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ENTERIC PATHOGENS AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE AMONG PEDIATRIC PATIENTS WITH DIARRHEA: A CROSS SECTIONAL STUDY IN SELECTED HEALTH FACILITIES, ADDIS ABABA, ETHIOPIA

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ABBREVIATIONS

AMR -Antimicrobial resistance

AST - Antimicrobial susceptibility testing

ATCC - American Type Culture Collection

CSA -Central Statistical Agency

E. coli - *Escherichia coli*

EHEC -Enterohemorrhagic *Escherichia coli*

HIV/AIDS -Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome

IMCI - Integrated Management-of Childhood illness

NCCLS -National Committee on Clinical Laboratory Standards

ORS -Oral Rehydration Salt

PFSA -Pharmaceuticitical Fund and Supply Agency

SMAC -Sorbitol MacConkey agar

Spp -Species

SPSS -Statistical Package for Social Science

SS -Shigella –Salmonella medium

TASH -Tikur Anbesa Specialized Hospital

TCBS -.thiosulfate citrate bile salt sucrose medium

WHO -World Health Organization

XLD -Xylose-Lysine-Deoxycholate medium

ABSTRACT

Background: Diarrheal disease remains a major public health problem in developing countries including Ethiopia. The current study was designed to isolate medically important enteric pathogens and assess the antimicrobial susceptibility testing of bacteria causing diarrhea in pediatrics for those antibiotics were prescribing in Integrated Management of Childhood illness (IMCI).

Methods: Across-sectional study to determine enteric pathogenic microorganisms that cause diarrhea and antimicrobial susceptibility profile was carried out in selected health facilities of Addis Ababa, Ethiopia from November 2016 to May 2017. Stool specimens from pediatric patients aged 0-14 years were collected randomly from two health centers and one specialized hospital to identify enteric pathogens. Antimicrobial susceptibility tests were performed on all bacterial isolates using the Kirby-Bauer disc diffusion method.

Results: In this study, the major etiologic agents of diarrhea in pediatrics were intestinal parasites and bacterial infection accounting for 93(32%) and 42(14.5%) respectively. Out of 290 study patients complain of diarrhea examined, *E.histolytica/dispar* 75(25.8%), *G.lamblia* 13(4.5%) *and H.nana* 4(1.4%) were identified. The majority of bacterial enteropahogens isolated in the study were *Shigella* spp 22(7.6%) followed by enterohemorrgic *E.coli* O157:H7 13(4.5%), *Salmonella* spp 7(2.4%). The overall co-infection rate between parasite-parasite, parasite-bacteria and bacteria-bacteria was observed in 12(4.1%) children. All the bacterial isolates from diarrheal patients were 100% susceptible to meropenem, cefepime, azithromycin and showed antimicrobial resistance to ampicillin, Augmentin, trimethoprim-sulphamethoxazole and ciprfloxacillin. *Salmonella* spp showed resistance to trimethoprim-sulphamethoxazole and chloramphenicol, 42.9% and 14.3% respectively. Another enteric bacteria *Shigella* spp were resistant to 77.3%ampicillin, 68.2% trimethoprim-sulphamethoxazole and36.4%Augmentin whereas *E.coli*O157:H7 resistance anti-biogram showed 69.2%ampicillin,46.1% trimethoprim-sulphamethoxazole,38.5%Augmentin, 23.1%ciprfloxacillin and Amikacin, ceftriaxone and gentamycin were resistant with the same rate of 15.4%..

Conclusion: The results showed that *E.histolytica*, *G.lamblia* and *H.nana* and bacterial isolates *Salmonella* spp, enterohemorrhagic *E.coli* O157:H7, *Shigella* spp were the most frequently isolated pathogens in Children. The most frequently prescribing drugs ampicillin, amoxicillin+clavulic acid and trimethoprim-sulphamethoxazole showed high resistance for *Salmonella* and *Shigella* isolates in the study. It was found that ciprofloxacin was the best drug of choice for the treatment of diarrhea caused by *Salmonella* and *Shigella*. Chloramphenicol was a drug of choice for the treatment of shigellosis. So it calls more attention to conduct extensive continuous surveillance to revise and update the prescribing policy in Integrated Management of Childhood illness and Clinicians should rely on stool culture and antimicrobial susceptibility testing.

Key words: Enteric pathogens, diarrhea, antimicrobial susceptibility, pediatrics, Addis Ababa, Ethiopia

1. INTRODUCTION

Diarrhea is the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual) [WHO, 2017]. Bloody diarrhea refers to any diarrhea episode in which the loose or watery stools contain visible red blood. Compared with watery diarrhea, bloody diarrhea generally lasts longer, associated with more complications, more likely to adversely affect a child's growth, and has a higher case fatality rate [Eldaif *et al.*, 2015].

A wide variety of bacterial, viral, and protozoan pathogens excreted in the faeces of humans and animals are known to cause diarrhea. Among the most important of these are *Escherichia coli*, *Salmonella spp*, *Shigella spp*, *Campylobacter jejuni*, *Vibrio cholera*, Rotavirus, Norovirus, *Giardia lamblia*, *Cryptosporidium sp.*, *and Entamoeba histolytica* [Akinnibosun*, et al*, 2015, Mikoleit, 2010 and Onyango *et al.*, 2010].

Among the viruses, rotavirus seems to be the most common viral infections. The contribution of the various pathogens of diarrhea may differ substantially between regions depending on local meteorological, geographic and socio-economic conditions. Food intolerance, reactions to medicines such as antibiotics and antacids containing magnesium may also contribute to diarrhea. Chronic diarrhea can be caused by chronic ethanol ingestion, though this kind of diarrhea is typical among children under 5 years old. The main cause of death from acute diarrhea is dehydration, which results from loss of fluid and electrolyte in stool [Akinnibosun *et al*, 2015].

The evolution of antibiotic resistance in bacteria is a topic of major medical importance. Antimicrobial resistance in enteric pathogens is of great importance in the developing world where the rate of diarrheal diseases is highest. The progressive increase in antimicrobial resistance among enteric pathogens in developing countries is becoming a critical area of concern. The resistance of enteropathogenic bacteria to commonly prescribed antibiotics is increasing both in developing as well as in developed countries. Resistance has emerged even to newer, more potent antimicrobial agents [Pavani, 2012].

Some of the factors leading to increased risk of diarrhea include failure to breast-feed exclusively for the first 4-6 months of life. Diarrhea has been noted to be far greater in non-breastfed than exclusively breastfed infants. Host susceptibility to infection is determined by the child's age,

presence of protective maternal factors (transplacental antibodies), nutritional and immunological status, prior exposure and acquired immunity and genetic susceptibility. Diarrhea as an opportunistic infection may result from immunological impairment due to current illness or immunodeficiency in persons with HIV/AIDS. In rare cases, overuse of antibiotics leads to overgrowth of commensal *Clostridium difficile* which releases a toxin that causes diarrhea. Moreover, some of the antiretroviral medications in HIV patients, particularly protease inhibitors, cause diarrhea as a side-effect. Children with diarrhea are at risk of dehydration and therefore early and appropriate fluid replacement is a main intervention to prevent death [RonoSalinah *et al.*, 2014].

1.1 Statement of the problem

Diarrhoeal disease is the second leading cause of death in children under five years old. It is both preventable and treatable. Each year diarrhea kills around 525 000 children under five [WHO, 2017]. It has been recognized since the beginning of civilization and remains one of the most prevalent public health problems of today. About two thirds of the world population lives in areas regarded as underdeveloped and it is estimated that over 1.3 billion cases of diarrheal illness occur each year in the underdeveloped countries, of these over 2.7 million deaths occur in children [Pavani, 2012].

The proportion of deaths attributed to diarrhea among children aged less than 5 years is estimated to be approximately 15% worldwide, and as high as approximately 25% in Africa and 31% in South East Asia. More than two dozen enteric pathogens, belonging to diverse branches of the tree of life, are known to cause diarrhea and can be tested for in a clinical setting. However, it is likely that additional pathogens remain to be identified among the enteric micro biota [Pop *et al.*, 2014]. In our country Ethiopia, diarrhea in children is the second leading top ten cause of death accounted 8% [Global health/CDC, 2016].

Although diarrhea mortality rate among children under 5 years of age in sub-Saharan Africa has been decreasing annually by about 4% since 2000, mortality remains high. Of the estimated 3.6 million child deaths in 2010, 12% were attributed to diarrheal diseases. The incidence of childhood diarrhea in Africa has also decreased from 4.2 to 3.3 episodes per child-year from 1990 to 2010, but sub-Saharan Africa still accounts for one third of diarrheal episodes yearly (500 million of 1.7 billion worldwide), with the highest incidence among children 6–11 months of age [Langendorf *et al*., 2015].

Acute diarrhea as a gastrointestinal related symptom may have some different causes such as infection. Infectious diarrhea leads to approximately three million deaths worldwide and 516 deaths in Iranian children younger than 5 years per year. The rate of enteropahogens isolation in acute diarrhea varied in different studies depending on the sampling methods and microbiological techniques. Some of them, the most common bacterial pathogen is diarrheagenic E. coli [Dooki *et al.*, 2014].

In Sao Paulo, Brazil, the etiologic profile of acute diarrhea in 154 children aging less than 5 years indicated that intestinal pathogens were Rotavirus; 32(20.8%), bacteria; 53(34.4%), both; 25 (16.2%), and 2(1.4%) with *Giardia* intestinal is (in one case associated with *Rotavirus* and in another one associated with bacteria). Altogether, there were 105 bacterial isolates; 90 were *Escherichia coli* (27 EPEC , 24 Diffuse adhering *E. coli* (DAEC) , 21 ETEC and 18 EAEC), 12 were *Shigella* spp., 2 were *Salmonella* spp., and one was *Yersinia spp*. Children with mixed infections (viral and bacterial) had increased incidence of severe vomiting, dehydration and hospitalization [Eghdami and Islami, 2014].

In a multicenter European study, 16 pathogens were identified in 65% of stool samples from children with acute diarrhea, a rate similar to that reported in developing countries. Many viruses and bacteria pathogenic to the intestine have been identified, of which rotavirus and pathogenic *Escherichia coli* are the most common. Rotavirus infections account for up to 60% and 40% of all diarrheal episodes in developing and developed countries, respectively, and an estimated 870 000 deaths in children every year. Rotaviruses most commonly cause diarrhea between the ages of 6–24 months, with severe infection occurring at a younger age in developing than in developed countries. Other important ones are *Campylobacter* spp, *Salmonella* spp, *Shigella* spp, and *Yersinia* spp. *Shigella* spp are the most important causes of acute bloody diarrhea (dysentery) and account for about 15% of all deaths attributable to diarrhea in children younger than 5 years. *Vibrio cholera* remains a major cause of epidemic diarrhea, especially where sanitation is compromised after a disaster. Non-agglutinating or non-O1 strains of *V cholerae*, previously thought to be non-pathogenic, have been identified as responsible for outbreaks of diarrheal disease [Paesi e*t al.*, 2012 ,Thapar and Sanderson, 2004].

Since most diarrheal diseases are treated empirically, it is important to know the susceptibility pattern of the prevalent pathogens. The problem of antimicrobial resistance in bacterial pathogens causing diarrheal diseases continues to be alarming. Emergence and spread of antimicrobial resistance to newer and more potent agents used in treatment have been described for *Salmonella, Shigella* and *Vibrio cholerae* [Kansakar *et al.*, 2011].

The antibiotic resistance patterns and also multidrug resistance rates of *Shigella* isolates in Iran among nine different commonly applied antibiotics were as follows: Trimethoprim-sulphamethoxazole (80.5%), ampicillin (63.8%), tetracycline (58.3%), gentamycin (36.1%),

chloramphenicol (33.3%), nalidixic acid (27.7%), and cefixime (16.6%). All the Shigella strains were susceptible to ciprofloxacin and ceftriaxone. Of the *Shigella* isolates, 47.2% were resistant to two or more antibiotic. The most common multidrug resistance pattern was to trimethoprim-sulphamethoxazole, ampicillin, and tetracycline [Jomezadeh *et al.*, 2014].

Non-typhoid *Salmonella* are re-emerging as one of the most important etiological agents of infectious diseases in the world. Multi-antibiotic resistance in non-typhoid *Salmonella* has been associated with enhanced virulence and excess mortality in patients compared with infection with sensitive strains. High rates of resistance to multiple antimicrobial agents (resistance to three or more classes of antibiotics) by enteric pathogenic were previously reported from Libya [Rahouma *et al.*, 2011].

While stool cultures and antimicrobial testing of the isolates are the best way to select the most adequate antimicrobial regimen, the results are only available after 72 hours or more. In some instances, it is possible to wait for the result; often cases improve substantially during this interval and the use of antibiotics is no longer required when the results become available, even if enteropathogenic bacteria are identified. In severe cases, however, it is advisable to start antimicrobials empirically as soon as stools are collected for culture [Diniz-Santos *et al.,* 2006].

In Ethiopia, stool culture is not well organized in health facilities and dominated by empirical treatment of pediatrics with diarrhea leads to the overuse of antibiotics and change in epidemiology and antimicrobial resistance of bacterial agents, so the results of this investigative work would provide useful data for choosing the appropriate antibiotics for empirical treatment. Thus, this study was designed to identify common bacterial and parasitic enteropahogens and antimicrobial susceptibility pattern for the efficacy of locally prescribed antimicrobial substances for pediatric patients in low routine culture isolation settings.

1.2 Significance of the Study

Since the isolation and antibiotic susceptibility not uniform throughout the health facilities for pediatric patient empirical treatment should be updated to minimize antimicrobial resistance. In brief the study will

- Increases the level of understanding on the common enteric pathogens and expand them in routine microbiology laboratory activity
- Provides updated information on susceptibility pattern of the isolates to avoid extensive use and misuse of antimicrobial drugs which have favored the emergence and survival of resistant strains of micro-organisms
- Increases awareness towards enteric pathogens and antibiogram for empiric treatment
- Can be used as a baseline for next studies in this line
- Can be a source of information for policy makers or decision makers in this area.

1.3 Literature review

13.1 Epidemiology of pediatric diarrhea

Diarrhea is a leading killer of children, accounting for 9 per cent 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. [WHO/UNICEF, updated 2017]. Globally, pneumonia and diarrhea are among the leading causes of child mortality. Together, these diseases account for 29% of all child deaths, causing the loss of more than 2 million young lives each year. In 2010, the number of child deaths from pneumonia and diarrhea was almost equal to the number of child deaths from all other causes after the neonatal period - that is, nearly as many children died from pneumonia and diarrhea as from acquired immunodeficiency syndrome (AIDS), malaria, measles, meningitis, injuries and all other post-neonatal conditions combined [UNICEF, updated 2017].

1.3.2 Pathogenesis

Microbial agents cause diarrhea by a number of mechanisms, several of which are Considered below [WHO, 1992].

Viruses

- Viruses, such as rotavirus, replicate within the villous epithelium of the small bowel, causing patchy epithelial cell destruction and villous shortening. The loss of normally absorptive villous cells and their temporary replacement by immature, secretory, crypt-like cells causes the intestine to secrete water and electrolytes. Villous damage may also be associated with the loss of disaccharides enzymes, leading to reduced absorption of dietary disaccharides, especially lactose. Recovery occurs when the villi regenerate and the villous epithelium matures.

Bacteria

- *Mucosal adhesion.* Bacteria that multiply within the small intestine must first adhere to the mucosa to avoid being swept away. Adhesion is through superficial hair-like antigens, termed pili or fimbriae that bind to receptors on the intestinal surface; this occurs, for example, with enterotoxigenic E. *col;* and *V: cholerae 01.* In some instances, mucosal adherence is associated with changes in the gut epithelium that may reduce its absorptive capacity or cause fluid secretion (e.g. in infection with enteropathogenic or

enteroaggregative E. *coli). Toxins that cause secretion.* Enterotoxigenic *E. coli,* V: *cholerae01* and some other bacteria produce toxins that alter epithelial cell function. These toxins reduce the absorption of sodium by the villi and may increase the secretion of chloride in the crypts, causing secretion of water and electrolytes (see Unit 2). Recovery occurs when the affected cells are replaced by healthy ones after 2-4 days. *Mucosal invasion. Shigella, C. jejuni,* entero- invasive *E. coli* and *Salmonella* can cause bloody diarrhea by invading and destroying mucosal epithelial cells. This occurs mostly in the colon and the distal part of the ileum. Invasion may be followed by the formation of micro abscesses and superficial ulcers; hence the presence of red and white blood cells, or visible blood, in the stool. Toxins produced by these organisms cause tissue damage and possibly also mucosal secretion of water and electrolytes.

Protozoa

- *Mucosal adhesion. G. larnblia* and *Cryptosporidiurn* adhere to the small bowel epithelium and cause shortening of the villi, which may be how they cause diarrhea.

1.3.3 Common etiologic agents of pediatric diarrhea

Salmonella species - is a member of the family enterobacteriaceae, facultative anaerobic Gram-negative rod which colonizes the intestinal tracts of vertebrates. Some serotypes, including Salmonella enterica subsp. Enterica serotype Typhi (Salmonella Typhi), are only found in human hosts. The majority of Salmonella cases occur as the result of ingesting contaminated food or water. Salmonella can also be acquired by contact with domestic animals and their food products, farm animals or animals in petting zoo, and exotic pets like turtles, hedgehogs, and iguanas [Humphries *e*t al., 2015]. Salmonella can also be transmitted from person to person via the oral-fecal route.

Shigella species- are host adapted to humans but have been documented in rare instances from dogs and primates. They can be acquired from ingestion of a variety of foods or water contaminated with human feces, sexually during oral-anal sex, or by laboratory workers. The four species of Shigella are Shigella dysenteriae, Shigella flexneri, Shigella boydii, and Shigella sonnei. Transmission by person-to-person contact is common for Shigella spp. because of a low infectious dose of 10 to 100 organisms [Humphries *e*t al., 2015].

A prospective cross sectional conducted in a teaching hospital in Abadan, Iran, during June 2011 to May 2013, Stool specimens were collected from pediatric age group. *Shigella* species were isolated from stool specimens of 36/705 (5.1%) pediatric age group, admitted in pediatric ward. The predominant serogroup was S. *flexneri* 19 (52.7%) followed by S. *sonnei* 11(30.5%), S.*boydii*4 (11.1%), and S. *Dysenteriae* 2 (5.5%). generally 392 (55.6%) of patients were female and 313 (44.4%) of them were male [Jomezadeh *et al.*, 2014].

Escherichia coli- *E. coli* O157 are small (0.5 x 2.0 μm), non-motile, Gram-negative, facultative anaerobic rods. Unlike most *E. coli*, *E. coli* O157 does not grow well at 44.5 °C, which requires modified detection. *E. coli* O157 causes non-bloody diarrhoea that may progress to bloody diarrhea and hemolytic uremic syndrome (HUS) with acute renal failure. Infected persons shed about 108 bacteria per gram faeces; the duration of shedding is 7-13 days [de Roda Husman *e*t al., 2010]. Numerous types of *E. coli* that cause diarrheal disease have been described, including enterotoxigenic strains, Enteropathogenic strains, enteroinvasive strains, enterohemorrhagic strains, and enteroaggregative strains. Of these different types, only enterohemorrhagic strains of serotype 0157:H7 can be routinely detected in most clinical microbiology laboratories because a specific selective medium, sorbitol-MacConkey agar is widely available [Hines and Nachamkin, 1996]. E. coli O157:H7 was first recognized as a pathogen in 1982 during an investigation into an outbreak of hemorrhagic colitis associated with consumption of hamburgers from a fast food chain restaurant [Riley et al., 1983].

The geographic distribution of these strains varies, and media for detection may be available in some laboratories and not in others. Sorbitol-negative strains can be further identified with specific serotyping reagents or toxin assays and *E. coli* 0157 was reported to be the most frequently isolated organism at the University of Calgary Clinical Microbiology Laboratory (Calgary, Alberta, Canada), accounting for 34% of all positive stool cultures[Hines and Nachamkin, 1996].

The study conducted on diarrheagenic *Escherichia coli* in high income country Israel shows that, fifty nine (19%) children tested positive for DEC, EAEC and atypical EPEC were most common, each detected in 27 (46%), followed by ETEC (n = 3; 5%), EHEC and typical EPEC (each in 1 child; 1.5%). Most EAEC isolates were resistant to cephalexin, cefixime, cephalothin and

ampicillin, and genotypic characterization of EAEC isolates by O-typing and pulsed-field gel electrophoresis showed possible clonal relatedness among some. The likelihood of having > 10 loose/watery stools on the most severe day of illness was significantly increased among patients with EAEC and rotavirus co-infection compared to children who tested negative for both pathogens [Tobias *et al.*, 2015].

Rotavirus - infections account for up to 60% and 40% of all diarrheal episodes in developing and developed countries, respectively, and an estimated 870 000 deaths in children every year. The genus Rotavirus, first identified in the duodenal mucosa of children with gastroenteritis by electron microscopy in 1973, is divided into groups A–E and further into serotypes G and P. Group A rotaviruses and specifically the G1, G2, G3, G4, and G9 serotypes are responsible for most infections. Rotaviruses most commonly cause diarrhea between the ages of 6–24 months, with severe infection occurring at a younger age in developing than in developed countries [Paesi e*t al.*, 2012, Thapar and Sanderson, 2004]. The faeces of an infected person can contain more than 10 trillion infectious particles per gram; only 10–100 of these are required to transmit infection to another person. Rotavirus is transmitted by the faecal-oral route. It infects cells that line the small intestine and produces an enterotoxin, which induces gastroenteritis, leading to severe diarrhea and sometimes death through dehydration [de Roda Husman *e*t al., 2010].

***Clostridium difficile*-**is an obligate anaerobic, spore forming Gram-positive rod. The spores of C. difficile are resistant to stomach acid, heat, and many commercial disinfectants used in hospitals [Humphries *e*t al., 2015]. It a major nosocomial pathogen that causes intestinal disease from uncomplicated antibiotic-associated diarrhea to severe, possibly fatal, antibiotic-associated colitis. In the last 5–7 years, a change in the epidemiologic pattern of *C. difficile* infection characterized by an increasing incidence and severity of infection has been observed. A few epidemiological studies recently conducted in the pediatric population demonstrated a two fold increase in the incidence of *C. difficile* infection in the last 5 years, but with no increase in the incidence of severe complications, such as the need for colectomy or mortality. The clinical presentation of *C. difficile* associated disease can range from asymptomatic carriage in the gastrointestinal tract and mild diarrhea to potentially fatal pseudomembranous colitis. Diarrhea is watery and usually non-bloody, but approximately 5%–10% of patients have bloody diarrhea. Fecal material typically contains excess mucus, and pus or blood may also be noted. The disease

may progress to a pseudomembranous colitis, possibly including intestinal perforation and toxic mega colon. Neonatal infections by *C. difficile* can be asymptomatic, but usually display fever, diarrhea, and irritability within 48 hours after production of the toxins [Ciccarelli *et al*., 2013].

The study conducted in India, of 280 children frequency of diarrhoeagenic bacteria isolated from the samples showed that *Escherichia coli* was recorded as the predominant bacteria with 44.2% of prevalence followed by *Shigella, Salmonella, Klebsiella* and *Campylobacter* with 28.2%, 13.6%, 7.8% and 6.1% respectively. Patients falling in the age group of 1-3 years were the major sufferers of diarrhea due to all etiologies except *Klebsiella* which mainly had impact on the patients below six months. Fever and vomiting were predominant symptoms. All 100% patients with *Salmonella* as etiology presented with fever. The maximum number of patients had frequency of diarrhea less than five times a day (52.5%). And among the patients who presented with frequency of more than 10 times a day *Salmonella* was a major causative agent found. Maximum patients presented with some dehydration (67.1%) and the patients who presented with severe dehydration *Salmonella* was identified as a major etiological agent [Rathaur *et al.*, 2014].

Another study conducted on bacterial and viral etiology of childhood diarrhea in Ouagadougou, Burkina Faso shows that at least one pathogen was detected in 64% of the 283 patients and in 8% of the 60 controls, Rotavirus was found in 30% of the patients, followed by diarrhoeagenic *Escherichia coli* (24%), *Salmonella enterica ssp*. *enterica* (9%), *Shigella spp.* (6%), adenovirus (5%) and *Campylobacter spp.* (2%). Multiple pathogens were found in 11% of the patients and in 2% of the controls. Viruses were found mainly in children of ≤ 2 years of age, whereas bacteria were equally prevalent among all the age groups. Viral infections occurred mostly during the cool dry season and the bacterial infections during the rainy season. Fever (64%) and vomiting (61%) were the most common symptoms associated with diarrhea [Bonkoungou *et al.*, 2013].

Other cross-sectional survey of intestinal parasitic and bacterial infections in relation to diarrhea in Vhembe district and the antimicrobial susceptibility profiles of isolated bacterial pathogens were conducted. Stool samples were collected from 528 patients attending major public hospitals and 295 children attending two public primary schools and were analyzed by standard microbiological and parasitological techniques. *Entamoeba histolytica/E. dispar* (34.2%) and

Cryptosporidium spp. (25.5%) were the most common parasitic causes of diarrhea among the hospital attends while *Giardia lamblia* (12.8%) was the most common cause of diarrhea among the primary school children. *Schistosoma mansoni* (14.4%) was more common in non-diarrhoeal samples at both hospitals (16.9%) and schools (17.6%).*Campylobacter* spp. (24.9%), *Aeromonas* spp. (20.8%), and *Shigella* spp. (8.5%) were the most common bacterial causes of diarrhea among the hospital attendees while *Campylobacter* (12.8%) and *Aeromonas* spp. (12.8%) were most common in diarrheal samples from school children. *Vibrio* spp. was less common (3%in the hospitals) and was all associated with diarrhea [Samie *et al*, 2009].

In our country Ethiopia, a cross sectional study was conducted in three selected health facilities (two health center and one teaching hospital), Addis Ababa between August-December 2012.The overall prevalence of enteric bacterial infection was isolated among (41.1%) of the patients. The predominant isolated organisms were *E. coli* (24.1 %) species, followed by (9.1 %) of *Shigella species, Salmonella species* (3.95 %), and *Citrobacter species* (3.95%). A total prevalence (34%) of parasites was identified. The most frequently identified protozoan parasites were *E. histolytica* (17.8%), followed by *G. lamblia* (10.3%), *H. nana* (3.6%), *A. lumbricoides* (2.0%), and *S. stercoralis* (0.4%). Co-infections were found in (7.1%) of the patients, of these (5.1%) were bacteria/ parasite and (1.9%) were parasite/parasite co-infection [Mamuye *et al.*, 2015].

Other study in Ethiopia, a systematic review and meta-analysis, the pooled prevalence estimates of *Salmonella* in stool samples of diarrheic children, diarrheic adults and carriers were 8.72%, 5.68%, and 1.08% respectively. Non-typhi isolates accounted for 57.9% of the isolates from patients. Serogroup D occurred more frequently than serogroups C and B. *S. Concord*, *S. Typhi*, *S. Typhimurium* and *S. Paratyphi* were dominant and accounted for 82.1% of the serotypes isolated from patients [Tadesse, 2014].

Similar study in northern Ethiopia, Bahir Dar town , a Cross -sectional prospective survey was conducted among diarrheal children less than five years of age to determine the prevalence of *Salmonella* spp. Out of the total 422 stool samples collected, 33 (7.8%) showed positive results for *Salmonella* species. From the 33 *Salmonella* isolates 29 (87.9%) were *Salmonella enterica* subspecies arizonae and 4 (12.1%) were *Salmonella* group A [Yemane *et al.*, 2014].

The cross-sectional study conducted in Jimma, west Ethiopia *Campylobacter* species were isolated from 11.6% of the total patients. The isolation rates of *Salmonella* and *Shigella* species

were 5.8% and 4.9% respectively. Sixty five percent (283/430) of the children were found to be infected by one or more parasites. Close contact with cats or dogs, duration and consistency of diarrhea were associated with the isolation of *Campylobacter* species [Beyene and Haile-Amlak, 2004].

A hospital-based Surveillance for Rotavirus Gastroenteritis in Children Younger Than 5 Years of age in Ethiopia conducted in the three sentinel sites, Black Lion, Yekatit- 12 and Bete-Zata hospitals, a total of 1841 fecal specimens were collected from children of <5 years of age who were hospitalized with acute gastroenteritis from August 2007 to March 2012.. Based on the surveillance period of each hospital, a total of 233 (20%), 120 (19%) and 35 (81%) positive cases were observed, respectively. Unlike the other hospitals, the observed high rate of rotavirus positive cases at Bete -Zata hospital is because surveillance was only conducted for 6 months (October2011 through March 2012) during the peak of rotavirus activity [Abebe *et al.,*2014

1.3.4 Antimicrobial resistance

The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents. Antimicrobial agents are often categorized according to their principal mechanism of action. Mechanisms include interference with cell wall synthesis (e.g., β-lactams and glycopeptide agents), inhibition of protein synthesis (macrolides and tetracyclines), interference with nucleic acid synthesis (fluoroquinolones and rifampin), inhibition of a metabolic pathway (trimethoprim-sulfamethoxazole), and disruption of bacterial membrane structure (polymyxins and daptomycin). Bacteria may be intrinsically resistant to ≥1 class of antimicrobial agents, or may acquire resistance by de novo mutation or via the acquisition of resistance genes from other organisms. Acquired resistance genes may enable a bacterium to produce enzymes that destroy the antibacterial drug, to express efflux systems that prevent the drug from reaching its intracellular target, to modify the drug's target site, or to produce an alternative metabolic pathway that bypasses the action of the drug. Acquisition of new genetic material by antimicrobial-susceptible bacteria from resistant strains of bacteria may occur through conjugation, transformation, or transduction, with transposons often facilitating the incorporation of the multiple resistance genes into the host's genome or plasmids [Davies *e*t al., 2010, Tenover *e*t al., 2006].

The study conducted in Iran, the resistant patterns of nine different commonly applied antibiotics were trimethoprim-sulphamethoxazole (80.5%), ampicillin (63.8%), tetracycline (58.3%), gentamycin (36.1%), chloramphenicol (33.3%), nalidixic acid (27.7%), and cefixime (16.6%). All the Shigella strains were susceptible to ciprofloxacin and ceftriaxone. Of 36 *Shigella* isolates, 47.2% were resistant to two or more antibiotic. The most common multidrug resistance pattern was to trimethoprim-sulphamethoxazole, ampicillin, and tetracycline [Jomezadeh *et al.*, 2014].

The study conducted in India among 280 children, majority of isolated bacterial agents were resistant to Co-trimoxazole and *Shigella was* being highly resistant enteropahogens isolated. *Salmonella* species were least resistant isolates. None of the isolates were resistant to Cefotaxime, Cefuroxime and Azetronam [Rathaur *et al.*, 2014].

In Burkina Faso, Only one Salmonella strain was resistant to nalidixic acid and ciprofloxacin. Of the *Shigella* strains, one was resistant to nalidixic acid but 81% to trimethoprim-sulphamethoxazole, 63% to streptomycin and 50% to ampicillin. Most of all the other Salmonella and *Shigella* strains were sensitive to all antimicrobials tested [Bonkoungou *et al.*, 2013].

Another study in South Africa, Antimicrobial resistance was common among the bacterial isolates but ceftriaxone (91%) and ciprofloxacin (88.6%) showed stronger activities against all the organisms [Samie *et al*, 2009].

In our country Ethiopia, the study conducted in Bahir Dar town among 33 Salmonella isolates were highly resistant to ampicillin (93.9%) followed by Augmentin (75.8%) and trimethoprim-sulphamethoxazole (48.5%). However, the isolates showed high susceptibility to ciprofloxacin and norfloxacin (93.9% each) followed by gentamicin (87.9%). Likewise, the *Salmonella* isolates showed 90.9% of multidrug-resistance. *Salmonella enteric* subspecies *arizonae* were the dominant strains of *Salmonella* isolated from children with acute diarrhea in this study [Yemane *et al.*, 2014].

Another study conducted in western Ethiopia, Jimma, and the antimicrobial sensitivity study findings showed that all tested isolates were sensitive to chloramphenicol, gentamicin and kanamycin. A majority of the strains of *Campylobacter* species were sensitive to tetracycline and erythromycin. The majority and half of the isolates were resistant for trimethoprim-sulphamethoxazole and ampicillin, respectively [Beyene and Haile-Amlak, 2004].

2. OBJECTIVES

2.1. General Objective

To determine the enteric pathogens and antimicrobial susceptibility pattern among pediatric patients with diarrhea in selected government facilities of Addis Ababa.

2.2. Specific Objectives

- To isolate the major enteric pathogens causing diarrhea in children
- Assess antimicrobial resistance of the bacterial isolates in pediatrics
- To identify the predisposing factors for enteropahogens causing diarrhea

3. METHOD AND MATERIAL

3.1. Study design

A cross-sectional study was conducted to identify the enteric pathogens and antimicrobial susceptibility profile among pediatrics with acute diarrhea illness in selected government health facilities in Addis Ababa that includes Teklehaimanot Health Center, Beletshachew Health Center and Tikur Anbesa Specialized Hospital in Addis Ababa.

3.2. Study period and area

The study was conducted from November 2016 to May 2017 in Addis Ababa, capital city of Ethiopia and seats of African Union. The city was founded by Emperor MinilikII in 1886; it is the largest city in Ethiopia, with a population of 3,384,569 (dencity5, 165.1/km$^{2)}$; all of the population is urban inhabitants. For the capital city 662,728 households were counted living in 628,984 housing units, which results in an average of 5.3 persons to a household according to the 2007 population census with annual growth rate of 3.8%. The city is populated by people from different regions of Ethiopia – the country has as many as 80 nationalities speaking 80 languages and belonging to a wide variety of religious communities. All Ethiopian ethnic groups are represented in the city [CSA of Ethiopia. Census 2007, preliminary].

3.3. Population

3.3.1. Source population

All pediatrics attending the health facilities seeking medical care during the study period

3.3.2. Study population

All pediatrics with diarrhea visiting the health facilities during the study period.

3.3.3. Inclusion criteria

Pediatrics aged 0-14 years (WHO recommended age group) with any type of diarrhea.

3.3.4. Exclusion criteria

Children age >14 years

Non-diarrheal pediatrics

Pediatrics on antibiotic treatments during data collection time

3.4. Sampling technique

A convenient sampling technique was employed for that pediatrics fulfills the inclusion criteria during the study period.

3.4.1. Sample size determination

The amount of sample size that infer the target population in this study was calculated for estimating a single population proportion at 95% confidence interval (CI) (Zα/2 = 1.96), 5% margin of error, and 10% non-response rates.

Using the formula $n=\frac{z^2 * p * q}{d^2}$ Where q= 1-p, z=1.96, p=0.219, q=0.781, d=0.05

$$n = \frac{1.96^2 * 0.219 * 0.781}{0.05^2}$$

n=263

The prevalence was taken from the previous study in Ethiopia (Balakrishnan *e*t al., 2015) and then the total the sample size was 290including 10% non-response rate.

3.5 Study Variables

3.5.1 Dependent Variable

- Enteric pathogens
- Antimicrobial susceptibility pattern

3.5.2. Independent Variable

- Socio-demographic variables (age group,sex,educational status of mother ,marital status of mother ,family size and economic status)
- Clinical data(previous history of diarrhea ,type of diarrhea , treatment taken , duration of diarrhea ,sign and symptoms and HIV status)
- Environmental variables (type of water source, food/drink taken)
- Behavioral variables (hand washing habit , feeding habit)

3.6 Operational definition

Enteric pathogens – are microbes that are able to cause enteric ~~–d~~isease of which ≥3 or more unformed stools per day and any documented intestinal infection associated with disrupted intestinal absorptive and/or barrier function.

Pediatric age – cutoff for admission to hospital pediatric services was 14 years (Age Limits of Pediatrics, American Academy of Pediatrics, Council on Child Health, Pediatrics,).

Multidrug resistance –resistance to more than one antimicrobial agent.

3.7 Data Collection

3.7.1 Demographic and clinical data collection

A Face-to-face interviews based on the questionnaire were conducted on mothers or care taker of the children who complained of diarrhea. Interviewer nurses informed interviewees that participation in the study was voluntary and confidential. The interviewer nurse took appropriate physical examination and history of the pediatric in medical registration including treatment history, HIV status .in addition the interviewer nurse collected source of drinking water and hand washing habit before and after meal, toilet usage.

3.7.2 Laboratory data collection

Stool samples were collected from pediatrics who fulfill the inclusion criteria with a sterile clean and leak-proof plastic container. The laboratory technologist instructed baby's mother or care taker to collect the stool specimen in container with the spoon attached to the lid. The specimen should contain at least 5gm of faces free from contamination of urine. Once the specimen placed on the container it should be sealed and was brought to the laboratory. The laboratory personnel transferred part of the specimen to Cary Blair medium and examined the rest for parasitological examination. The specimens were transported to Tikur Anbesa microbiology laboratory with Cary Blair transport media at 2-8^{o}C.

3.7.2.1 Microscopic examination

A wet mount was prepared and examined for each sample and observed under the light microscope for cyst, ova and trophozoites of various parasites. The stool specimen was also being observed for inflammatory cells, WBC and RBC associated with diarrhea.

3.7.2.2. Culture identification method

The samples were cultured on differential and selective media for bacterial cultivation in order to isolate bacterial enteropahogens. All stool specimens were placed into Carry Blair transport medium & transported to Tikur Anbesa specialized Hospital microbiology laboratory, where they were inoculated into Salmonella-Shigella medium (SS agar), Xylose-Lysine-Deoxycholate (XLD) agar /Hektoen Enteric (HE)agar, Sorbitol MacConkey medium(10ug),thiosulfate citrate

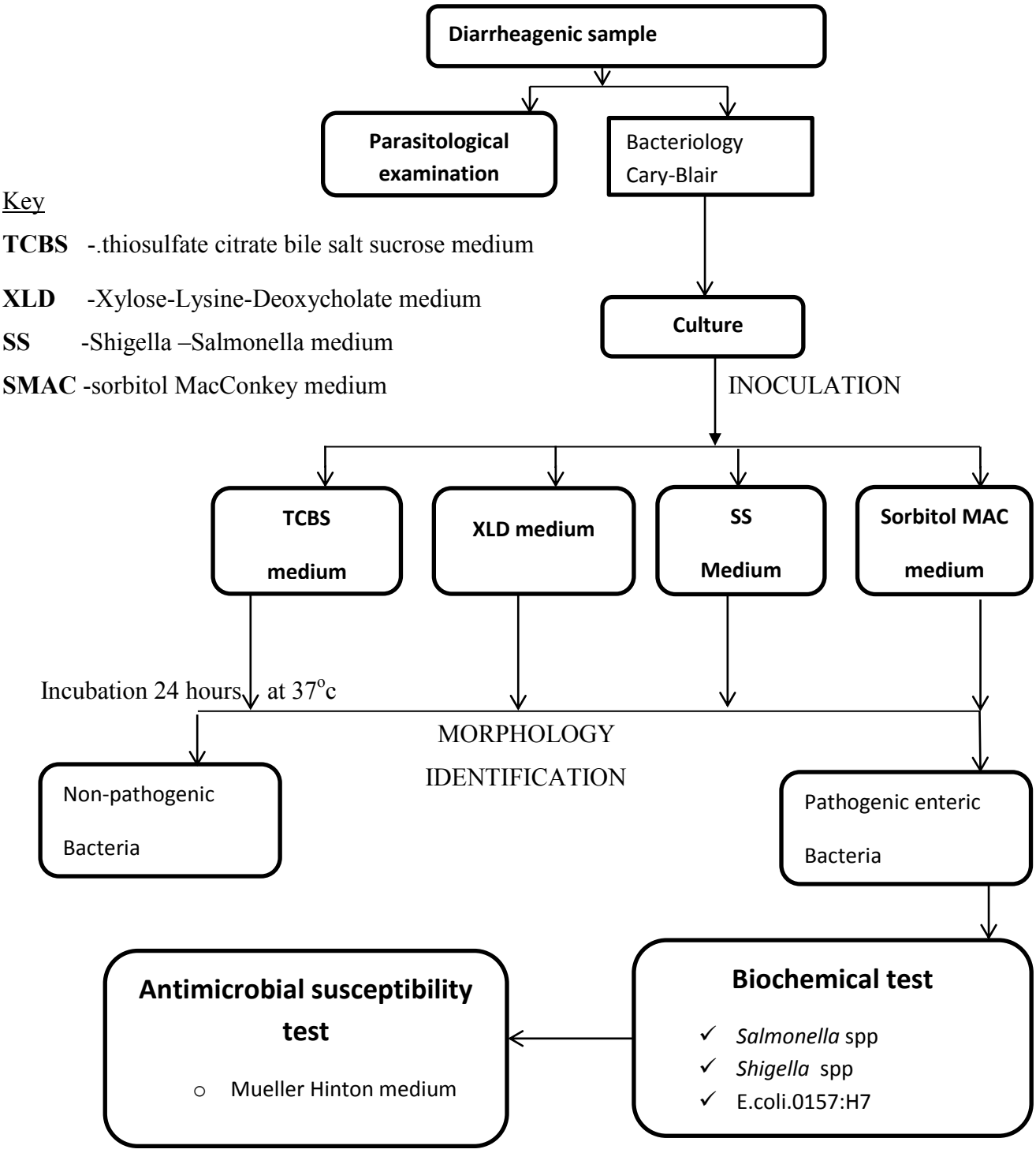
bile salt sucrose agar TCBS agar. The culture plates were incubated aerobically at 37^{o}C for 18-24 hours. The significant growth colonies were examined morphologically for size, shape, and ability to ferment lactose and sorbitol. Further identification of enteric bacterial pathogens was done by subculture of single colony to multiply which were used for biochemical test and antimicrobial susceptibility test. Control strains were E. coli ATCC25922, *Salmonella*spp ATCC13076; *Shigella*sppATCC12022 were performed parallel to test to assure the isolation .The isolated pathogens were identified using conventional biochemical tests.

The isolates were grouped into lactose fermenting (LF) and non-lactose fermenting (NLF) colonies which were then characterized based on the following standard biochemical tests by (Cheesbrough, 2009). Indole Test , Urease test ,manitol broth, hydrogen sulphide production and gas production test (using triple sugar iron agar), Citrate Utilization Test, Motility test, Carbohydrate fermentation test, malonate test-, lysine decarboxylase test(LDC).oxidase test was used to differentiate Shigella which is oxidase negative from pseudomonas ,oxidase positive after subculture pinkish colonies from XLD medium [annex I].

3.7.2.3. Antimicrobial susceptibility test

Pure Colony of isolated bacterial organism was mixed with normal saline and measured at 0.5 McFarland standards for susceptibility testing. Susceptibility was determined by using Mueller Hinton agar .The following antibiotics were used to screen for the susceptibility of the isolates; ciprofloxacin- CIP (5µg), Augmentin-AUG (30µg), gentamicin- GN (10µg), chloramphenicol- C (30µg), and trimethoprim-sulphamethoxazole - SXT (30µg),Ampicillin -AM(10µg), meropenem-MEM(10µg), Amikacin-AMK(30µg), cefepime- FEP(30µg), azithromycin AZT(30µg) and Piperacillin tazobactam -tzp(10µg). After incubation, the diameter of each inhibition zone was measured with a pair of calipers, and recorded in mm. The results then interpreted according to CLSI guidelines antimicrobial susceptibility breaking points 2016 recorded as sensitive (s), intermediate (I) or resistance(R) [CLSI, 2016 update].

Sample collection and method of stool culture technique steps



Key

TCBS -.thiosulfate citrate bile salt sucrose medium

XLD -Xylose-Lysine-Deoxycholate medium

SS -Shigella –Salmonella medium

SMAC -sorbitol MacConkey medium

Figure 1: Flow chart for identification of enteric pathogens and antimicrobial susceptibility among pediatrics patients with diarrhea in selected health facilities facilities, Addis Ababa, 2017

3.8 Quality assurance

All specimens were properly labeled as parallel to the questioner and the media used with the same identifier, media were sterilized before inoculation throughout the experiment. Cary-blair transport media was checked for its viability for one week by inoculating control strains and successful to store enteric pathogens up to one week 2-8^{o}C. Used control organisms for each isolated enteropahogens Control strains were E. coli ATCC25922, *Salmonella*sppATCC13076; *Shigella* spp ATCC12022 .The isolated pathogens were identified using conventional biochemical tests. All microbiological media and antimicrobial disks were purchased from BIOMARK *laboratories ltd* and Abtek *biological Ltd* with expiration date 2020.

3.9 Data analysis

Data was entered to statistical package for the social science (SPSS versions 20) and was analyzed to make inferences on the frequency of occurrence of enteric pathogens associated with diarrhea and to show bacterial resistance pattern to locally prescribe antibiotic substances. Descriptive statistics to analysis by using frequency, proportions graphs, crosstabs and odds ratio. Bivariate analysis was performed for each factors associated with enteric pathogens in pediatrics diarrhea. Regression analysis was conducted to identify associated factors and how they are associated with dependent variables .The strength of association was presented by odds ratio and 95% confidence interval and p-value of <0.05 was considered as statistical significant association between risk factors and enteric pathogens causing diarrhea and antimicrobial resistance of bacterial infection.

3.10 Ethical consideration

The study was conducted after it was approved by Addis Ababa University, Medical Faculty Department of Immunology, Parasitological and Microbiology Ethical Review Committee. It was also be approved by AHRI/ALERT Ethical Review Committee and Addis Ababa city administration health bureau. The purpose of the study was explained to each participant and informed consent was given prior to sample collection. The result was recorded and reported to physicians for patient management.

3.11 Dissemination and utilization of results

The test result was recorded on logbook and disseminated to clinicians for patient treatment

After the completion of the study, the research will be disseminated to Ministry of Health, Addis Ababa University, Addis Ababa Health Bureau and funder of the project, AHRI. It will also be submitted for scientific publication.

4. RESULTS

4.1 Socio-demographic variable

From November 2016 to May 2017, a total 290 pediatric patients from three health facilities were enrolled in this study. The study participants comprised of 43.1% from Teklehaimanot Health Center, 34.5% from Tikur Anbesa Specialized Hospital, and 22.4% from Beletshachew Health Center with 100% response rate. As shown in the Socio-demographic characteristics Table of patients (Table 1) the mean age of pediatrics participated in this study was 5.8±3.75 (SD) years and the majority of pediatrics n=140(48.3%) with diarrhea were under five years followed by 5-9 years n= 91(31.4%) and those with 10-14years account for n= 59(20.3%). Among the study patients, the ratio of males to females was 1.15:1. Concerning the pediatrics' mother or care taker educational status , most of them (86.6%) were educated ranging from read and write to university graduate level, with regard to marital status 79.0% were married, 11.0% were divorced, 8.6% were single, and only 1.4% were widowed. Most of the study participants have large family size with relatively low income (55% earned <1500 per month).

Table 1: Distribution of Socio-demographic characteristics of pediatric diarrheal patients from selected health facilities of Addis Ababa, Ethiopia, 2017

Category	Frequency n (%)
Health facilities	
Tikur Anbesa Specialized Hospital	100 (34.5)
Teklehaimanot Health Center	125(43.1)
Beletshachew Health Center	65(22.4)
Pediatric age group(years)	
0-4	140(48.3)
5-9	91(31.4)
10-14	59(20.3)
Sex	
Male	155(53.4)
Female	135(46.6)
Educational background of mother/caretaker	
Illiterate	39(13.4)
Read and write	47(16.2)
Elementary	78(26.9)
High school	86(29.7)
College /university	40(13.8)
Marital status of mother/guardian	

Single	25(8.6)
Married	229(79.0)
Divorced	32(11.0)
Widowed	4(1.4)
Family size	
2-3	119(41.0)
4-5	136(46.9)
≥6	35(12.1)
Monthly income(ETB)	
<500	52(17.9)
500-1000	63(21.7)
1001-1500	44(15.2)
>1500	131(45.2)
Total	**290(100)**

4.2 Clinical history, features, environmental, and behavioral variables

Among the study participants 59.0% contracted diarrhea previously and the type of diarrhea observed was bloody in 8.3%, watery in 21.4%, mucoid in 49.0% and semi liquid in 21.4 % as shown in Table 2.

Table 2: Clinical features, environmental, and behavioral variables of diarrheal pediatric patients from selected health facilities of Addis Ababa, Ethiopia, 2017

Variables	**Frequency n (%)**
Previous history of diarrhea	
Yes	171(59.0)
No	119(41.0)
Type of diarrhea	
Bloody	24(8.3)
Watery	62(21.4)
Mucoid	142(49.0)
Semi-liquid	62(21.4)
Have you take Treatment	
Yes	198(68.3)
No	92(31.7)
Types of treatment administered	
Antibiotics only	104(52.0)
ORS only	41(20.5)
Anti-pain	19(9.5)

Antibiotic+ ORS	18(9.0)
Antibiotic +ORS+ antipain	9(4.5)
Probiotics/prebiotics	9(4.5)
Duration of diarrhea	
1-2 day	156(53.8)
3-4 day	79(27.2)
5-6 day	45(15.5)
≥7 day	10(3.4)
Associated Clinical features	
Dehydration	46(15.9)
Fever	41(14.1
Vomiting	62(21.4)
Frequent diarrhea	67(23.1)
More than one symptom	66(22.7)
History of stool examination by laboratory	
Yes	260(89.7)
No	30(10.3)
Types of laboratory test (n=260)	
Microscopy	213(82.0)
Culture	6(2.3)
I do not know	41(15.7)
Frequency of prescribing similar medication /antibiotics	
Many times	40(13.8)
Some times	79(27.2)
Never	171(69.0)
HIV serological status known	
negative	190(65.5)
positive	3(1.0)
Not tested	97(33.4)
Source of drinking water	
Boiled water	47(16.2)
Bottled water	44(15.2)
Treated Pipe water	45(15.5)
Pipe water	154(53.1)
Habit of washing pediatric hand after defecation	
Always	137(47.2)
Sometimes	139(47.9)
Never	14(4.8)

Types of food/drink taken before illness	
Raw meat	17(5.9)
Raw vegetable	102(35.2)
Ground beef	5(1.7)
Raw milk	26(9.0)
Cooked food and drink	140(48.3)
Mother hand washing before and after feeding	
Yes	239(82.4)
No	51(17.6)
Total	**290**

4.3 Etiologic agents of diarrhea

The major etiologic agents of diarrhea in pediatrics in this study were intestinal parasites mainly protozoa infection and bacterial infection which accounts 93(32%) and 42(14.5%) respectively in 290 the study pediatric patients. Of these patients with diarrhea *Entamoeba histolytica/dispar* was identified from 75(25.8%), *Giardia lamblia* from 13(4.5%), and *Hymenolepis nana* from 4(1.4%) among the parasitic infections. The majority of bacterial enteropahogens isolated in the study were *Shigella* spp from 22(7.6%) followed by enterohemorrgic *Escherichia coli* O157:H7 from 13(4.5%), and *Salmonella* spp from 4 (2.4%) patients. However, no *Vibrio cholerae* spp has been isolated as shown in Table 3.

Table 3: Distribution of etiologic agents of diarrhea among diarrheal pediatric patients attending the selected health facilities of Addis Ababa, Ethiopia, 2017

Enteropahogens	**Pediatric Age group**			Total
	0-4 years (n=140)	5-9 years (n=91)	10-14 years (n=59)	0-14 years
Parasite				
Entamoeba histolytica/dispar	26(18.6%)	32(35.2%)	17(28.8%)	75(25.8%)
Giardia lamblia	7(5.0%)	2(2.2%)	4(6.7%)	13(4.5%)
Hymenolepis nana	2(1.4%)	2(2.2%)	-	4(1.4%)
Total	35(25.7%)	36(39.6%)	21(35.5%)	93(32.0%)
Bacteria				
Salmonella spp	4(2.8%)	3(3.3%)	-	7(2.4%)
Shigella spp	11(7.8%)	8(8.8%)	3(5.1%)	22(7.6%)
*E.coli*O157:H7	8(5.7%)	2(2.2%)	3(5.1%)	13(4.5%)
Total	23(16.3%)	13(14.3%)	6(10.2%)	42(14.5%)
Co-infection				
E.histolytica/dispar and *G.lamblia*	3(2.1%)	1(1.1%)	2(3.4%)	6(2.0%)
E.histolytica/dispar and *Salmonella* spp	1(0.7%)	1(1.1%)	-	2(0.6%)
E.histolytica/dispar and Shigella spp	2(1.4%)	1(1.1%)	-	3(1.0%)
Shigella and E.coli O157:H7	-	1(1.1%)	-	1(0.3%)
Total	6(4.2%)	4(2.8%)	2(3.4%)	12(4.1%)

The identified enteric pathogens were composed of 56% *Entamoeba histolytica/dispar followed by* 16% *Shigella* spp ,10% *E.coli* O157:H7 ,10% *Giardia lamblia ,5% Salmonella*spp and 3% *H.nana* as shown in figure 2 .

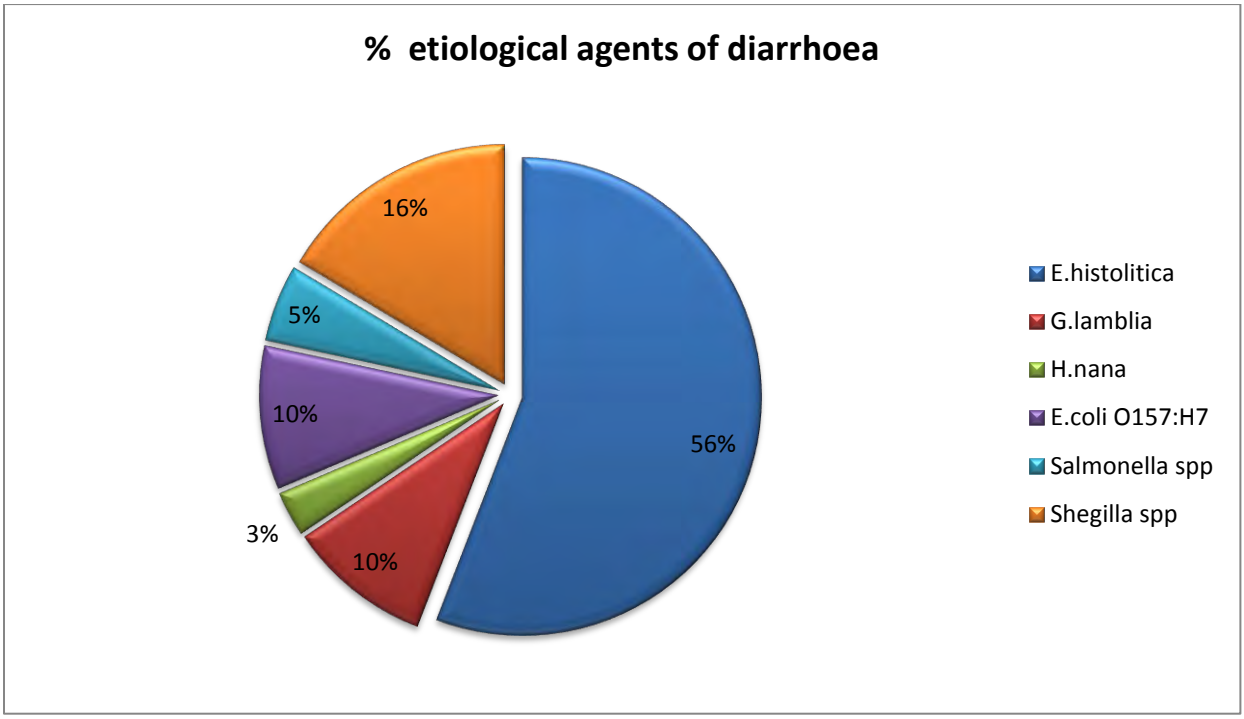


Figure 2: Distribution of etiologic agents of diarrhea among study participant attending selected health facilities of Addis Ababa, Ethiopia, 2017.

4.5 Antimicrobial susceptibility

All the bacterial isolates from diarrheal patients (table 4) were 100% susceptible to meropenem, cefepime, Piperacillin tazobactam, azithromycin and showed variable antimicrobial resistance to ampicillin, Augmentin, trimethoprim-sulphamethoxazole, ciprofloxacin and ceftriaxone. The rate of isolation of resistant strains from patients attending Tikur Anbesa Specialized Hospital and Health Centers were also shown to be different.

Table 4: Antimicrobial susceptibility pattern of enteric bacterial pathogens identified from patients attending in selected health facilities of Addis Ababa, Ethiopia, 2017.

Antibiotic discs	*Salmonella* spp *n*=7			*Shigella* spp n=22			*E.coli 0157 H7* n=13		
	S	I	R	S	I	R	S	I	R
AMP(10µg)	*2(28.5)*	1(14.3)	4(57.1)	4(18.2)	1(4.5)	17(77.3)	4(30.8)	0(0.0)	9(69.2)
AUG (30µg)	1(14.3)	*2(28.5)*	4(57.1)	13(59.1)	1(4.5)	8(36.4)	5(38.5)	3(23.1)	5(38.5)
GN (10µg)	-	-	-	-	-	-	10(76.9)	1(7.7)	2(15.4)
C (30µg)	6(85.7)	0(0.0)	1(14.3)	22(100.0)	0(0.0)	0(0.0)	12(92.3)	0(0.0)	1(7.7)
TMP-SXT (1.25/23.75µg)	4(57.1)	0(0.0)	3(42.9)	6(27.3)	1(4.5)	15(68.2)	6(46.1)	1(7.7)	6(46.1)
MEM(10 µg)	7(100.0)	0(0.0)	0(0.0)	22(100.0)	0(0.0)	0(0.0)	13(100.0)	0(0.0)	0(0.0)
AMK(30 µg)	-	-	-	-	-	-	10(76.9)	1(7.7)	2(15.4)
tzp(10 µg)	7(100.0)	0(0.0)	0(0.0)	22(100.0)	0(0.0)	0(0.0)	13(100.0)	0(0.)	0(0.0)
FEP(30 µg)	7(100.0)	0(0.0)	0(0.0)	22(100.0)	0(0.0)	0(0.0)	13(100.0)	0(0.0)	0(0.0)
AZT(30 µg)	7(100.0)	0(0.0)	0(0.0)	22(100.0)	0(0.0)	0(0.0)	13(100.0)	0(0.0)	0(0.0)
CRO(30 µg)	7(100.0)	0(0.0)	0(0.0)	22(100.0)	0(0.0)	0(0.0)	10(76.9)	1(7.7)	2(15.4)
CIP(5 µg)	7(100.0)	0(0.0)	0(0.0)	21(95.4)	1(4.5)	0(0.0)	10(76.9)	0(0.0)	3(23.1)

Key S-Susceptibility I- Intermediate, R-Resistance, TMP- SXT -trimethoprim-sulphamethoxazole, Ampicillin-AMP, Augmentin-AUG, chloramphenicol- C, Meropenem –MEM, Amikacin-AMK, Piperacillin tazobactam, Cefepime FEP, Azithromycin-AZT, Ceftriaxone-CRO, Ciprofloxacin-CIP

Salmonella spp isolates were 100% susceptible to meropenem, Piperacillin tazobactam azithromycin,cefepime,ciprofloxacin,ceftriaxone,85.7% chloramphenicol,57.1% trimethoprim-sulphamethoxazole,28.5% ampicillin,14.3% Augmentin and were resistant to ampicillin and Augmentin with the same rate 57.1% and showed resistant to trimethoprim-sulphamethoxazole and chloramphenicol 42.9% and 14.3% respectively .

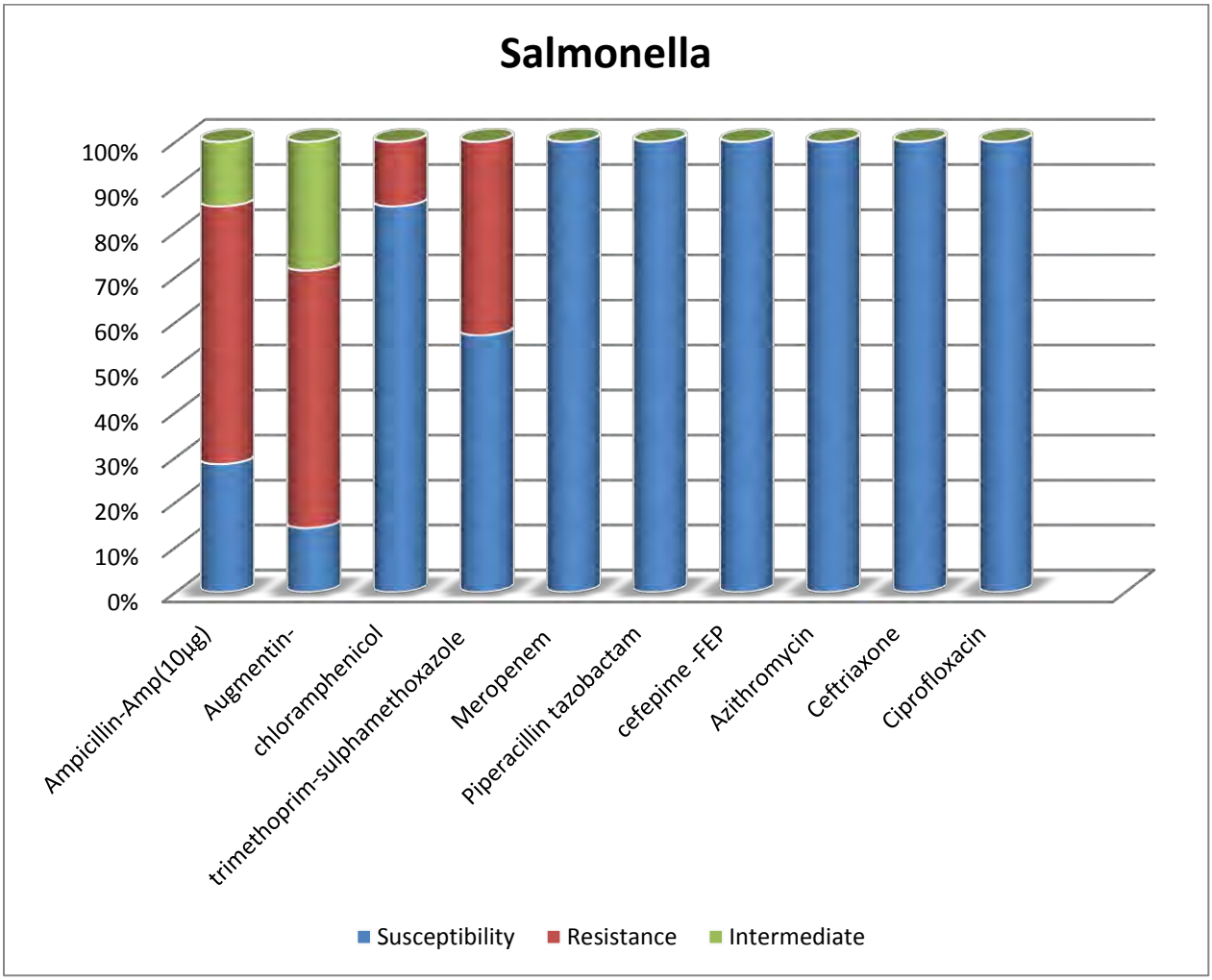


Figure 3: Antimicrobial susceptibility profile of *Salmonella* spp isolated from stool specimen in diarrheagenic pediatric patients from selected health facilities of Addis Ababa, Ethiopia, 2017.

Shigella spp isolates were susceptible 100% to meropenem. Piperacillin tazobactam, azithromycin, ceftriaxone, chloramphenicol, 95.4% ciprofloxacin, 59.1% Augmentin, 27.3% trimethoprim-sulphamethoxazole, 18.2% ampicillin and were resistant to 77.3% ampicillin 68.2% trimethoprim-sulphamethoxazole and 36.4% Augmentin.

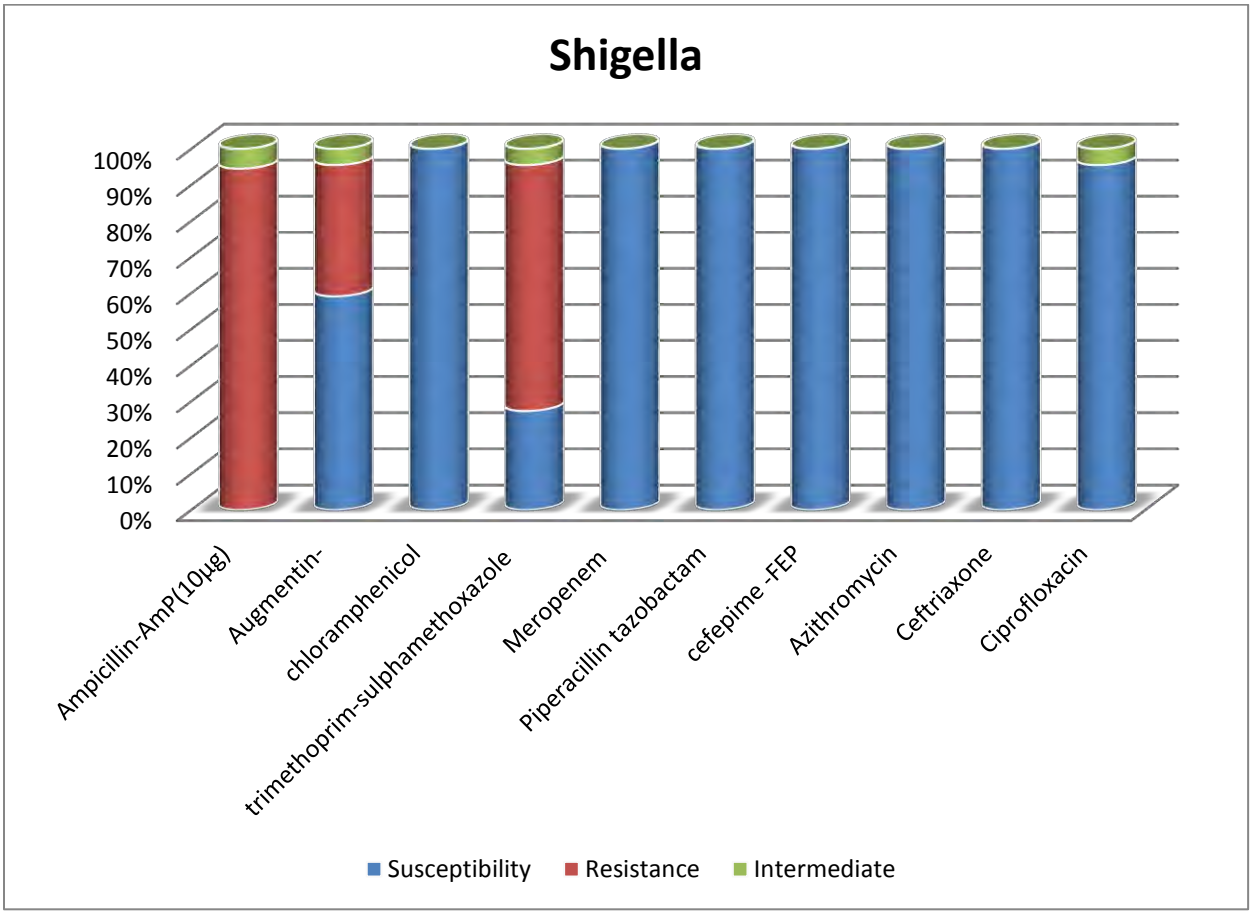


Figure 4: Antimicrobial susceptibility profile of *Shigella* spp isolated from stool specimen in diarrheagenic pediatric patients from selected health facilities of Addis Ababa, Ethiopia, 2017.

Enterohemorrhagic coli 0157; H7 isolates were 100% susceptible to meropenem, Piperacillin tazobactam, azithromycin,. However, 92.3% of the isolates were susceptible to chloramphenicol and 76.9% of the isolates were susceptible to Ciprofloxacin, Amikacin, ceftriaxone and gentamycin whereas trimethoprim-sulphamethoxazole was susceptible for 46.1% of the isolates. The *Enterohemorrhagic coli 0157;H7* isolates showed 69.2% resistance to ampicillin, 46.1% to trimethoprim-sulphamethoxazole ,38.5% to Augmentin, 23.1% to ciprofloxacin and the isolates had similar resistance (15.4%) to Amikacin, ceftriaxone and gentamycin were resistant with the same rate.

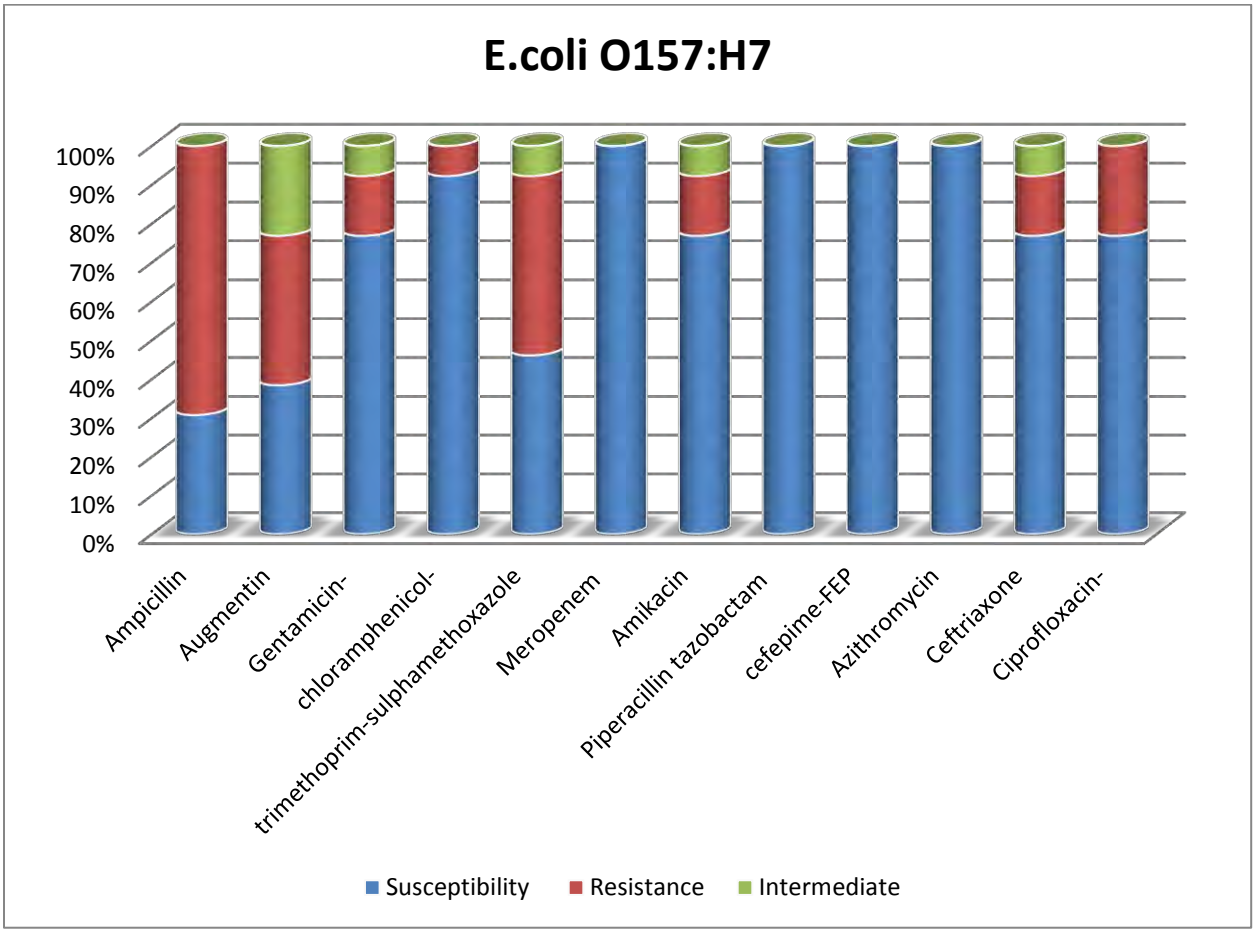


Figure 5: Antimicrobial susceptibility profile of *E.coli 0157; H7* isolated from stool specimen in diarrheagenic pediatric patients in selected health facilities of Addis Ababa, Ethiopia, 2017.

4.5.1 Isolation of antibiotic-resistant strains by health facilities

The isolation rate of antibiotic-resistant strains by health facility was showed to be different. Accordingly, the isolation rate of antibiotic-resistant strains in 30 ampicillin resistant isolates six of them were from health centers and twenty four of them were from TASH. The remaining resistant antibiotics from health center were 2/17 Augmentin, 1/4 gentamycin, 7/18 Cotrimethoxazole were analyzed whereas in TASH 15/17 Augmentin, 2/2 Chloramphenicol, 2/2 Amikacin 3/4 gentamycin, 11/18 Cotrimethoxazole, 1/1 azithromycin, 3/3 ciprofloxacin and 2/2 ceftriaxone were identified. A proportion higher from TASH compared to the two health centers. This might be referred patients from health centers diagnosed at hospitals.

4.5.2 Multi-drug resistant isolates

Three (43%) of Salmonella isolates were multidrug resistant, 29% of them showing resistance to three antimicrobials and 14% of them resistance to four antimicrobials. Regarding, Shigella *isolates,* sixteen (72.7 %%) of the isolates showed multidrug resistance of which twelve (54.5%) of the isolates showed resistant to two antimicrobials and the remaining four (18.2%) were resistant to three antimicrobials. The other enteric bacteria, *E coli* O157:H7 also showed multidrug resistance for nine (69.2%) isolates in which five (38.5%), one (7.7%), two (15.4%) and one (7.7%) were multidrug resistance for two, three, four and six antimicrobials respectively as it is summarized on table 5.

Table 5: resistance antibiogram of isolates from pediatrics patients in selected health facilities of Addis Ababa, Ethiopia, 2017.

Number of antimicrobial resistance	Resistance antibiogram	Resistance isolates n (%)		
		Salmonella spp(n=7)	Shigella spp (n=22)	*E coli* O157:H7 (n=13)
RO	None	2(29)	3(13.6)	3(23.1)
R1	Amp	1(14)	1((4.54)	1(7.7)
	AUG	1(14)	-	-
	SXT	-	1(4.54)	-
R2	Amp ,AUG	-	4((18.2)	2(15.4)
	Amp,SXT	-	8(36.4)	2(15.4)
	Amp ,CIP	-	-	1(7.7)
R3	Amp,AUG,SXT	2(29)	4(18.2)	-
	Amp,SXT,CRO	-	-	1(7.7)
R4	Amp ,AUG,SXT,CRO	-	-	1(7.7)
	Amp,AUG,SXT,C	1(14)	-	-
	Amp ,AGU,CIP,GEN	-	-	1(7.7)
R6	AUG,CIP,SXT,C,AZT,GEN	-	-	1(7.7)

Key:Amp-Ampicillin,C-Chloramphenicol,SXT-Cotrimethoxazole.AUG-Agumentin,AZT-azthyromycin, GEN-Gentamycin, CRO-ceftriaxone, CIP-ciprfloxacillin, RO-none resistance,R1-resistance for one antimicrobial,R2-resistance for two antimicrobial,R3-resistance for three antimicrobial ,R4-resistance for four antimicrobial ,R6- resistance for six antimicrobial

4.4 Bivariate analysis of potential risk factors associated with diarrhea

Following the description of identified etiologies of diarrhea in the pediatric patients participated in this study we have analyzed potential risk factors associated with these infections using bi-variant and multivariate regression models. Our bivariate analysis showed some of socio-demographic factors (family size); among the clinical features previous history of bloody or watery diarrhea, prolonged duration of diarrhea and/or fever; and among behavioral and environmental variables consumption of raw vegetable and meat were associated with re-infection and/or diarrheal disease as shown in tables 6, 7 and 8. All the other variables studied had no association in our bi- and multi-variant regression models.

4.4.1 Socio-demographic characteristics

The socio-demographic characteristics of pediatrics patients analyzed include age and sex and that of mothers/ care takers or family includes mother's educational and marital status, family size and economic status. The association of these variables with detection of parasitic or bacterial enteropahogens was assessed and large family size (>6) was significantly associated with diarrhea (p=0.013, AOR=2.85, 95%CI [1.25, 6.67]) as shown in the table below (Table 6).

Table 6: Socio-demographic and associated risk factors for diarrhoeagenic enteropahogens among study participant attending selected health facilities of Addis Ababa, Ethiopia, analyzed at Tikur Anbesa Specialized Hospital, 2017

Socio demographic variables	Enteric pathogen	No enteric pathogen	p-value	COR(95%CI)	p-value	AOR(95%CI)
Pediatric age group(years)						
0-4	58	82	0.69	0.88(0.47,1.64)	0.84	1.06(0.56,2.03)
5-9	49	41	0.56	1.22(0.63,2.38,1)	0.55	0.81(0.40,1.61)
10-14	27	32		1		1
Sex						
Male	64	91	0.74	1.08(0.68,1.72	0.56	1.15(0.71,1.87)
Female	60	75				
Educational status of mother/caretaker						
Illiterate	19	20	0.58	1.28(0.53,3.12)	0.69	0.82(0.31,2.15)

Read and write	20	27	0.84	1.08(0.46,2.56)	0.66	0.82(0.33,2.02)
Elementary	34	44	0.80	1.11(0.51,2.38)	0.55	0.78(0.35,1.75)
High school	35	51	0.94	0.98(0.45,2.22)	0.90	1.04(0.47,2.32)
College /university	16	24	1		1	
Marital status of mother/guardian						
Single	10	15	0.82	1.27(0.15,10.53)	0.72	1.49(0.16,13.46)
Married	103	126	0.87	1.18(0.16,8.53)	0.74	1.41(0.17,11.33)
Divorced	10	22	0.46	2.20(0.27,17.92)	0.39	2.57(0.29,22.86)
Widowed	1	3	1		1	
Family size						
2-3	40	79	1		1	
4-5	68	73	0.07	0.64(0.38,1.05)	0.17	1.42(0.84,2.43)
≥6	21	14	0.006	2.70(1.23,5.88)	0.013	2.85(1.25,6.67)
Monthly income(ETB)						
<500	18	71	0.14	1.64(0.84,3.20)	0.12	1.78(0.86,3.67)
500-1000	29	27	0.73	0.90(0.49,1.64)	0.65	0.86(0.46,1.61)
1001-1500	17	34	0.51	1.26(0.63,2.51)	0.53	1.26(0.61,2.58)
>1500	60	34	1		1	

*Significance <0.05

4.4.2 Clinical data features

The other associated risk factor for development of diarrhea and isolation of enteric pathogens analyzed were clinical history and feature of the patients. Of these variables having bloody and/or watery diarrhea were statistically associated with identification enteropahogens and

diarrheal disease with p value (p=0.03) and AOR=4.5, 95%CI [1.11, 20.0] and AOR=3.2, 95%CI [1.06, 10.0] respectively. Further the identification of enteropahogens was found to be 4.5 and 3 times more likely to be associated with bloody and watery than semi-liquid diarrhea. The duration of diarrhea 5-6 days has significantly associated with identification and isolation of enteric pathogens (p=0.02 AOR=2.9, 95CI [1.12, 7.7) and was found to be 2.9 times more likely associated compare to the duration of 1-2 days. Among the clinical manifestations of the study pediatrics patients, fever was statistically significant association for the detection of enteropahogens from diarrhea stool (p=0.001, AOR=9.1,95%CI[1.88,33.3]) ,and found to be nine times more likely to associated with diarrhea than compare to those who have mild stomach ache and nausea.

Table 7: Clinical data risk factors for diarrhea enteric pathogens among study participant attending selected health facilities of Addis Ababa, Ethiopia, 2017

Clinical data Variables	Enteric pathogens	No enteric pathogens	p-value	COR(95%CI)	p-value	AOR(95%CI)
Previous history of diarrhea						
Yes	68	103	0.28	1.29(0.80,2.07)	0.19	1.84(0.74,4.61)
No	56	63	1			
Type of diarrhea						
Bloody	12	12	0.10	2.27(0.85,5.88)	0.03*	4.54(1.11,20.0)
Watery	33	31	0.32	1.45(0.71,2.94,)	0.03*	3.22 (1.06,10.0)
Mucoid	53	88	0.56	1.19(0.65,2.20)	0.90	1.06(0.40,2.83)
Semi-liquid	26	35	1		1	
Have you take Treatment						
Yes	83	115	0.72	1.09(0.66,1.79)	0.65	2.07(0.08,49.56)
No	41	51	1		1	
Types of treatment administered						
Antibiotics only	44	60	0.51	1.570.40,6.20)	0.35	2.26(0.40,12.66)
ORS only	21	20	0.81	1.19(0.28,5.07)	0.57	1.71(0.26,10.96)

Antipain	6	13	0.23	2.70(0.53,13.85)	0.16	4.10(0.57,29.48)
Antibiotic+ORS	5	13	0.45	2.50(0.37,16.88)	0.19	4.35(0.46,40.40)
Antibiotic+ORS+antipain	5	4	0.16	3.25(0.61,17.28)	0.11	5.84(0.66,51.49)
Probiotics/prebiotics	3	6	1		1	
Duration of diarrhea						
1-2 day	61	95	1		1	
3-4 day	38	41	0.18	0.69(0.40,1.19)	0.34	0.65(0.27,1.55)
5-6 day	26	19	0.02*	2.13(1.08,4.16)	0.02*	2.94(1.12,7.70)
≥7day	2	8	0.24	2.56(0.52,12.50)	0.84	0.81(0.09,6.8)
Clinical manifestation						
Dehydration	15	32	0.18	1.60(0.79,3.21)	0.62	1.30(0.46,3.67)
Fever	24	17	0.08	1.88(0.93,3.85)	0.001*	9.1(2.44,33.30)
Vomiting	28	34	0.76	0.91(0.49,1.66)	0.17	0.53(0.21,1.31)
Mild Stomach ache and nausea	60	80	1		1	
Frequency of prescribing similar medication /antibiotics						
Many times	17	23	0.90	1.04(0.52,2.09)	0.48	1.43(0.52,3.90)
Sometimes	37	43	0.68	0.89(0.52,1.52)	0.43	0.72(0.31,1.64)
Never	74	96	1			
Have you examined diarrhea by laboratory						
Yes	111	149	0.26	1.53(0.72,3.27)		
No	16	14	1		1	
Types of laboratory test (n=260)						
Microscopy	95	119	1		1	
Culture	1	4	0.30	3.19(0.35,29.04)	0.69	1.79(0.09,34.65)
I do not know	15	26	0.35	1.38(0.69,2.76)	0.24	1.87(0.65,5.38)

HIV serological status						
Negative	83	107	1		1	
Positive	2	1	0.44	0.38(0.03,4.35)	1.00	NA
Not tested	43	3	0.92	0.97(0.59,1.59)	0.55	1.25(0.59,2.65)

*Significance <0.05,*COR-crude odd ratio in bivariate logistic regression ,AOR-adjusted odd ratio in multivariate logistic regression and 95% confidence interval for association of risk factors to dependent variable in the study. Reference category is denoted by number 1 selected due to less association factor to dependent variable*

4.4.3 Environmental and behavioral variables

Detection of enteric pathogens was significantly associated with pediatric patients who consumed raw meat (p=0.04) and vegetables (p=0.02). Eating raw meat was about 3 times more likely to associate to enteric pathogens detection as compare to those eating cooked food or drink AOR=3.12, CI [1.06,9.1] and eating raw vegetables was about 2 times more likely to associate with enteric pathogens detection as compared to those eating cooked food or drink of AOR=1.85, CI [1.08,3.12]. However, there was no statistical significant association between source of drinking water, habit of hand washing before and after defecation and habit of hand washing before and after meal with the detection of enteric pathogens.

Table 8: Environmental and behavioral risk factors for diarrhoeagenic enteric pathogens among study participant attending selected health facilities of Addis Ababa, 2017.

Environmental and behavioral variables	Enteric pathogen	No enteric pathogen	p-value	COR(95%CI)	p-value	AOR(95%CI)
Source of drinking water						
Boiled water	21	26	1		1	
Bottled water	25	19	0.25	0.61(0.26,1.40)	0.41	0.69(0.29,1.64)
Pipe treated water	21	24	0.54	1.23(0.64,2.38)	0.46	1.29(0.65,1.59)
Pipe water	61	93	0.85	0.92(0.40,2.09)	0.91	0.95(0.40,2.23)
Habit of washing pediatric hand after defecation						
Always	61	76	1		1	
Sometimes	62	77	0.98	0.99(0.62,1.60)	0.81	1.06(0.64,1.77)
Never	5	9	0.52	1.44(0.46,4.53)	0.52	1.46(0.45,4.75)
Types of food/drink taken before illness						
Raw meat	11	6	0.02*	3.33(1.16,10.0)	0.04*	3.12(1.06,9.1)
Raw vegetable	52	50	0.01*	1.88(1.12,3.12)	0.02*	1.85(1.08,3.12)
Ground beef	1	4	0.48	2.22(0.24,20.42)	0.40	2.64(0.27,24.87)
Raw milk	44	12	0.08	0.47(0.20,1.01)	0.15	0.53(0.22,1.26)
Cooked food and drink	50	90	1		1	
Have you washed before and after feeding						
Yes	103	136	1		1	
no	25	26	0.44	0.78(0.43,1.44)	0.27	0.69(0.36,1.33)

*Significance <0.05,*COR-crude odd ratio in bivariate logistic regression ,AOR-adjusted odd ratio in multivariate logistic regression and 95% confidence interval for association of risk factors to dependent variable in the study. Reference category is denoted by number 1 selected due to less association factor to dependent variable.*

5. DISCUSSION

In this cross-section study, the overall prevalence of parasitic and bacterial enteropahogens in pediatrics age of 0-14 years were 32.0% and 14.5% respectively. As a result, children were more affected with parasitic infection compared to bacterial infection .This difference could be other potentially pathogenic bacteria were not isolated due to shortage of supplies and controls. Among the parasitic infections, *E. histolytica/dispar* was the most frequently identified protozoa causing diarrhea in children with an overall rate of 25.8% which was comparable with another study in Addis Ababa accounting 19 % [Eguale *e*t al., 2015]. However, the present result is higher than a study conducted in western Ethiopia; Jimma which was 0.8% [Beyene and Tasew, 2014]. This big difference might be due to geographic variation, variation in age group of which, this study includes pediatrics age 5-14 years in which parasitic infection relatively high, sampling technique, metrological differences between the studies, in Jimma continues community education might improve in control of the disease.

The predominant enteric bacterial pathogen isolated in this study was *Shigella* spp accounting for 7.6% which was comparable to a study done in southern part of Ethiopia, Hawassa and Eastern part of Ethiopia, Harar which showed 7.0% [Getamesay *et al.,* 2014] and 6.9% [Reda *et al.*, 2011]respectively. On the other hand, a study done in Northern Ethiopia, Mekele hospital with a total of 216 participants showed that *Shigella* was isolated from 15 (6.9 %) of the participants. Ten (66.7%) of the positive isolates were from children <15 years [Gebrekidan *et al.,* 2015]. Other studies conducted in neighboring countries in Sudan 8.0% [Saeed and Sandstrom, 2015] and in Djibouti 7.7% [Mikhail *et al.*, 1990] of Shigella spp was reported. In high burden countries like Iran the prevalence of Shigella spp was 8.5% [Samie *et al.*, 2009]. However, the result was higher compared to the prevalence found in Jimma 0.9% (2/218) [Teshale *et al.*, 2015], 2.3% by Beyene, and Tasew, 2014 and 4.9% by [Beyene, and Haile-Amlak, 2004]. On the other hand, similar study conducted in Butajira, Central Ethiopia showed 4.5% [Mengistu *et al.,* 2014]. The possible difference could be due to differences in implementation of personal and environmental hygiene in the community from the continuous interventions made by the health extension workers.

Our finding is also higher compared to other studies including Kenya 4.0% [Shah *et al.,* 2016], China 1.4% [Qu *et al.*, 2016] and South Africa 5.1% [Jomezadeh *et al.,* 2014.]. This difference could be due to sample size and study design.

The study showed low prevalence isolation rate of *Shigella* spp compared to the study conducted in Addis Ababa, Ethiopia done from August-December 2012 in St. Paul Millennium Medical College 9.1% *Shigella* spp was reported [Mamuye *et al*, 2015]. This difference could be due to seasonal variation of data collection time. Other studies done in India and Zambia reported 14.4% of *Shigella* spp from stool sample of 118 [Manikandan and Amsath, 2013] and 271 [Chiyangi *et al*, 2017] respectively. The difference might be due to variation in age group, geographical distribution, and endemicity of the disease.

The prevalence for the isolation of *Salmonella* spp from pediatric patients in this study was 2.4%, which is in line with other studies conducted in South region, Hawassa which showed 2.5% [Getamesay *et al.,* 2014] and in Addis Ababa, Ethiopia 3.95% [Mamuye *et al.*, 2015]. The prevalence was comparable with a study done in neighboring countries, Sudan 4.0% [Saeed and Sandstrom, 2015], central Kenya 3.4% [Shah *et al,* 2016]. On the other hand, bacterial pathogens isolated from childhood diarrhea in Beijing, China indicated that 4.3 % of *Salmonella* spp were isolated [Qu *et al,* 2016]. However, our study is lower compared to the study conducted in Bahir Dar town, among diarrheal children less than five years of age. Out of the total 422 stool samples collected, 7.8% showed positive results for *Salmonella* species [Yemane *et al,* 2014] whereas in Jimma health center [Beyene and Tasew, 2014] and Addis Ababa [Eguale *et al.,* 2015] the rate was 6.2%. This difference might be age group, sample size, geographical variation and epidemicity of the disease. Among high burden countries of the disease, a study in India on 280 children, the frequency of diarrhoeagenic bacteria isolated *Salmonella* spp was 7.8% [Rathaur *et al.,* 2014]. The present study was much lower than a report from Lusaka, Zambia which showed prevalence 25.5% in under five children [Chiyangi *et al.*, 2017]. This is due to the probability of relative improved hand washing habit after defecation as well as before and after feeding, mother/care taker education status in Addis Ababa where 86.6% showed in this study with direct interview. This finding was comparably higher than the study conducted in Gondar reported that 1.6% of *Salmonella* spp isolated [Huruy *et al.*, 2011]. The possible difference might be due to variable geographical and climate conditions

Numerous types of *E. coli* that causes diarrheal disease have been described, including enterotoxigenic strains, Enteropathogenic strains, enteroinvasive strains, enterohemorrhagic strains, and enteroaggregative strains. Of these different types, only enterohemorrhagic strains of serotype O157:H7 can be routinely detected in most clinical microbiology laboratories because a specific selective medium, Sorbitol-MacConkey agar is widely available. The geographic distribution of these strains varies, and media for detection may be available in some laboratories and not in others. Sorbitol-negative strains can be further identified with specific serotyping reagents or toxin assays and *E. coli* O157 was reported to be the most frequently isolated organism at the University of Calgary Clinical Microbiology Laboratory (Calgary, Alberta, Canada), accounting for 34% of all positive stool cultures [Hines and Nachamkin, 1996]. It was well documented that humans can acquire *E. Coli* O157*:H7* infection when consuming animal products, raw meat and raw milk.

The present study isolated *E. coli* O157:H7 from children in Addis Ababa, Ethiopia using standard method by sorbitol-MacConkey media for recovering serotype *E. coli* 0157:H7 in 4.5% which is comparable to study findings in the world [Effler *e*t al., 2001, Hines and Nachamkin, 1996, Lim *e*t al., 2010**,**]. This is the first study in Addis Ababa with pediatric age to my knowledge even though only few studies have been reported in Ethiopia. The study conducted in Bahir Dar town showed an overall isolation rate of 48.3% *E. coli* in children aged under five with acute diarrhea of which 28.9% *E. coli* O157:H7 was isolated [Adugna *et al*, 2015]. In a study conducted in Jimma town isolated 1.8% *E. coli* O157:H7 food handlers [Teshale *et al.*, 2015], but other similar study in Gondar didn't isolate the serotype using the similar method [Huruy *et al.*, 2011]. This gap might be due to the population of Addis Ababa is reactively more dense and at high risk for transmission due to consumption of raw meat and vegetables

Our finding was in line with the study conducted in Nigeria, Lagos with the prevalence of 5.1% of EHEC associated with watery diarrhea, hemorrhagic colitis and the hemolytic uremic syndrome was described [Ogunsanya *e*t al., 1994].

Double infection among parasites and bacteria were observed in 12 of 290 (4.1%) patients. Of these *E. histolytica* was the predominant protozoa pathogen which occurs in co-infection with *G. lamblia*, *Salmonella* spp and *Shigella* spp. A concurrent infection with *Shigella* spp and *E. coli*

O157:H7 was observed in one patient with bloody diarrhea. Similar concurrent infections rate of 5.4 % were reported from Jimma. However, in these co-infections there were no bacterial-bacterial combinations [Beyene and Tasew, 2014].

The emergences of increased antimicrobial resistances are global challenges, particularly in developing countries. The cuases of the antimicrobial resistance (AMR) in developing countries are more complex and may be rooted in the practices of health care and patients' behavior towards the use of the antimicrobials as well as supply chains. Some of these factors include misuse of antimicrobial agents, inappropriate prescriptions practices, inadequate patient education, limited diagnostic facilities, unauthorized sale of antimicrobials, and lack of appropriate drug regulatory mechanisms. In this study, the susceptibility pattern for all bacterial strains showed resistance to at least two drugs as shown in (table 4). The majority of the bacterial pathogens were resistant to two or more drugs tested, with ampicillin, cotrimoxazole and Augmentin being the most ineffective drugs similar to the study conducted in Zambia [Chiyangi *et al.*, 2017]. The main reason could be the frequent use of these antibiotics. The antimicrobial resistance pattern of *Shigella* spp. against ampicillin, trimethoprim-sulphamethoxazole, Augmentin, gentamycin and ciprfloxacillin were 77.3%, 68.2, 36.4%, 9.0% and 0% (intermediate 4.5%), respectively. The highest resistance of ampicillin (77.3%) and trimethoprim-sulphamethoxazole (68.2%) were comparable to the report from Gondar 79.9%,73.4% [Yismaw *et al*, 2009] and Meklele 86.7%, 66.7% [Gebrekidan *et al,* 2015] respectively. Study in Butajira, Ethiopia also showed 47.1% resistance to Ampicillin, 76.5% to trimethoprim-sulphamethoxazole [Mengistu *et al,* 2014]. This indicates that the resistance of ampicillin increasing through time. While a study done in Tikur Anbesa, Addis Ababa showed resistance level of 78.7% to ampicillin and 45.3% to trimethoprim-sulphamethoxazole of *Shigella* spp. [Asrat, 2008] which indicated that trimethoprim-sulphamethoxazole resistance was increased by 12.9% within the last nine years .This study showed relatively low rate of ampicillin resistance compared to other findings in other parts of Ethiopia Harar 100% [Reda *et al.,* 2011**]** and Jimma 100% [Beyene, and Tasew, 2014]. The *Shigella* isolates were 100% susceptible to meropenem, tazobactam, azithromycin, Amikacin, ceftriaxone, and chloramphenicol.

Another enteric bacterial pathogen *Salmonella* spp. also showed antimicrobial resistance to ampicillin (57.1%), Augmentin (57.1%), trimethoprim-sulphamethoxazole (42.9%) and chloramphenicol (14.3%) comparable to others in Jimma 62.5% [Beyene *et al.,* 2014] and in Butajira 60% [Mengistu *e*t al., 2014]. However, the finding was lower compared to other studies from Addis Ababa [Asrat, 2008] showing ampicillin resistance of (81.2%), trimethoprim-sulphamethoxazole (75.7%) and chloramphenicol (83.7%). This might be due variation in number of strains and different batch of antimicrobial disk used.

The antibiogram of *E. coli* O157:H7 showed resistance of 69.2% to ampicillin, 46.1% trimethoprim-sulphamethoxazole, and 38.5% to Augmentin, 23.1% to ciprofloxacin. The isolates had similar resistance pattern (15.4%) to Amikacin, ceftriaxone and gentamycin. The study showed difference with the study done in Bahir Dar in which high levels of antimicrobial resistance to ampicillin (86.8%), tetracycline (76%) and cotrimoxazole (76%) was documented and low levels of resistance to ciprofloxacin (6.9%) was included [Adugna *et al*, 2015]. On the other hand, the study conducted in Jimma town on the resistance showed the following level of resistances to the antibiotics ampicillin (50%), Augmentin (75%), trimethoprim-sulphamethoxazole(50%), ceftriaxone(25%) unlikely to the present study indicated no resistance was documented for ciprofloxacin [Teshale, 2015].

The risk of diarrhea was associated with having large family size (≥6) and was found to be 2.85 times more likely to be infected with enteropahogens compared to those having small family size (2-3). The presence of fecal leucocytes and red blood cells was high in co-infected individuals as compared to in a single pathogen associated diarrhea. Other risk factors assessed on the type of diarrhea in which bloody and watery were 4.5 and 3 times more likely associated with detection of enteropahogens compared to semi-liquid diarrhea respectively. With regard to clinical manifestation dehydration, fever, vomiting, and mild stomach ache, fever was statistically significant associated with identification of enteric pathogens in diarrheic stool. Moreover, enteric pathogens causing diarrhea were also associated with the duration of diarrhea in which relatively prolonged diarrhea 5-6 days of illness were 9 times more associated for detection compared to short duration of 1-2 days of illness. From habit of feeding, eating raw meat was about 3 times more likely associated to enteric pathogens compared to those eating cooked food or drink in pediatrics. The possible reason for this in direct interview mother replied that children

older than 7 years took grind under cooked meat and cross contamination between elders'. On the other hand, eating raw vegetables was about 2 times more likely to associate with enteric pathogens compared to those eating well cooked food or drink.

5.1 Limitations of the study

Due to lack of control strains and selective media, primary bacterial enteropahogens such as *Yersinia entricolitica,* microaerophilic *Campylobacter* spp and anaerobic *Clostridium difficle* which are common causes of diarrhea were not isolated. Another most important causative agent of diarrhea, viruses like Rotavirus was not assessed due to shortage of supplies in the country. Further confirmation of *E. coli* O157:H7 with serology and molecular PCR method were not done.

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Parasitic and bacterial infections are common public health problems among pediatric patients under 15 years of age. Children with fever, bloody and watery diarrhea had the highest incidence of enteric pathogens. Duration of diarrhea elapsed 5-6 days, and consumption of raw meat and vegetables were associated with the detection of enteric pathogens causing diarrhea. So, feeding the children proper cooked food and drink could minimize the problem. Assessment finding about utilization culture showed that only 2.3% of participant had used stool culture and AST which is very low and most clinicians treat diarrhea empirically with broad spectrum antibiotics in IMNCI clinic. This was the main reason for increment of antimicrobial resistance in the country.

The most frequently prescribing drugs ampicillin, amoxicillin+clavulic acid and trimethoprim-sulphamethoxazole showed high resistance for *Salmonella* and *Shigella* isolates in the study. But it was found that ciprofloxacin was the best drug of choice for the treatment of diarrhea caused by *Salmonella* and *Shigella*. Chloramphenicol was a drug of choice for the treatment of shigellosis.

6.2 Recommendation

Adhere routine stool culture for enteropathogenic bacteria including *E. coli* O157:H7 using Sorbitol MacConkey in microbiological laboratory and appropriate referral linkage between hospitals and health centers is necessary for early identification and treatments. It is better to expand routine stool culture for as many as enteropathogenic bacteria isolation and antimicrobial susceptibility for better management and control of diarrhea in the country. Since the gastrointestinal infections are caused by food borne pathogens, environmental sanitation and continuous community education are mandatory. The alarming of antimicrobial resistance in enteric bacterial pathogens was a threat for children, so it is important to continue surveillance on these organisms in terms of prevalence, clinical epidemiology and antimicrobial susceptibility patterns obtained from hospitals and health centers in the country. In general, further study is necessary for isolation of potential enteric pathogens and periodic monitoring and evaluation of antimicrobials' potency as well as cross-checking the en-vitro susceptibility of antimicrobials with the in-vivo efficacy.

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8. ANNEXES

Annex I: Flow chart for gram negative rods biochemical testcheesbrough, 2009

I	Lactose	Indol	Urea	Manitol	H_2S	Gas glu	Citrate	Motility	Lysin	**Organism**	Additional
LACTOSE OR ONPG (ortonitrophnilegalactosidase) **POSITIVE**			+	+	+	+	+	+	-	Citrobactor	Urea +
		-		+			+	+	-	Entrobactor cloacae	
					-	+		-	+	Klebsiella pneumonia	Malonate+
							-/+	-	-/+	Klebsiellaozenae	
							+/-	+	-	Ent. agglomerans (Erwinia)	
						-	-	-	-	Klebs.rihinoscleromitus1 Shigellasonnei (2)	
NB:-					-		+	+	+	Serratia (3)	
	+		-	+		+	+	+	+	Ent.arogens (4) or Hafnia (5)	
1. Ornithine (-) 2. Ornithine (+) 3.Gas variable week. 4.Additional inositol (+) 5.Additional inositol (-) 6.MR^-, VP^+ 7.MR^+, VP^-									_	Ent.Cloacae (6) or Citrobactor (7)	
					+	+	+	+	+	Citrobactor	
									+	Arizona	Malonate +
			+	+	-	+	+	-	+/-	Klebsellaoxytoca	
		+					+	+	+	Citrobactordiversus	
				+	-	+	-	+	+	E. Coli	
			-					-	+	E. Coli	
						-	-	-	-	E. Coli (A—D)	
				-	-	-	-	-	-	Sh. Dysente or E. Coli A-D	

II	Lactose	Indol	Urea	Maitol	H_2S	Gas glu	Citrate	Motility	Lysin	**Organism**	Additional
LACTOSE AND ONPG NEGATIVE				+/-	-	+	+	+	-	Providenciarettgeri	PAD (+)
			+	_	-	+	-	+	-	Morganellamorganii	PAD (+) LDC(-)
		+			+	+	-/+	+	-	Proteus vulgaris	PAD (+)
						+	+	+	-	Provideniaalkalifaciens 4	PAD (+)

NB:- + 90% or more positive -90%or more negative +/- majority negative -/+majority negative	-			-	-	-	+	+	-	ProvidinicaStuartii	PAD (+)
							-	-	-	Shegelladysentriae	
			-		+	+	-	+	+	Edwardsiella	
							-	-	+	E. coli (A—D)	
				+	-	-			-	Shigella spp.	
							+	+	-	Provideniciastuartii (B)	PAD (+)
		-		-	-	-	-	-	-	Shigelladysentriae	
			-		-	-	-	-	-	Shigella spp.	
				+		+	-	+	_	SalmonellaGroup A	
					+	+	+	+	+	Salmonellaor Arizona	
						-	-	+	+	Salmonellathyphi	VC (+)
			+	-	+	+	+/-	+	-	Proteus mirabilis	PAD (+)
	d	-	d	+	-	+d	+	+	+	Seratiamarcescens	Ox +/-

III	Lactose	Indol	Urea	Maitol	H_2S	Gasglu	Citrate	Motility	Lysin	**Organism**	Additional
NON FERMENTATIVE	-	-	d	+/-	-	-	+	+/-	+/-	Pseudomonas Aeruginesa	Cat + Oxi +
	+/-	-	-s	-	-	-	+/-	-	-	Acinetobacter	Cat + Oxi -
	-	-	-	-	-	-	d	+	-	Alcaligens spp.	Cat + Oxi -

AnnexII:Antimicrobial Susceptibility Breaking Point CLIS, 2016

Zone Diameter Interpretive Standards for *Enterobacteriaceae*, in mm Testing conditions

Media: Mueller-Hinton agar.

Use maximum 12 disks on a 150 mm plate;

Use maximum 6 disks on a 100-mm plate. Disks should be placed no less than 24 mm apart, center to center. Number of disks to test = 12 Inoculum: direct colony suspension equivalent to 0.5 McFarland standards

Incubation: 35+/- 2 oc ,ambient air 16-18 hr

Antimicrobial Agent		Disk content	Zone diameter nearest whole mm			
Test? #			R	I	S	Comments
A	Ampicilin	10 µg	≤ 13	14-16	≥ 17	Report amoxicillin with S/I/R result from ampicillin
A	Gentamicin	10 µg	≤ 12	13-14	≥ 15	Do not report for Salmonellaand Shigella spp.
A	Tobramycin	10 µg	≤ 12	13-14	≥ 15	Do not report for Salmonellaand Shigella spp.
A	Amikacin	30 µg	≤ 14	15-16	≥ 17	Do not report for Salmonellaand Shigella spp.
A	Cefotaxime	30 µg	≤ 22	23-25	≥ 26	
A	Ceftriaxone	30 µg	≤ 19	20-22	≥ 23	
A	Ceftazidime	30 µg	≤ 17	18-20	≥ 21	
A	Trimethoprim+Sulfamethoxazole	1.25/23.75µg	≤ 10	11-15	≥ 16	
A	Ciprofloxacin (breakpoint for Salmonellaonly)	5 µg	≤ 20	21-30	≥ 31	
A	Ciprofloxacin (breakpoint for non-Salmonella)	5 µg	≤ 15	16-20	≥ 21	
A(Salm)	Nalidixic acid	30 µg	≤ 13	14-18	≥ 19	Only test for Salmonellaor if requested (drug N/A)
AU	Nitrofurantoin (PO only)	300 µg	≤ 14	15-16	≥ 17	
1	Amoxicillin+clavulanic acid (PO only)	20/10 µg	≤ 13	14-17	≥ 18	
7	Cefuroxime	30 µg	≤ 14	15-17	≥ 18	Do not report for Salmonellaand Shigella spp.
4	Cefepime	30 µg	≤ 18	19-24	≥ 25	
5	Cefixime (PO only, only for uncomplicated UTI)	5 µg	≤ 15	16-18	≥ 19	Do not test or report Morganella spp. with cefixime
6	Imipenem or meropenem	10 µg	≤ 19	20-22	≥ 23	If R: Notify leader of Hospital Infection Control Team
2	Norfloxacin (PO only)	10 µg	≤ 12	13-16	≥ 17	URINE ONLY &oOnly test if ciprofloxacin is N/A
9	Trimethoprim (PO only)	5 µg	≤ 10	11-15	≥ 16	Only test and report for urine isolate (drug N/A))
8	Aztreonam	30 µg	≤ 17	18-20	≥ 21	
3	Chloramphenicol	30 µg	≤ 12	13-17	≥ 18	DO NOT TEST IN URINE. ALWAYS TEST IN CSF.

Annex III: Description the study

My name is Zeleke Ayenew I am MScgraduate in Addis Ababa University College of health science department of bacteriology, immunology and parasitology. I would like to interview you few questions about risk factors for the cause of diarrhea in pediatrics .the objective of this study is identify enteric pathogens profile and antimicrobial susceptibility pattern in pediatrics with diarrhea for choosing suitable antimicrobial which minimize drug resistance in empirical treatment .

You are kindly requested to be included in the study which will have importance for management of childhood diarrhea treatment and your cooperation and willingness for about 30 minute of interview and providing specimen will be very helpful in identifying the problems related to the issue.

The specimen that you provide will be stool with leak proof plastic container about 5ml within short time after child defecates. Giving to these specimens does not affect your health. I assure you all the information regarding to the result being confidential. your participation is voluntary and you are not obligate to answer any questions and give any specimens that you are not volunteer to answer .i would like to appreciate if you give me a few minutes to answer my questions but if you are not confortable feel free to stop it any time .

Are you volunteer to continue? If yes, continue to next page for interview .if no, continue next participant

Thank you for your cooperation!!!

Address: investigator; zeleke Ayenew, cellphone +251923482264

E-mailzelekeayenew377@gmail.com .Department of bacteriology, immunology and parasitology, Addis Ababa University.

Annex IV: Consent Form

I have read the information sheet concerning this study (or have understood the verbal explanation) and I understand what will be required of me and what will happen to me if I take part in it. I also understand that any time I may withdraw from this study without giving a reason whenever discomfort me about my child.

May I continue the interview?

1. Yes _____Continue the interview

2. No _____Stop the interview and thank the respondent

Witness's signature certifying that the informed consent has been given

Witness's signature _____ Date _____

Introduction to the Interview

Thank you for deciding to participate in the interview and for coming to this session. Previously (on the statement of consent form), we have discussed briefly on the purpose of the research, how you were identified, and your part in the research study. Now I am going to have discussion with you on the relevant topic items. Before going to the discussion, would you tell me important backgrounds such as age, educational background etc.? There is no right or wrong answers. All answers /responses/ ideas you provide are equally important and you are requested to respond honestly from your experiences and beliefs. I may interrupt and probe your ideas. Once again I would like to tell you that what we are going to discuss is very confidential and it will be used only for the research.

Thank you!

Annex V: Questionnaire

SN	**Socio- demographic information**	Alternatives
1	Pediatric age group :	1 ☐ 0-4 2 ☐ 5-9 3 ☐ 10-14
2	Sex:	1☐ male 2☐ female
3	Mother's educational status:	1☐illustrate 2☐ read and write 3☐ elementary school 4☐ High school 5☐ collage/university
4	Mothers marital status ;	1☐ single 2☐ married 3☐ divorce 4☐ widowed
5	Family size	1. ☐2-32. ☐4-5 3. ☐6-7 4. ☐≥8
6	Monthly income birr/ETB	1.☐<500 2.☐500-100 3.☐1001-1500 4. ☐>1500
	Risk factor assessments	
7	Is your child being contracted with diarrhea before this study?	1 ☐ yes 2 ☐ no
8	the type of diarrhea ?	1 ☐ bloody 2 ☐ watery 3 ☐mucoid 4 ☐ semi-fluid
9	Frequency of diarrhea per month?	1☐once 2☐ twice 3☐ three and above

10	Have you taken any treatment for diarrhea case ?	1☐ yes 2☐ no
11	If yes what type of treatment have you take for?	1 ☐ antibiotic 2 ☐ ORS 3 ☐antipain (Painkiller) 4 ☐ probiotics/prebiotics 5 ☐antibiotic+ ORS 6☐ antibiotic+ antipain (Painkiller 7☐antibiotic+ORS +antipain
12	For how long have your child/children been sick of dirrhorea during the study period?	1 ☐ 1-2 days 2 ☐ 3-4 day 3 ☐ 5-7 days 4 ☐ more than a week
13	What symptoms have you observe on your child?	1 ☐ dehydration 2 ☐ fever 3 ☐ vomiting 4☐frequent diarrhea 5☐more than one symptoms
14	Have you confirm the cause of diarrhea with laboratory ?	1 ☐ yes 2 ☐ no
15	For Q14 if yes what method of confirmation you ordered? …	1 ☐ microscopy 2 ☐ stool culture 3 ☐ I do not know
16	How often did your child have taken the same antibiotic treatment while s/he was sick of diarrhea?	1 ☐ many times 2 ☐ sometimes 3 ☐ never the same
17	Is your child free of HIV infection?	1 ☐ yes 2 ☐ no 3 ☐ unknown

18	What is your drinking water source?	1 ☐ boiled water 2 ☐ bottled water 3 ☐ pipechlorinated water 4 ☐ pipeunchlorinated water
19	Do you have a habit of hand washing of your child after toilet?	1 ☐ daily 2 ☐ sometimes 3 ☐ never
20	What was the food/drink taken before illness of your child?	1 ☐ raw meat 2 ☐ raw vegetable 3 ☐ ground beef 4 ☐ unpasteurized milk 5 ☐ cooked food or drink
21	Do you wash your child's hand before and after meal?	1 ☐ yes 2 ☐ no

Laboratory data

Lab code----------

Laboratory result (for PI)

Type of examination		Result										
1 microscopic result												
2 isolated microorganism and antimicrobial susceptibility	trimethoprimsulph amethoxazole	Gentamicin	Ciprofloxacin	Ceftriaxone	Augmentin	Amikacin	Azithromycin	Ampicillin	Chromaphenicol	Meropenem	Cefepime	Piperacillin

R-resistance, S-Sensitive, I-Intermediate

ቅጽI:ስለጥናቱማስተዋወቂያናበጥናቱለመሳተፍፈቃደኝነትመጠየቂያየአማርኛቅጽበህፃናትላይተቅማጥንየሚያመጡረቂቅተህዋ ሥያንናየመድሀኒትፈዋሽነትላይየሚደረግጥናት

ነ. ስለጥናቱማስተዋወቂያቅፅ

ጤናይስጥልኝ? እኔ ስሜ_____ይባላል፡፡የምማረው በአዲስአበባ ዩኒቨርቲ ህክምና ትምህርት ቤት ሲሆን ጥናቴን የምሰራው በህፃናት ላይ ተቅማጥን የሚያመጡ ረቂቅ ተህዋሥያንና የመድሀኒት ፈዋሽነት በሚል ነው፤የጥናቱ አላማ በህፃናት ላይ ተቅማጥን በሚያመጡ ረቂቅ ተህዋስያንና የመድሀኒት ፈዋሽነት ማጥናት ነው፡፡ጥናቱ የሚካሄደው በተክለሀይማኖት ጤና ጣቢያ፣ በለጥሻቸው ጤና ጣቢያ እና በጥቁር አንበሳ ሪፈራል ሆስፒታል በሚታከሙ ህፃናት ይሆናል፡፡እርሰዎንም ከዚህ ጋር ተያያዥነት ያላቸውን ጥያቄዎች እንጠይቀዎታለን፡፡ ጥናቱ ለእርሰዎ የህፃናት ተቅማጥ መንስኤች፣ህክምና እና መከላከያው በሚታዘዙ ፀረተህዋሥያን የፈሽነት ደረጃ ግንዛቤ ለማገኘት ጥንቃቄ ለማድረግ ይረዳል፡፡የሰገራ ናሙና በላብራቶሪ ሲመረመር ምንም አይነት ችግር የማያስከትል ሲሆን የመድሃኒት ትእዛዝ ና የባለሙያ ምክር ይሰጥዎታል፡፡እርሰዎንም በዚህ ጥናት እንዲሳተፉ በትህትና እንጠይቀዎታለን፡፡በዚህ ጥናት በመሳተፈዎ የምናገኘው መረጃ ለጥናታችን ውጤታማነት እነዲሁም በጥናቱ ውጤት ላይ ከፍተኛ አስተዋፅዎይ ኖረዋል፡፡ስለዚህም በዚህ ቃለ-መጠይቅ በመሳተፈዎ ምስጋናዬ የላቀ ነው፡፡በጥናቱ በመሳተፈዎ ምክንያት የሚመጣበዎት ምንም አይነት ችግር አይኖርም፡፡ ነገር ግን 5 ሚሊሊትር የሰገራ ናሙና ለመዉሰድ በታዘዘልወት ፕላስቲክ እቃ ላብራቶሪ ካስመረመሩ በኋላ ከሚተርፍ የሰገራ ናሙና ለጥናቱ እንዲሰጡ እጠይቅዎታለሁ፡፡ በጥናቱ ውስጥ የሚሠጡት መረጃ ሙሉ በሙሉ ሚስጢራዊነቱ የተጠበቀ ነው፡፡ስለዚህ በጥናቱ ለመሳተፍ የእርሠዎ ሙሉ ፈቃድ አስፈላጊ ነው፡፡በተጨማሪም ለመመለስ የማይፈልጓቸው ጥያቄዎች ካሉ ጥያቄዎችን ለመመለስ አይገደዱም፡፡ አንዲሁም በጥናቱ ላለመሳተፍ ከፈለጉ በማንኛውም ጊዜ ማቋረጥ ይችላሉ፡፡በጥናቱ ባለመሳተፈዎ በርሰዎ ላይ የሚያስከትለው ወይም የሚያመጠው ምንም አይነት ጉዳት የለውም፡፡ ቃለመጠየቁን ለማካሄድ እስከ 30 ደቂቃ ይወስዳል፡፡ቃለመጠየቁን በተመለከተ ወይም አጠቃላይ ስለጥናቱ ማንኛውንም አይነት ጥያቄና አስተያየት ቢኖረዎት በሚከተሉት አድራሻዎች መጠቀም ይችላሉ፡፡

ዘለቀ አየነው፡ አዲስ አበባ ዩኒቨርስቲ ህክምና ት/ቤት ስልክ +251923482264

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ዶ/ር. ታምራት አበበ፡አዲስ አባበዩኒቨርስቲ ህክምና ት/ቤት

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ቅጽII ከመጠየቁ በፊት የተጠያቂውን ስምምነት ማረጋገጫ ቅፅ

ከላይ በመግቢያው ላይ የተጠቀሰውን መረጃ አንብቢያለሁ ወይም በቃል የተሰጠኝን ማብራሪያ ተረድቻለሁ፡፡በዚህ መሰረት ከእኔ የሚጠበቅብኝን ድርሻ በሚገባ አውቄያለሁ እና በዚህ ጥናት ላይ በመሳተፌ ሊከሰቱ የሚችሉትን ሁኔታዎች ተገንዝቢያለሁ፡፡ ጥናት በማንኛውም ሠዓት ያለምንም ቅድመ ሁኔታና ምክንያት እራሴን ከተሳታፊነት የማግለል ሙሉ መብት እንዳለኝ ተረድቻለሁ፡ይህን ውሳኔዬን ተከትሎ በእኔም ሆነ በቤተሰቦቼ ላይ በምንፈልገው የጤና አገልግሎት ላይ ምንም አይነት አሉታዊ ተጽዕኖ እንደማይደርስብኝ ተረድቻለሁ፡፡

ፊርማ ---------- ቀን ----------

ጥናቱን በተመለከተ የቃል ማብራሪያ የተሰጠ መሆኑን የሚያረጋግጥው የቃለመጠይቁ አድራጊ ስም ና ፊርማ

የጠያቂው ስም----------

ፊርማ----------

ቀን----------

መጠይቁን እንድቀጥል ፈቃደኛነዎት;

1.አዎ ፈቃደኛ ናቸው ---------- ቃለ መጠይ ቁይቀጥላል

2. አይ ፈቃደኛ አደለሁም---------- ቃለ መጠይቁን በማቆም አመስግነው ይለያዩ

የመጠየቁ ውጤት መግለጫ

ሀ.ሙሉ በሙሉ የተሞላለ. በከፊል የተሞላ ሐ.ተጠያቂው ፈቃደኛ አይደለም መ. ሌላካለ------

መጠየቁን የሞላው ሰው ስም----------ፊርማ----------ቀን----------

ቅጽIII መጠይቅ መመሪያ አንብበው /አድምጠው መልሱን ከተሰጡት አማራጮች አንዱንያክብቡ ወይም በክፍት ቦታው ላይ ይሙሉ፡

ተቀ	1 **የህፃኑ/ኗመረጃ**	ምርጫ
1	ህፃኑ/ኗየሚገኝበት/የምትገኝበትየዕድሜክልል	1 ☐ 0-4 2☐5-9 3 ☐ 10-14
2	በቤትዎውስጥተቅማጥየያዛቸውህፃናትብዛት_____	ግለፅ----------
3	ፆታ:	1☐ወንድ 2☐ሴት
4	የህፃኑ/ኗእናትየትምህርትደረጃ፡	1☐ያልተማረች 2☐ማንበብናመጻፍ 3☐1ኛደረጃ 4☐ከፍተኛደረጃትምህርት 5☐ኮሌጅ/ዩኒቨርስቲ
5	የጋብቻሁኔታ፡	1☐ያላገባች 2☐ያገባች 3☐የፈታች 4☐በሞትምክንያትባሏንያጣች
6	የቤተሰብብዛት_____	ግለፅ----------
7	ወርሃዊየቤተሰብገቢበኢትዮጵያብር	ግለፅ----------
	2 የህፃንተቅማጥመንስኤዳሰሳ	
8	ህፃንዎከዚህበፊትበተቅማጥበሽታታሞ/ማያውቃል/ታውቃለች ?	1 ☐አዎ 2 ☐የለም
9	የተቅማጡዓይነትምንነበር ?	1☐ደምየተቀላቀለ 2 ☐ውሀመሳይ 3 ☐ንፍጥመሳይ

		4 ☐በከፊልፈሳሽ
10	በተራቁጥር ~~3~~” የጠቀሱትየተቅማጥዓይነትበወርውስጥለምንያህልጊዜተደጋገመ ?	1☐ለአንድጊዜ 2☐ለሁለትጊዜ 3☐ከሶስትጊዜበላይ 4☐አልተደጋገመም
11	ለተቅማጡመድሀኒትወስደውያውቃሉ ?	1☐አዎ 2☐የለም
12	በጥያቄ ~~1~~0”መልስዎአዎከሆነየወሰዱትመድሀኒት፡	1 ☐ፀረተዋስያን---------- 2 ☐ኦርኤስ 3 ☐ማስታገሻ 4☐በፋብሪካየታሸገየተቅማጥመድሀኒት 5 ☐አላውቅም
13	ተቅማጡበልጅዎለምንያክልጊዜቆየ ?	1 ☐1-2 ቀን 2 ☐3-4ቀን 3 ☐5-6ቀን 4 ☐ከሳምንትበላይ
14	ህፃንዎተቅማጥሲታመም/ስትታመምያዩትምልክትምንነበር ?	1 ☐መከሳት 2 ☐ትኩሳት 3 ☐ትውከት 4 ☐ፑሎፑሎማስቀመጥ 5☐ሌላካለይግለፁ_____
15	ሀኪምዎየልጅዎንሰገራእንዲያስመረምሩወደላቦራቶሪተልከውያውቃሉ ?	1 ☐አዎ 2 ☐የለም
16	በጥያቄ ~~1~~5” መልስዎ ~~አ~~ዎ” ከሆነየላቦራቶሪምርመራዓይነት ?	1 ☐በማይክሮስኮፕእንዲታይ 2 ☐ካልቸር 3 ☐አላውቅም
17	ህፃንዎበተቅማጥሲታመምተመሳሳይመድሀኒትለምንያህልጊዜይታዘዝለ ወታል ?	1 ☐ለብዙጊዜ 2☐አንዳንዴ 3☐ተመሳሳይመድሀኒትታዝዞአያውቅም 4☐አላስታውስም

18	ህፃንዎከኤችአይቪነፃነው ?	1 ☐አዎ 2 ☐የለም 3☐አላስመረመርሁትም/ኋትም
19	ቤትውስጥየሚጠጣውውሀምንጭ ?	1 ☐ፊልቶየቀዘቀዘ 2 ☐በፋብሪካየታሸገ 3☐የታከመየቧንቧውሀ 4 ☐ያልታከመየቧንቧውሀ
20	ህፃንዎከተጸዳዳ/ችበኃላእጁን/ጇንበሳሙናየማጠብልምድአለዎት?	1 ☐ሙሉበሙሉ 2 ☐በከፊል 3 ☐የለኝም
21	ህጻንዎከመታመሙበፊትየመገቡትየምግብአይነት ?	1 ☐ጥሬስጋ 2 ☐ያልበሰለአትክልት 3 ☐ያልበሰለአሳ 4 ☐ጥሬወተት 5 ☐የበሰለምግብ /መጠጥ
23	ህፃንዎንምግብከመመገብዎበፊትናከመገቡበኃላእጁን/ጇንበሳሙናየማጠብልልምድአለዎት?	1 ☐አዎ 2 ☐የለም

የናሙናኮድ----------ካርድቁጥር ----------

አመሰግናለሁ!!!

Laboratory result (for PI)

Type of examination		Result										
1 microscopic result												
2 isolated microorganism and antimicrobial susceptibility	trimethoprimsulphamethoxazole	Gentamicin	Ciprofloxacilin	Ceftraxone	Augementin	Amikacin	Azitromicin	Ampicilin	Chromaphenico	Meropenem	Cefepime	Piperacillin tazobactam

R-resistances-Sensitive, I-Intermediate