseases caused by these pathogens in the hospitals. This study also revealed that the enteric pathogen infection was significantly associated in poor hand washing practice, drinking unprotected water and usage of unclean utensils for feeding of children. Therefore, improving hygiene status of under five children and implementation work on identified associated factors with regular drug susceptibility test is important to reduce the problem. The government should imply and ease the supply of getting protective water and improve the sanitary conditions of the peoples.

Researchers are recommended to conduct other analytical study designs like case control to overcome the chicken egg dilemma that can be the issue of this study

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10. List Of Annexes

Annex 1. Information sheet in English Version

I. English version of participant information sheet

Department of Medical Labratory Science, college of Allied Health Sciences,Addis Ababa University, Addis Ababa,Ethiopia,2020.

Title of the Research Project: Prevalence of Enteropathogen and Antibiotic Susceptibility Patterns among under-five children with diarrhea in Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia

Principal Investigator: Seada Bereka(BSc, MSc candidate)

Name of the Organization: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University

Introduction

You are invited to participate as a study subject in a research conducted by MSc candidate, from medical Laboratory Sciences department, Addis Ababa University. Your participation is voluntarily. The research teams will include one principal investigator, two advisors from Addis Ababa University microbiological department. Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to study the prevalence of entropathogens in under 5 children with diarrhea.

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis, monitoring of response to treatment, epidemiological surveillance, prevention as well as research. Especially there is a shortage of information about the risk factors of diarrhea that's the reasons for children to visit health-care centers, but knowledge of the causative agent of these diarrhea cases is also limited. Therefore, the purpose of this proposed study is to know the exact causative agents and risk factors of diarrhea in under 5 years of children in Addis Ababa, Ethiopia. You have been chosen for this study. Therefore, we invite you to take part in this study and contribute to the establishment of indigenous reference values. The values are needed for providing quality laboratory service. Thus, result from this study is anticipated to

improve the health status of the children's at large in Ethiopia.

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimen collected from your child will be used for the research purpose, and the results of the sample will be exposed to some concerned professional staffs as it is needed. You are requested to give your consent to the sample collector. There will be a face-to-face interview for additional questions.

Procedures: After agreeing that you can take part, one or more of our research staff will ask you some questions which will take up to 5 minutes. Your child weight, height and vital signs will be measured. You will be asked to provide fresh stool on a particular container we provide. We will conduct laboratory examination to determine different parasitological and microbiological parameters.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. However, based on the diagnosis result your child will be treated in view of that. In addition, the result of the study will be beneficial for the decrease in child morbidity and mortality rate which is caused by diarrhea. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result free.

Contact information

If you have any questions about this study you can contact the following principal investigators and advisors for further information.

Name Seada Bereka

Phone: +251911306513

E-mail: seadabereka@gmail.com

Annex 2. Amharic version of information sheet

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል፡፡እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉም ሃሳብ ይጠይቁ፡፡

መግቢያ

የጥናቱ ርዕስ "**ከ አምስት አመት በታች ያሉ ህጻናትን የሚያጋጥማቸው ተቅማጥ መንስኤውን ለይቶ ለማወቅና ስላለው ስርጭት ምክንያት ለማወቅ የሚል ነው**".

የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡ በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም፡፡በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል፡፡

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ናሙናዎ ለጥናቱ እንዲሚዉል መስማማት ይጠበቅብዎታል፡፡ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል፡፡ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቸን አይጨምርም፡፡ ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል እርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡በተጨማሪም ስለስለ ልጅዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይኖርብዎትአል፡፡

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ችግሮች ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም፡፡

የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ፡፡ ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸው፡፡ከዚያም በላይ ስለ እርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል ፡፡

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኘው ውጤት ግን ተጠቃሚ ነዎት፡፡

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸው ?

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም ::ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለዎት::የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ:: ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን::

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ::

ሰአዳ በረካ

ሞባይል፡ +251-911306513

ኢሜል፡seadabereka@gmail.com

Annex 3. Informed consent form in English version

Card no…..……

I had been informed that the objective of this study is to know the causative agents and risk factors of under 5 children diarrhea. The results of this study have an importance to treat my child and other patients, and to be used as an input for the future development of strategies or guidelines for diagnosing of diarrhea in Ethiopia. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include stool sample, and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation results.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Signature: _____

Date_____

Annex 4. Informed consent form in Amharic version

የተሳታፊዎች ስምምነት ማረጋገጫ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ “prevalence of entropathogen in under five children with diarrhea at Yekatit 12 hospital, Addis Ababa, Ethiopia” ጥናት ላይ በቂ ገለጻ ተደርጎልኛል፡፡ ለጥናቱም የሰገራ ናሙና እንደሚያስፈልግ ተገልጾልኛል፡፡የጥናቱንም አላማዎችም ተረድቻለሁ፡፡

በቃለ መጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል ፡፡በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡፡

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ ፡፡የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡፡

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ፡፡ ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

ፊርማ---------- ቀን ----/---/----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የአማካሪ ነርስ ስም ---------- ፊርማ ----------

ቀን----------

Annex 5 English version of Questionnaire

I. Demographic and Socio-Economic Information

1 Identification number: …….

2. Address:……

3. Your age: ……years. The child's age: ……

4. The child's sex Male Female

5. Education status of the guardian:

1. Illiteracy
2. Primary school
3. secondary school
4 collage/University

6 Marital statuses of the guardian:

1 Single
2. Married
3 Divorced
4 Widowed

7.Occupation status of the guardian:

1. Unemployed
2. Employed
3 Self-employed

8. Monthly income per month of the guardian:

1. < 500 Birr
2. 500-1000 Birr
3. > 1000 Birr

II. Clinical data

1. Hospitalized on: ……/……. / 2020
2. Weight: ……kg. Height: ……cm.
3. Temperature: ……. 0 C.
4. Number of days with diarrhea: ……. Days.
5. Stool frequency per day: ……
6. Is content of the stool? Bloody Mucoid Watery
7. Has the child vomited? ☐ Yes No ☐

If yes, state vomiting frequency per day:

8. Patient's dehydration status: 1. None
2. Mild
3. Moderate
4. Severe

9. Did the child contact to any diarrheal patient for the last 7 days? 1 Yes 2 No

10 weaning age 1 One Breast feeding 2) <2 year 3)>2 year

11 Child immunization 1 Complete 2 In Complete

10. Did the child eat any food sold by street vendors for the last 7 days? 1 Yes 2 No

If yes state what food the child ate.....

11.Treatment before hospitalization.....

12.Treatment during hospitalization.....

13.Stool sample collected on: …../……/2021.

III. Environmental and behavioral factors

1 Drinking water source

1 Protected 2 Un protected

2 Hand washing Facilities presence

1 Yes 2 No

3 Presence of latrine

1 Yes 2 No

4 If yes for question no. 4 1 Traditional pit latrine 2 Ventilated pit latrine

5 Care giver hygienic status

1 Poor 2 Good

6 Care giver hand washing practice during critical time

1 Yes 2 No

7 Cleaning of utensil for child feeding

Poor good

IV laboratory data

Stool microscopic examination (Result)

A) Presence of enteropathogen Yes no

B) If yes for question 14A

Bacteria Parasite

If Bacteria specify…….

If Parasite specify……

Antibiotic Sensitivities test

Resistant for ----------

Susceptible for----------

Intermediate for……

Annex 6: Amharic version of Questionnaire

ከ አምስት አመት በታች ያሉ ህጻናትን የሚያጋጥማቸው ተቅማጥ መንስኤውን ለይቶ ለማወቅና ስላለው ስርጭት ምክንያት ለማወቅ በሚል የተዘጋጀ ቃለ መጠይቅ ::

የተሳትፎመሙያ

1.መለያ ቁጥር.....

2.አድራሻ.....

3.የልጅ እድሜ.....

4.ጾታ ወ ሴ ☐ ☐

5.የትት ደረጃ 1.መጀመርያ ደረጃ

2 .ሁለተኛ ደረጃ

3.ዲግሪ

6.የጋብቻ ሁኔታ 1.ያገባ

2.ያላገባ

3.ፍቺ የፈጸመ

7.የስራ ሁኔታ 1.የለም

2. ተቀጣሪ

3.የግል

8.የወር ገቢ 1.<500

2.500-1000

3.>1000

የህክምና መረጃ:

1.ሆስፒታል የመጣበት ቀን...../...../2020

2.ክብደት ..... ቁመት.....

3.የሙቀት መጠን.....

4.ተቅማጥ የጀመረበት ቀን.....

5.የ ሰገራ ድግምግሞሽ በቀን.....

6.ደም አለ ሰገራው ላይ አዎ የለም ☐ ☐

7.ልጅ ያስመልሰዋል? አዎ የለም ☐ ☐

አዎ ከሆነ በቀን ምን ያህል ጊዜ.....

8.የልጁ የመጠማት ሁኔታ 1.ምንም

2.ትንሽ

3.መካከለኛ

4. ከፍተኛ

9.በተቅማጥ ከተያዘ ታማሚ ጋራ ተገናኝቷል ላለፉት ሰባት ቀናት?

10.ልጆ መንገድ ላይ ያለ ምግብ ተመግቧል አዎ ☐ የለም ☐

አዎ ከሆነ ምን አይነት ምግብ.....

11.ሰገራ ናሙና የተወሰደበት ቀን...../...../2020

Annex 7: Declaration

I, the undersigned agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the research publications office.

M.sc.

Candidate: Seada Bereka

(b.sc)

Signature:

Date of submission .....

This thesis has been submitted with our approval as advisors.

Advisor: Dr.Abraham Tesfaye

(PhD)

Signature .....

Date .....

Place: Addis Ababa,Ethiopia

Advisor: Sosina Walelign

(MSc,PhD candidate)

Signature .....

Date: .....

Place:Addis Ababa,Ethiopia