Addis Ababa University

College of Health Science

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Magnitude of common enteric bacteria and their Antimicrobial susceptibility pattern among governmental school children in a de-worming setting at Sululta, North Shewa, Oromia, Ethiopia.

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This is to certify that the thesis prepared by Abiyot Mesfin entitled "**Magnitude of common enteric bacteria and their Antimicrobial susceptibility pattern among governmental school children in a de-worming setting at Sululta, North Shewa, Oromia, Ethiopia**" submitted in partial fulfillment of the requirements for the Degree of Masters of Sciences in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of abbreviations

AMR	Antimicrobial resistance
AST	Antibiotic susceptibility tests
ATCC	American type of culture collection
CI	Confidence interval
CLSI	Clinical and Laboratory Standards Institute
E.coli	*Escherichia coli*
EPHI	Ethiopian Public Health Institution
GI	Gastrointestinal
HUS	Hemolytic Uremic Syndrome
LF	Lactose fermenters
MAC	MacConkey
MIC	Minimum inhibitory concentration
NLF	Non lactose fermenting colonies
QC	Quality control
SOPs	Standard operating procedures
SPP	Species
SPSS	Statistical Package for Social Sciences
STEC	Shiga toxin-producing *E. coli*
STH	Soil-transmitted helminthes
WHO	World Health Organization
XLD	Xylose-Lysine-Deoxycholate

Abstract

Back ground: Current de-worming programs focus solely on reducing the morbidity caused by helminthes and often fail to monitor for indirect consequences of treatment on other pathogens. As human health reflects integrated impact of infectious agents to which hosts are exposed, it is crucial to monitor for unintended effects of mass drug treatments in co-infected populations.

Objective: To assess the magnitude of common enteric bacteria and their antimicrobial susceptibility patterns among governmental school children in a setting of mass de-worming **Methods**: A school based cross sectional study was conducted in three primary schools from April to June 2017 in Sululta, Oromia, Ethiopia. A structured questionnaire was used for data collection on socio-demographic characteristics of parents as well as for children. A total of 249 de-wormed and 88 non de-wormed school children were enrolled. Stool specimen was collected and cultured on primary isolation media and antimicrobial susceptibility patterns were done for bacteria with CLSI guideline. Data was entered and analyzed using SPSS version 21.

Results: Overall prevalence of enteric pathogen (intestinal parasite and bacterial) was 112(33.2%).From theses, 34(10.1%) were bacterial, 33(9.8%) were protozoa and 46(26.1%) were helminthes. 83 (33.3%) of the pathogens were from de-wormed group, of which 25(10%) were bacteria, 27(10.8%) protozoa and 31(12.4%) were of helminthes origins. The rest 29(32.9%) were detected from non de-wormed group which were 9(10.2%) bacteria, 5(6%) protozoa and 15(17%) helminthes). No statistically significant difference was noted between de-wormed and non-de-wormed groups with isolated bacteria. The dominant bacterial isolates were *Salmonella* spp. and *E.coli O157* from pathogenic one and the least one was *Shigella* spp. All isolates showed 100% resistance to Ampicilin (10µg), but susceptible to nalidixic acid, Ciprofloxacin and cefteriaxone.

Conclusion: Overall prevalence of enteric bacteria was 34(10.1%) and magnitude of the common enteric pathogens among de-wormed and non de-wormed school children was relatively similar**.** Antibiotics like nalidixic acid, Ciprofloxacin and cefteriaxone are active against isolated pathogenic bacteria. Broad surveillance is needed to see the effect of mass de-worming and rational use of antibiotics should be encouraged.

Keywords: de-worming, school children, AST, Enteric bacteria, Oromia, Ethiopi

1. Introduction

1.1 Background

World Health Organization (WHO) recommends that single-dose anthelmintics (400 mg albendazole or 500 mg mebendazole) be routinely administered to school children either once a year in areas with soil-transmitted helminthes (STH) prevalence between 20 % and 50 % or twice a year in areas with STH prevalence above 50 % because of the reduced in the burden of helminthes. National de-worming programs target children of school age, which the WHO defines as being between 5 and 14 (1). The strategy for control of infections STH is to control morbidity through the periodic treatment of at risk people living in endemic areas. People at risk are: preschool children, school-age children, women of childbearing age (including pregnant women in the second and third trimesters and breastfeeding women), Adults in certain high-risk occupations such as tea-pickers or miners (1).

Gastrointestinal (GI) infectious diseases are prevalent in economically developing countries**.** Estimates based on extrapolation fro6743m isolation of known diarrheal pathogens and the numbers of stool samples submitted for study suggest that there might be 38 million cases of acute gastrointestinal illness each year. (2)

The goals of clinical microbiology laboratory testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. The most widely used testing methods include broth microdilution or rapid automated instrument methods that use commercially marketed materials and devices. Manual methods that provide flexibility and possible cost savings include the disk diffusion and gradient diffusion methods. Each method has strengths and weaknesses, including organisms that may be accurately tested by the method. Some methods provide quantitative results (eg, minimum inhibitory concentration), and all provide qualitative assessments using the categories susceptible, intermediate, or resistant (3).

For many years, enteric infections have been diagnosed by analysis of bacterial cultures and microscopy to detect ova and parasites (4). Culture is named to describe the biological amplification of viable and cultivatable bacteria with manufactured growth media (5). Selective agars allow culture of specific *Salmonella*, *Shigella*, *Vibrio*, *Yersinia* and *Campylobacter* species.

Isolation of cultured organisms is still an invaluable tool for determining sensitivity to antimicrobial agents in clinical settings and for identifying specific strains, virulence factors or toxins during investigations of outbreaks (4).

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For many years, enteric infections have been diagnosed by analysis of bacterial cultures and microscopy to detect ova and parasites (4). Culture is named to describe the biological amplification of viable and cultivatable bacteria with artificial culture media (5). Selective agars allow identification of species like *Salmonella, Shigella, Vibrio, Yersinia* and *Campylobacter* species. Isolation of cultured organisms is still an invaluable tool for determining sensitivity to antimicrobial agents in clinical settings and for identifying specific strains, virulence factors or toxins during investigations of outbreaks (4).

1.2 Statement of the problem

Bacterial gastrointestinal infections continue to cause illness and death and contribute to economic loss in most parts of the world, including high-income countries that have developed surveillance and control programmes (6) .Diarrheal disease continues to be an important cause of morbidity and mortality among young children in developing countries. Children and young adults are the most affected, particularly in regions with limited resources and where hygienic measures are inadequate (7, 8).

Individuals who are infected by soil transmitted helminthes (STH) might harbor other infectious agents, including virulent viral and bacterial pathogens, which may be indirectly caused by anthelmintic treatment as stated in different studies, as well as in naturally co-infected animals (9). High rates of co-infections are expected, due to the overlapping distributions and similar routes of transmission of STH and diarrheal pathogens (9). The shared intestinal niche between these pathogens also suggests that direct and indirect interactions will occur within the human gut, potentially impacting the disease dynamics of each pathogen in unexpected ways (9).

Current de-worming programs focus solely on reducing the morbidity caused by helminth infections and often fail to monitor for indirect consequences of treatment on other pathogens (10)

From an applied perspective, identifying the health outcomes of co-infections involving helminth macroparasites and microparasites such as protozoa, bacteria, or viruses is of utmost importance to public and domestic animal health officials. This is, on one hand, because macroparasite control has become widely available and efficient for human populations and domestic animals. On the other hand, before any such macroparasite control programme is incorporated in the fight against microparasites, its potential effects on the focal microparasites should be carefully taken into account (10). Anthelmintic treatment resulted in a concomitant increase of another co-infecting parasite species, suggesting that the targeted helminths were suppressing these infections (11).

The mechanisms by which they do so are likely multifactorial probably include direct, as well as indirect effects on each other. An altered physical microenvironment elicited by helminth infection, result induction of a localTh2 immune response that leading to increased epithelial turn over, mucus production (leading to altered nutrients availability), epithelial barrier integrity, and

altered antimicrobial peptide secretion and also temporary or chronic epithelial barrier disruption by helminthes leading generation of micro biota specific immune cells (12).

As stated above, the World Health Organization currently recommends that school children in endemic areas are regularly treated with drugs which kill soil transmitted helminthes (STH). The recommended drugs are effective at eliminating or greatly reducing worm infections. But the question remains whether doing so will increase host susceptibility to other gastrointestinal infections (bacteria, viruses, or protozoa), potentially due to alterations to the host immune response and/or gut microbiota with helminth removal as has been claimed (13,14).

With the aim of reducing the magnitude of intestinal helminthes and improve the life of children, Ethiopia has launched school based mass de-worming (15). However, the effects of anthelmintic treatment on susceptibility to other diseases, including diarrheal pathogens, remain unknown. Such information could help to inform policy makers to effects of mass anthelmintic administrations if any. So, the aim of this study was to assess the magnitude of common enteric bacteria and their antimicrobial susceptibility pattern in a situation of mass de-worming among school children

1.3 Significance of the study

- The study would provide updated information about the magnitude of common intestinal bacteria and their antimicrobial susceptibility pattern among school children that were previously de-wormed or non- dewormed for soil transmitted helminthes.
- This study would help inform public health policy makers of potential indirect consequences of mass de-worming programs on other co-infecting pathogens
- For participants, it helps to control any change after de-worming and to see the magnitude of common enteric bacteria with de-worming strategy.

2. Literature review

2.1 Definition

Gastroenteritis is defined as an inflammation of the stomach, large and small intestines. It is one of the most common illnesses in humans worldwide. Although it can affect individuals of any age, it presents a significant health risk to those at extremes of age, the very young and the very old (16, 17).

2.2 Causes of gastroenteritis

Gastrointestinal infections are the most common diseases caused by enteric bacteria. Some examples are Salmonellosis (*Salmonella* spp.), cholera (*Vibrio cholerae*), dysentery (*Shigella* spp.) and other infections caused by *Campylobacter jejuni*, *Yersinia* spp. and *Escherichia coli* O157:H7 and many other strains. Viral causes (rotaviruses), protozoa cause; *Cryptosporidium, Giardia lamblia,* and *Entamoeba histolytica* cause acute diarrheal disease. (18-20).

Salmonella, a member of the family *Enterobacteriaceae*, is a facultatively anaerobic Gram-negative rod. *Salmonella* taxonomy is a complicated matter, with two species in the genus: *Salmonella enterica* and *Salmonella bongori***.** *Salmonella enterica* has six subspecies that can be further serotyped using the Kauffmann-White-Le Minor scheme, based on the properties of their somatic (O), flagellar (H), and capsular polysaccharide (Vi) antigens. There are over 2,500 serotypes of *S. enterica* . Because of the diversity of the genus, several isolates may be difficult to identify due to atypical biochemical reactions (21). All infections in warm blooded animal species and humans are due to one single *Salmonella* species (22).

Enterohaemorrhagic E. coli/STEC has >300 serotypes, the most pathogenic of which is E. coli STEC O157. The risk is greatest in children <5 years old. Person-to-person transmission occurs with a low infective dose (<100 organisms). Disease ranges from mild to grossly bloody diarrhoea with abdominal pain, usually without fever. In 8-15% of cases, STECO157:H7 is associated with life-threatening haemolyticeuraemicsyndrome (HUS). This causes acute renal failure, microangiopathic haemolytic anaemia and thrombocytopenia, mainly children aged <5 years, and is caused by a toxin homologous the Shiga toxin of Shigella dysenteriae. Antibiotics appear to increase toxin production in animal models of STEC, and, although retrospective studies have shown variable associations between antibiotic use and development of HUS in

humans, a prospective study demonstrated increased rates of HUS in patients given antibiotics (36%) compared with those who were not (12%). High leucocyte count and vomiting were also associated with higher risk of HUS (23).

*Shigella*s are also Gram-negative bacteria from the family Enterobacteriaceae; they are non-motile and rod-shaped, and were discovered by Dr.Kiyoshi Shiga in 1936(24). The only known natural reservoirs of *Shigella* are humans and large primates. Shigellosis is highly infectious, with minimum inoculums of only 10–100 organisms required to cause disease. Asymptomatic infection is associated with the spread of disease and prolongation of outbreaks due to silent transmission. Severe intestinal and extra-intestinal manifestations can occur with all four species of *Shigella*, but are most common with *S. dysenteriae* type 1, due in part to the production of Shiga toxin. Infections due to *S. dysenteriae* are often acquired by international travel and are often multidrug resistant (25-27).

2.3 Mechanisms gastroenteritis

Bacteria cause gastroenteritis by three main mechanisms, associated with distinctive but overlapping clinical syndromes

- Production of preformed toxin induces vomiting and abdominal cramps within a few hours of ingestion.
- Secretion of toxin after adhering to intestinal epithelium causes a syndrome of watery diarrhoea, without blood or mucus or associated fever (non-inflammatory).
- Invasion of intestinal mucosa causes dysentery, the passage of small-volume stools containing blood, mucus and pus associated with fever, lower abdominal pain and tenesmus (inflammatory (28).

2.4 Transmissions

Transmission is through the fecal oral route, with spread occurring person-to-person, and through consumption of water or food contaminated with feces from infected individuals. Not surprisingly, shigellosis seen more commonly under conditions that facilitate spread of bacteria through the fecal-oral route, such as in daycare centers or in areas without indoor plumbing. (25-27).

E. coli O157:H7 transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products, raw milk, and contaminated raw vegetables

and sprouts. Some STEC can grow in acidic foods, down to a pH of 4.4, and in foods with a minimum water activity of 0.95.STEC is destroyed by thorough cooking of foods until all parts reach a temperature of 70 °C or higher. *E. coli* O157:H7 is the most important STEC serotype in relation to public health; however, other serotypes have frequently been involved in sporadic cases and outbreaks (29).

2.5 Magnitude of enteric bacteria

A cross sectional study by Dhital *et al* (30) on the prevalence of enteric pathogens in children under 15 years of age in Kathmandu, Nepal found that, out of total 600 stool samples collected, pathogenic bacteria were isolated from 51 samples (30). Of which, 29 samples contained *Shigella* spp. and 22 samples contained *Salmonella* spp. Moreover, out of 177 parasite positive samples, protozoa were isolated in 92.09 %, helminths were isolated in 6.21 % and both helminths and protozoa were isolated from 1.69 % of the samples. Among protozoa, *Giardia lamblia* was most common followed by *Entamoeba histolytica*. Among helminths, *Ascaris lumbricoides* was most common followed by *Hymenolepis nana* (30).

Johargy *et.al*.in Saudi Arabi studied that, from a total of 270 stool samples tested in preschool children for various causative agents of gastroenteritis, 39% were positive samples. Of these, thirteen (5%) were of bacterial origins of which 9 (3%) were Salmonella species, 4(2%) were Shigella species and only 3 (1%) of the samples were positive for *Giardia lamblia*. (31).

A study conducted by shebib ZA *et al* in 2003 involving 200childern with bloody diarrhea and 100 age matched controls at two Baghdad hospital showed that,*E.coli*O157 was isolated in 23(11.5%) of 200cases and 3%,1% were *salmonella* spp and *shigella* spp respectively. Within this finding, all *E.coli*O157 isolates were resistant to more than one antimicrobial agent and sensitive to ciprofloxacin, nalidixic acid (32).

As similar study done in Gaza at kindergarten children by Laham NA *et al* in 2015 described that out of the 150 study samples, the overall percentage of positive stool samples with a known enteric pathogen was 60.6%.The prevalence of different enteric pathogens causing community gastroenteritis among symptomatic cases (88.5%) was significantly higher than the prevalence in asymptomatic carriage (11.1%). The most prevalent isolated enteric pathogens were *Entamoeba histolytica* (28.0%) and *Giardia lamblia* (26.7%).Regarding the prevalence of bacterial pathogens in cases and control samples, *Shigella* spp. and *E. coli* O157:H7 were isolated from 3

(2%) samples; all of them were reported in symptomatic cases. Meanwhile, Salmonella was not isolated. *E. coli* O157:H7 was only isolated (6.4%) from cases in the 5-year-old group. Therefore, all the asymptomatic control samples (100%) in all age groups were negative for bacterial infections (33).

A study conducted by Silvestro L. *et al* on Asymptomatic carriage of verocytotoxin-producing Escherichia coli O157(VTEC) in farm workers in Northern Italy shows that VTEC O157 was isolated from four (1.1%) of the farm workers from 350 Stool specimens were collected from 276 different dairy farms(34).

Similar study in Zahedan, Islamic Republic of Iran in 2008 stated that, from total of 322 stool samples from children referred with diarrhoea to a clinic in Zahedan, Islamic Republic of Iran, there were 21 sorbitol-negative *E.coli* isolated; serotyping revealed 4 (1.2%) strains positive for O157.Within this findings, the isolated *E. coli* O157:H7 strains were sensitive to amikacin, gentamicin, nitrofurantoin and tobramycin and resistant to chloramphenicol, cefalexin, cefalothin and co-trimoxazole(35).

Another study by Adwan K. *et al* (36) Shiga toxin producing Escherichia coli isolates from symptomatic and asymptomatic patients in northern Palestine in 1999 were screened for serotype O157. STEC was identified in 176 (70.4%) of 250 stool samples. Of the 176 STEC isolates, 120 were from symptomatic and 56 from asymptomatic patients. Of the 176 STEC isolates, 124 (70.5%) were of serotype O157. Of the seven antimicrobial agents tested, resistance to ampicillin and tazobactam was present in 83% of STEC isolates with resistance to piperacillin in 81%, norfloxacin 59%, gentamicin 55.1%, amikacin 48% and imipenem 26%. Resistance to five or more drugs was found in 49% of the isolates (36).

The prevalence of sorbitol-nonfermenting *Escherichiu coli* 0157:H7 (EHEC) was assessed in 100 patients with diarrhoea by stool culture on sorbitol MacConkey agar. Six of them (6%) harbored sorbitol-negative *E.coli* in their stool samples. All isolates were susceptible in vitro to gentamicin, ampicillin, tetracycline, nalidixic acid and nitrofurantoin, and resistant to sulphamethoxazole, co-trimoxazole and tetracycline (37).

A study conducted in three primary school Calabar, Nigeria in 2004 found that**,** of 593 stool samples obtained from randomly selected children ,123 (20.7%) of the samples isolated were Shigella spp. The infection rate was highest among the younger children with (64.2%) 79/123 of the isolates coming from children under 10 years of age. In all, 24 (19.5%) of the isolates were

sensitive to all of the nine antimicrobial agents tested while 67 (54.5%) were resistant to two or more antibiotics (38).

As a cross-sectional study conducted in South Africa; Vhembe district in 2009 by Same A *et al* , investigated 528 stool samples collected from hospital patients and 295 samples from children attending public primary schools, *Salmonella* spp were 7(2.4%) and *Shigella* spp 6(2%) from school samples. Within this finding, ceftriaxone, ciprofloxacin, ofloxacin, gentamicin, kanamycin, and lomefloxacin were more effective on all the bacterial pathogens tested with overall susceptibility of 91%, 89%, 86%, 81%, 78%, and 74% respectively. Resistance to more than two antibiotics was common and was observed in four (22.2%) *Vibrio* isolates, 22 (22.4%) *Campylobacter* isolates, 18 (24.3%) *Aeromonas* isolates 7 (18.9%) *Salmonella* isolates, and 16 (31.4%) *Shigella* isolates (39).

Cross sectional study was conducted from March to November 2012 in Jimma health center, Jimma southwest Ethiopia by Beyene and Tasew among diarrheal children whose age were less than 15 years found that, a total of 129 (49.6%) samples were positive for intestinal parasite107 (41.1%), Shigella 6 (2.3%) and *Salmonella* species16 (6.2%). Of these, *Shigella* species showed hundred percent resistances to ampicillin, amoxacillin, and cotrimoxazole. All Salmonella isolates were resistant against amoxicillin. All *Shigella* and *Salmonella* species were susceptible to ceftriaxone, ciprofloxacin and gentamycin (40).

Similarly, a cross-sectional study at selected public health facilities in Addis Ababa, Ethiopia by Mamuye Y. *et al* showed that, a total of 253 children 115 males and 138 females with acute diarrhoea were enrolled. From 190 enteric pathogens were isolated, Sixty one (24.1%) was *E. coli*, (9.1%) was *Shigella* followed by (3.95%) *Salmonella* and *Citrobacter* species each and 86 (34.0%) were parasites. The overall resistance rates of isolated *Shigella* and *Salmonella* spp were high for Ampicillin (95.7%, 80.0%) and Augmentin (91.4%, 80%) respectively. However, high sensitivity was observed among both isolates for Ciprofloxacin (91.3%, 100%) and Ceftraxion (91.4%, 100%). More than 87% of *Shigella* species and 70.0% for *Salmonella* species showed multiple resistances (resistance for two or more antibiotics). The prevalence of *Shigella* species was significantly varied among children with different employment parent's status. Raw meat consumption was an independent predictor variable for exposures of *Salmonella* infection ($p \leq 0.05$) (41).

According to a study conducted by Aklilu *et al* , among apparently healthy food handlers of Addis Ababa University student's cafeteria, Addis Ababa, Ethiopia in 2015, out of 172 food-handlers screened, stool cultures revealed only six (3.5%) *Salmonella* isolates. No *Shigella* species was isolated from any of the stool samples obtained. Within this finding, all of them showed multi-resistances (resistance to two or more than two classes of antimicrobials) to the antibiotics. Percentage of isolates that were resistant to ampicillin, clindamycin, amoxicillin and erythromycin were 100%, followed by sulphamethoxazole trimethoprim and cefotaxime which were 16.7%. All isolates were 100% susceptible to Ciprofloxacin, gentamicin and amikacin (42).

In addition, study conducted by Mulatu *et al*, revealed that from a total of 158 fecal samples collected in under-five children with diarrhea, 11 (7.0%) *Shigella* species and 4 (2.5%) *Salmonella* species were isolated, respectively. Moreover, they also noted that the Sero-grouping data indicating that all *Shigella* isolates were found to be *S. flexneri* whereas, *Salmonella* species were identified as serogroup B (3, 1.9%) and serogroup A (1, 0.6%). And all the four *Salmonella* species and 11 *Shigella* species were subjected to antimicrobial susceptibility tests. Accordingly, *Shigella flexneri* showed high resistance against Amoxicillin (100%), Erythromycin (90.9%) and Ampicillin (63.6%). However, low resistance rate was observed against gentamycin (27.3%) and Chloramphenicol (9.1%) and there was no resistance rate observed against Ciprofloxacin, Nalidixic acid and Cotrimoxazole (43).

Further more in the survey by Mengistu *et al*. On prevalence and antimicrobial susceptibility patterns of *Salmonella* serovars and *Shigella* species in Butajira, central Ethiopia, showed that 40 (10.5%) *Salmonella* and 17 (4.5%) *Shigella* strains were isolated from 382 patients. Besides this high frequency of resistance for both *Shigella* and *Salmonella* isolates was observed to tetracycline (82.4, 52.5%), co-trimoxazole (76.5, 37.5%) and ampicillin (47.1, 60%), respectively. All isolates were sensitive to ceftriaxone except 6 intermediate level *Salmonella* isolates. Fifty three percent of *Shigella* isolates were Multi-Drug Resistant as compared to 27.5% of *Salmonella* isolates (44).

3. Hypothesis

The magnitude of common enteric bacteria and their antimicrobial susceptibility patterns is similar between non de-wormed and de-wormed school children.

4. Objectives

4.1 General objective

- To assess the magnitude of common enteric bacteria and their Antimicrobial susceptibility pattern among governmental school children in a setting of mass de-worming at Sululta, North Shewa, Oromia, Ethiopia.

4.2 Specific objectives

- To assess the magnitude of common enteric bacteria from Sululta governmental primary school children.
- To determine drug susceptibility pattern for the isolated bacteria.
- To compare the magnitude of common enteric bacteria based on de-worming status.

5. Materials and methods

5.1 study area

The present study was conduct in Sululta from April to June 2017. Sululta is one of the woredas in the Oromia Region of Ethiopia. Part of the Oromia Special Zone Surrounding Finfinne, it is bordered on the south by the city of Addis Ababa, on the west by the Mulo and Mirab Shewa Zone, on the north by Semien Shewa Zone, and on the east by Bereh. It is located in North showa zone of Oromia regional state 21Km to Northwest of Addis Ababa. The area has an altitude of 2450m above sea level. Based on 2007 national census report, the total population for this woreda was 129,000, of whom 64,516 were men and 64,484 were women; 15,145 or 11.74% of its population were urban dwellers. There are 9 governmental primary schools.

5.2 study design and period

A cross sectional study was conducted from April to June 2017at Abdi Boro, Legedima and Weserbie primary schools in Sululta North Shewa Zone of Oromia Ethiopia.

5.3 Population

5.3.1 Source Population

- All governmental primary school children whose ages were between 5 and 14years and found in Sululta town.

5.3.2 Study population

- Selected school children from Sululta governmental primary schools that fulfill the inclusion criteria.

5.4 Inclusion and Exclusion criteria

5.4.1 Inclusion

- All students whose ages were between 5 and 14 years, willing to participate and where their parents/guardians consented were included.

5.4.2 Exclusion

- Students who were taking antibiotics for the last two weeks before specimen collection.

5.5 Study variables

5.5.1 Dependent variables

- Magnitude of common intestinal bacteria in stool specimen
- Magnitude of antibiotic susceptibility pattern

5.5.2 Independent variables

- Age
- Sex
- Sanitary facility at home/availability of latrine
- Hand washing habit before meal and after toilet
- De-worming status
- Grade level of the students
- Consumption of raw meat and milk

5.6 Measurement and data collection

5.6.1 Sample size determination

Using the formula a single population proportion at 95% confidence interval (CI), prevalence (p=20.7%) (38).

$N = Z\alpha/2^2 (p)(1-p)/d^2$

$=1.96^2(.207)(1-0.207)/0.05^2$

The sample size calculated is 253 students. By adding 10% non response rate, the sample size becomes 278.

Where,

P = prevalence from previous study

Z α/2=coefficient at level of significance

d = margin of error

5.6.2 Sampling method

From the total of nine primary schools, Abdi Boro, Legedima, and Weserbie were selected randomly by lottery method; samples were then taken conveniently from children whose parent/ guardian were signed the consent form.

5.6.3 Data collection procedures

Three schools were selected randomly from the total nine primary public schools. These were Abdiboro (contains1069 students), Legedima(1183), and Weserbie (941) which were in total 3193 students. After obtaining consent, parent/ guardian of the enrolled child and the child were asked to complete short questionnaire regarding the child's demographics, their daily habits, household information. After this, each child was provided with a clean, dry, disinfectant free, wide mouthed container to collect about 2 to 3g of stool specimen into the container and was kept in Cary-Blair transport media, and transported using ice box to Ethiopian Public Health Institute in Bacteriology and mycology department for culturing of stool samples on differential, selective and enrichment medium for isolation and identification of bacterial pathogens within six to eight hours of sample collection.

5.6.4 Laboratory method

On the same day of sample collection, specimens were plated or inoculated on MacConkey agar (MAC), xylose lysine deoxycholate agar (XLD) (Oxoid), sorbitol macConky agar and placed in Selenite F enrichment broth (Oxoid) media and incubated at 37°C for18- 24hrs. Stool specimens incubated in selenite F broth were re-cultured in XLD media for another18- 24 hours at 37ºC. After overnight incubation, colonies which exhibit characteristics of *Salmonella, Shigella* species and *E.coliO157:H7* were identified by conventional biochemical methods like Urease tests, Triple sugar iron agar (TSI), Indole, lysine Decarboxylase (LDC), Citrate and motility tests were done.(For detail principle and procedure refer Annex VIII).

Serological tests

Principle: serological confirmation involves the reaction in which the microorganism (antigen) reacts with its corresponding antibody. This *in vitro* reaction produces macroscopic clumping called agglutination. The desired homologous reaction is rapid, does not dissociate (high avidity) and binds strongly (high affinity).Because a microorganism (antigen) may agglutinate with antibodies produced in response to other species, heterologous reactions are possible. Such unexpected and, perhaps, unpredictable reactions may lead to some confusion in serological identification. Therefore, a positive homologous agglutination reaction should support the morphological and biochemical identification of the microorganism

Test Procedure of slide agglutination for *Salmonella O/shigella or E.coli* O157 polyvalent O

Use this procedure to test the isolate with each selected antiserum.

1. Dispense 1 drop (35 µL) of the antiserum to be tested on an agglutination slide.
2. Dispense 1 drop of 0.85% sterile NaCl solution on an agglutination slide as negative control
3. Positive control**:** Dispense 1 drop of each BD Difco *Salmonella O/shigella or E.coli* O157 Antiserum to be tested on an agglutination slide. Add 1 drop of an appropriate BD Difco QC Antigen species or stock cultures of known serological identification
4. From a solid agar medium, transfer a portion of a loopful of isolated colonies to each reaction area above and mix thoroughly.
5. Mix each reaction area with a separate applicator stick and rock for 1 minute.

Read and Interpretation: Slide agglutination should be strong and clearly visible within one minute. There should be no visible agglutination in the saline control; if agglutination is seen in the control, the suspension is not suitable for testing by this method.

4+ 100% agglutination (background is clear to slightly hazy)

3+ 75% agglutination (background is slightly cloudy)

2+ 50% agglutination (background is moderately cloudy)

1+ 25% agglutination (background is cloudy)

– No agglutination

Antibiotic susceptibility testing: The isolated bacteria were subjected to antibiotic susceptibility testing by Kirby-Bauer disc diffusion technique according to the Clinical and Laboratory Standards institute (CLSI) guidelines (46).

The antibiotic discs used and their concentrations were: ceftriaxone (CRO, 30 µg), ciprofloxacin (CIP, 5 µg), nalidixic acid (NA, 30 µg), trimethoprim-sulfamethoxazole/co-trimoxazole (SXT, 25 µg) ampicillin (AMP 10 µg). 0.5 McFarland standard suspension of the bacteria in 0.85% sterile saline was prepared and inoculated on Mueller-Hinton agar with a sterile cotton swab. A ring of disks containing single concentrations of each antimicrobial agent was place on to the inoculated plate. Following overnight incubation at 37 °C, clear zones produced by antimicrobial inhibition of bacterial growth were measured in millimeter (mm) using a straight-line ruler. The zone of diameter interpreted using an interpretive chart for zone sizes and the results recorded as susceptible (**S**), Intermediate (**I**) or resistance (**R**) based on CLSI (41).

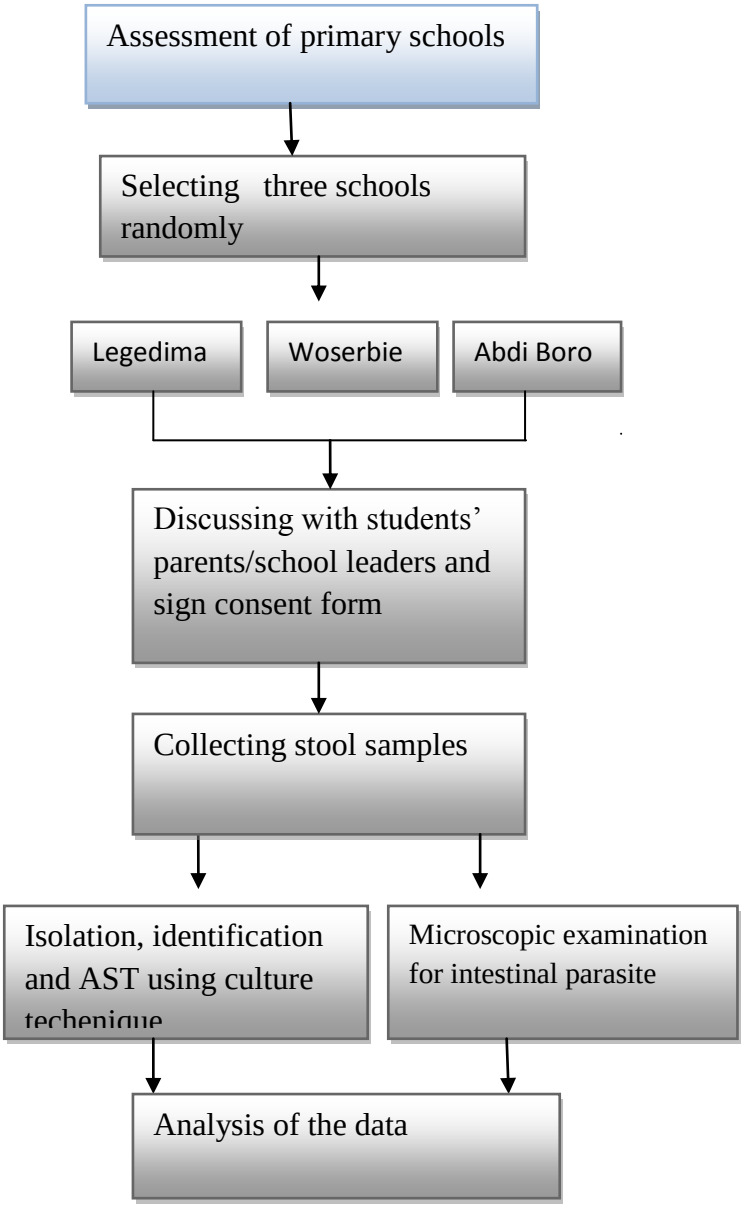

Figure 1.flow chart of the study

5.7 Data quality assurance

5.7.1 Pre analytical

Stool specimens were collected using right container, labeled correctly and kept in appropriate conditions until it processed. Each lot and shipment of media utilized was checked for quality control prior to use.

5.7.2 Analytical

For quality control of biochemical test, purity plate (prepared media for sterility, ability to support growth, and ability to produce appropriate biochemical reactions) were checked and for standardization of the antimicrobial susceptibility testing, control strain *Escherichia coli* (ATCC25922) was used.

5.7.3 Post analytical

- All results were recorded and kept in the appropriate prepared log sheet.
- Data cleaning

5.8 Data processing and analysis

Obtained data were entered, and analyzed by using SPSS (Version 21) software including univariate chi-square tests and binary logistic regression and P-value <0.05 were used to assess the magnitude of association and their significance level.

5.9 Ethical considerations

Before starting the research work, ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University College of Health Sciences, School of Allied Health Sciences, and Department of Laboratory Sciences. Official permissions from the study sites were obtained. To be eligible for enrollment into the study, participants were needed to be more than 4 and under 15 years of age and have written informed consent from a parent or guardian. Children over 12 years of age are required to provide written assent in order to participate in this study. The purpose of the study, the study procedures, possible risks/benefits, the rights and responsibilities of participants including their right to withdraw from the study any time was described The study was carried out after obtaining informed consent and confidentiality of the result was kept by locking hard copies and password protection of electronic data.

5.10 Dissemination of the result

This study on completion can serve as a reference material for any health professionals, researchers, experts and policy makers for intervention. To reach these bodies the finalized paper would be submitted to Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, and Department of Laboratory Sciences. So it can serve as a reference in the library. Additional effort will also be made to present on conferences to reach the medical/scientific community and publish the article on reputable Journals after the final reports.

5.11 Operational definitions

School age children: students whose ages are between 5 and 14 years old (1).

De-worming refers to anthelmintic treatment given by the health office free of charge without stool examination.

6. **Results**

6.1 Socio demographic characteristics of school children

There were 337 school children that participated in this study. Majority of the participants were under age category of 10-14,260(77.2%) and 77(22.8%) were in age category of 5-9 years old. From these, 193(57.3%) of students were female and the mean age was 11.2 years (SD ±2.37). Majority of the mothers were illiterate (52.8%) while majority of their father can read and write (40.7%). as shown in table 1

Table1: Socio demographic characteristics of school children and their guardians from selected government schools in Sululta, North Shewa, Oromia, Ethiopia from April to June 2017.

Characteristics	Frequency	Percent
Maternal occupation		
Civil servant	28	8.3
House wife	212	62.9
Private organization	28	8.3
Daily laborer	34	10.1
Merchant	35	10.4
Maternal education		
Illiterate	178	52.8
Rea and write	59	17.5
Primary	61	18.1
High school	25	7.4
Higher education	14	4.2
Paternal occupation		
Civil servant	68	20.2
Private organization	55	16.3
Merchant	68	20.2

	Daily laborer	71	21.1
	Farmer	56	16.6
	No work	19	5.6
Paternal education			
	Illiterate	59	17.5
	Read and write	137	40.7
	Primary	99	29.4
	High school	28	8.3
	Higher education	14	4.2
Address			
	Semi-urban	235	69.7
	Rular	102	30.3
Grade			
	Kindergarten	24	7.1
	1-4	181	53.7
	5-8	132	39.2
Sex of children			
	Female	193	57.3
	Male	144	42.7
Age category of children			
	5-9	77	22.8
	10-14	260	77.2

6.2 Magnitude of enteric pathogens

Overall burden of enteric pathogens were 112 (33.2%). From these, 34(10.1%), 46(14%) and 32(9.5%) were bacteria, helminthes and intestinal protozoa, respectively. From the total pathogens, 63(56.3%) were from females and 49(43.7%) were from males. Among bacterial isolates, *Proteus* spp. 20(5.9%) was the major species followed by *Salmonella* spp. 4(1.2%) and *E .coli O157:H7* 4 (1.2%) Table2.

Table2: Distribution of common enteric bacteria in Sululta, North Shewa, Oromia, Ethiopia from April to June 2017**.**

	Bacteria species	Frequency	Percent
	Proteus spp.	20	5.9
	Salmonella spp.	4	1.2
	E. coli O157	4	1.2
	Providencia spp.	2	0.6
	Shigella spp.	1	0.3
	Morganella morgani	1	0.3
	Pseudomonas spp.	1	0.3
	Edwardsiella	1	0.3
Total		34	10.1

The predominant intestinal parasite was *Entamoeba histolytica (E .histolytica/dispar)* 18(5.3%) followed by *Hymenolepis nana (H.nana)* and *Giardia lamblia(G.lamblia*)14(4.2%) each as showed in figure 2

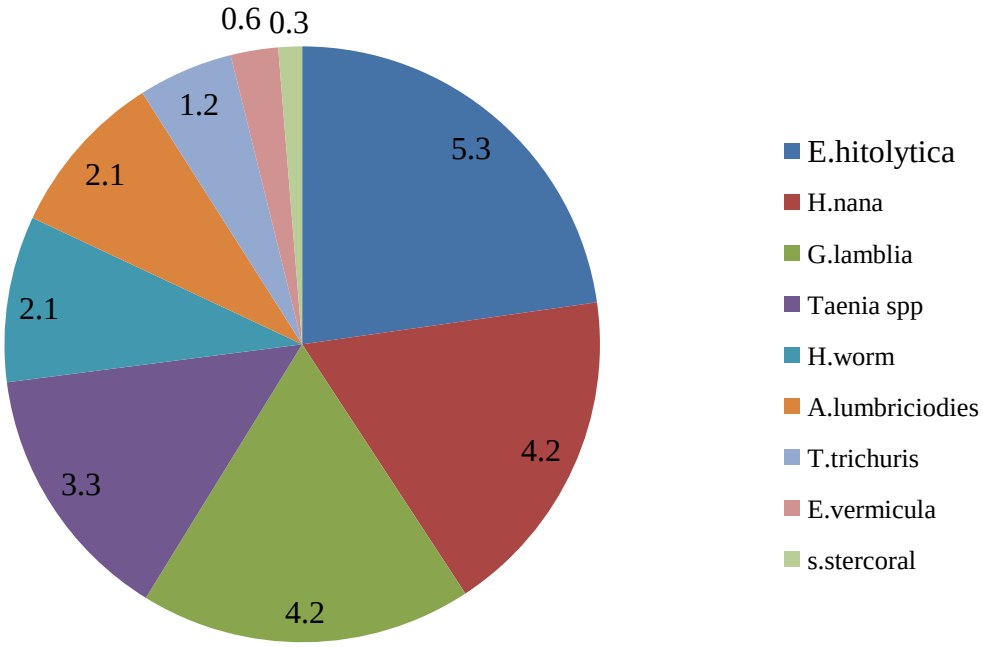

Figure 2: Distribution percent of intestinal parasites and protozoa in Sululta, North Shewa, Oromia, Ethiopia from April to June 2017.

Two hundred forty nine students (73.9%) were de-wormed during de-worming campaigns. From the total de-wormed students, 25(10%) had enteric bacteria, 27(10.8%) were positive for protozoa and 31(12.4%) were positive for one of the intestinal helminthes whereas 9(10.2), 5(6) and 15(17) were from non de-wormed group respectively as described in table 3.

Table 3: Frequency of enteric pathogens from de-wormed and non de-wormed school children in Sululta, North Shewa, Oromia, Ethiopia from April to June 2017

Organism	De-worming statues		
	De-wormed(n=249) N (%)	non de-wormed(n=88) N (%)	Total=337 N (%)
Isolated bacteria			
Proteus spp.	14(5.6)	6(7)	20(5.9)
*E.coli*O157	4(1.6)	0	4(1.2)
Salmonella spp.	2(0.8)	2(2.3)	4(1.2)
Shigella spp.	1(0.4)	0	1(0.3)
Providencia spp.	1(0.4)	1(1.1)	2(0.6)
Morganella morganii	1(0.4)	0	1(0.3)
Pseudomonas spp.	1(0.4)	0	1(0.3)
Edwardsiella	1(0.4)	0	1(0.3)
Total	25(10)	9(10.2)	34(10.1)
Protozoan			
Entamoeba histolytica	15(6)	3(3.4)	18(5.3)
Giardia lamblia	12(4.8)	2(2.3)	14(4.2)
Total	27(10.8)	5(6)	32(9.5)
Helminthes			
H. nana	12(4.8)	2(2.3)	14(4.2)
Taenia spp.	5(2)	6(6.8)	11(3.3)
H. worm	5(2)	2(2.3)	7(2.1)
A. lumbricoides	4(1.6)	3(3.4)	7(2.1)
T .tricuria	3(1.2)	1(1.1)	4(1.2)

E .vermicularies	1(0.4)	1(1.1)	2(0.6)
S. stercoralis	1(0.4)	0(0)	1(0.3)
Total	31(12.4)	15(17)	46(14)
Over all	83(33.3)	29(32.9)	112(33.2)

6.3 Antimicrobial susceptibility pattern of identified enteric pathogen

Antimicrobial susceptibility pattern of the pathogenic bacteria isolates are shown in table 4. All *Salmonella* spp. were resistant for Ampicilin other antibiotics including Nalidixic acid, Ciprofloxacin, Ceftriaxone were the most effective antibacterial agents against all isolates of *Salmonella* spp., *E.coli O157 and Shigella flexenerii* .One (25%) of *Salmonella* spp. was resistant for co-trimoxazole. *Shigella flexenerii* was resistant for both Ampicilin and co-trimoxazole. All *E.coli O157* isolates were resistant for Ampicilin, 2(50%) were resistance for co-trimoxazole and the remaining 2(50%) were susceptible. Four 4(44.4%) isolates were multi-drug resistant (i.e. resistant for Ampicilin and co-trimoxazole antimicrobial drugs).one *Salmonella* spp(25%),2(50%) were *E.coli* O157 and *Shigella flexenerii*

Table 4: Antimicrobial susceptibility pattern of *Salmonella spp, Shigella spp* and *E.coli* O157 isolates among school children in Sululta, North Shewa, Oromia, Ethiopia from April to June 2017.

Antibiotics	*Salmonella* spp. (4)		*Shigella flexenerii(*1)		*E.coli O157*(4)	
	Susceptible n (%)	Resistance n (%)	Susceptible n (%)	Resistance n (%)	Susceptible n (%)	Resistance n (%)
Amp (10µg)	0(0)	4(100)	0(0)	1(100)	0(0)	4(100)
NA(30µg),	4(100)	0(0)	1(100)	0(0)	4(100)	0(0)
SXT (5 µg)	3(75)	1(25)	0(0)	1(100)	2(50)	2(50)
CIP (10 µg),	4(100)	0(0)	1(100)	0(0)	4(100)	0(0)
CRO(30 µg)	4(100)	0(0)	1(100)	0(0)	4(100)	0(0)

N.B AMP-Ampicilin, NA- nalidixic acid, SXT-co-trimoxazole, CIP-Ciprofloxacin, CRO Ceftriaxone

6.4 **Association of some selected risk factors**

From crude analysis as shown in table 5, none of the variables including being de-wormed for soil transmitted helminthes (OR =0.98, with 95% CI 0.44-2.12), hand washing before meal without soap (OR =0.987, with 95% CI (0.43-2.28), having not trimmed finger nail (OR=0.885, with 95% CI 0.41-1.92) and hand washing after toilet without soap (OR =1.169, with 95% CI 0.484-2.82) were significantly associated with bacterial growth. In addition, consumption of raw meat (OR =1.48, with 95% CI 0.72-3.069), consumption of raw milk (OR =1.36, with 95% CI 0.63-2.9) also were not significantly associated with bacterial growth.

Table 5: Distribution of isolated enteric pathogens in bivariate analysis of some selected risk factor variables

Variables	Isolated enteric pathogen								
	Bacterial growth			Intestinal Protozoa			Helminthes		
	N (%)	p. value	Crude OR (95%CI)	N (%)	p. value	Crude OR (95%CI)	N (%)	p. value	Crud OR (95%CI)
De-worming status									
De-wormed(249)	25 (10)	0.96	0.98 (0.44-2.12)	27 (10.8)	0.279	1.66 (0.66-4.2)	31 (12.4)		1.00
Non De-wormed (88)	9 (10.2)		1.00	5 (6)		1.00	15 (17)	0.282	1.445 (0.74-2.83)
Hand washing before meal									
With soap(257)	26 (10.1)		1.00	28 (11)		1.00	34 (13.2)		1.00
Without (80)	8 (10)	0.976	0.987 (0.43-2.28)	4(5)	0.108	0.414 (0.14-1.22)	12 (15)	0.69	1.16 (0.56-2.36)
Hand washing after toilet									
With soap(275)	27 (9.8)		1.00	30 (11)		1.00	39 (14.2)		1.00
Without (62)	7 (11.3)	0.728	1.169 (0.484-2.82)	2(3.2)	0.072	0.262 (0.06-1.13)	7(11.3)	0.550	0.770 (0.327-1.81
Age category									
5-9(77)	8 (10.4)	0.92	1.04 (0.45-2.4)	5(6.5)	0.273	0.575 (0.21-1.55)	9 (11.7)	0.569	0.798 0.367-1.74
10-14(260)	26 (10)		**1.00**	27 (10.4)		1.00	37 (14.2)		
Consumption of raw meat									
Yes(179)	21 (11.7)	0.289	1.48 (0.72-3.069)	19 (10.6	0.733	1.13 (0.56-2.31)	20 (11.2)	0.446	0.78 (0.41-1.49
No (158)	13 (8.2)		**1.00**	13 (8.2)		1.00	22 (13.9)		1.00
Consumption of raw milk									
Yes(90)	11 (12.2)	0.434	1.36 (0.63-2.9)	5 (5.6)	0.122	0.46 (0.17-1.23)	12 (13.3)	0.919	0.964 (0.48-1.96)
No (247)	23 (9.3)		**1.00**	27 (11)		1.00	34 (13.8)		1.00
Fingernail									
Not Trimmed (107)	10 (9.3)	0.757	0.885 (0.41-1.92)	13 (12.1)	0.323	1.45 (0.69-3.04)	18 (16.8)	0.249	0.685 (0.36-1.30)
Trimmed (230)	24 (10.4)		**1.00**	19 (8.3)		**1.00**	28 (12.2)		1.00

As shown in table 6 below, all mentioned variables were not significantly associated with *E.coli*o157 and *Salmonella* spp. findings (p. value >0.05).

Table 6: Frequency of *E.coli* O157, *Salmonella* spp. in association with some risk factors

Variables	*E.coli*O157			*Salmonella* spp.		
	N (%)	P. value	crude OR (95%CI)	N (%)	p. value	crude OR (95%CI)
De-worming						
Yes (249)	4(1.6)		NA	2(0.8)	0.295	0.35 (0.048-2.51)
No (88)	0			2(2.3)		1.00
Consumption of raw milk						
Yes(90)	1(1.1)	0.938	0.91 (0.94-8.9)	0		
No (247)	3(1.2)		1.00	4(1.6)		NA
Consumption raw meat						
yes(179)	4(2.2)		NA	3(1.7)	0.369	2.68 (0.28-25.9)
No (158)	0			1(0.6		1.00
Fruits washed before eat						
Always(65)	1(1.5)		1.00	1(1.5)		1.00
Sometimes(236)	3(1.3)	0.868	0.82 (0.084-8.06)	3(1.3)	0.868	0.82 (0.084-8.06)
Never (36)	0					
Age category						
5-9(77)	0			1(1.3)	0.918	1.13 (0.12-10.9)
10-14(260)	4(1.5)		NA	3(1.2)		1.00
Hand washing before meal						
With soap(257)	3(1.2)		1.00	3(1.2)		1.00
Without(80)	1(1.3)	0.95	1.07 (0.11-10.45)	1(1.3)	0.95	1.07 (0.11-10.45)
Hand washing after toilet						

With soap(275)	4(1.5)		NA	3(1.1)		1.00
Without (62)	0			1(1.6)	0.73	1.49 (0.15-14.53)
Finger nail						
Trimmed(230)	2(0.9)		1.00	3(1.3)		1.00
Non trimmed(107)	2(1.9)	0.44	2.2 (0.30-15.63)	1(0.9)	0.771	0.71 (0.073-6.9)
Grade						
kindergarten Kg (24)	0			0		
1-4 (181)	2(1.1)	0.751	0.73 (0.101-5.22)	2(1.1)	0.751	0.73 (0.101-5.22)
5-8 (132)	2(1.5)		1.00	2(1.5)	1.00	

7. Discussions

In Ethiopia, most researches conducted in different areas are related to adults and there is a scarcity of data in young children especially in school age children. Even if there is, focus on prevalence of intestinal parasite, and those associated with common enteric bacterial pathogens is few. More importantly, mass de-worming strategies do not consider potential effect of de-worming on the host immune response and the gut microbiota composition. As a result, it is hard to find such studies in the literature. Because of such gaps, the literatures used here were not matched with the study unit even if the procedures are more or less similar.

In this cross sectional study, the relationship between the de-worming statues of children with the magnitude of enteric pathogens among school children was investigated.

In this finding, the overall burden of enteric pathogens was 112 (33.2%).When we saw from pathogenic isolated bacteria, *Salmonella* spp. 4 (1.2%) were one of the dominant findings. This finding was lower than the finding reported in Kathmandu, Nepal which was 22 (4%), in Saudi Arabia 9(3%), from preschool children, and 3% from a study done by Shibib ZA *et al.* in Baghdad(28), the finding is also lower from studies done in Ethiopia like 16(6.2%) in Jimma health center South West Ethiopia from diarrheal patients whose age were less than 15 years, and 3.95% by Mamuye Y *et a*l. done at selected health facilities in Addis Ababa40,41). But, it was higher than study done in Gaza at kindergarten *Salmonella* wasn't isolated during study time (33). This difference might be due to sample size, symptomatic study subjects in other studies unlike ours which were apparently healthy.

The magnitude of *Salmonella* spp. in the current finding was relatively comparable with a study done in South Africa by Same A *et al*. that was 7(2.4%) and study conducted by Aklilu *et al*. which was 6(3.5) among apparently health food handlers (40,42). Our finding also consistence with study conducted by Mulatu et al. from 158 fecal samples collected in under five children with diarrhea which was 4(2.5%)(43).

The magnitude of *Shigella flexenerii* in this study was 0.3% which was the only isolate from one student. This result was lower compared to other studies; 20.7% in Nigeria, 9.1% by Mamuye Y *et al*., 6(2.3%) by Beyene *et al*, 6(2%) by Same A *et al*, 4.83% by Dhital *et al*, 4(2%) by Johrgy *et al*., 3(2%) by Laham NA *et al*, results from Mulatu *et al* 11(7%) and 17(4.5%) by Mengistu *et*

al in Butajira(30,31,33,38,39,40,41,43,44). This might be due to study unit difference (symptomatic vs. asymptomatic) The magnitude of *shigella* isolate in our study was relatively higher than study conducted in Addis Ababa university cafeteria from apparently healthy food handler that found no *shigella* isolate and this result was in line with study by Shibib ZA *et al* which reported 1% prevalence (32).

In our finding *E.coli* O157 isolated were 1.2% which were consistent in study done by Silvestro L. *et al* 4(1.1%) from 350 farm workers and it also similar finding 1.2% from 322 stool samples in study by Fard AHM *et al*. (34, 35) Whereas other researchers have reported differently; for instance, in Lagos,Nigeria, they found 6% from 100 patients, in north Palastine by Adwan K. in 1999 that were 70.5% of the 176 STEC isolates(335,37). This might be due to time of sample collection; it was during an outbreak of diarrhea in the area.

In another case control study was 6.4% from case in the 5 years old group and it was 23(11.5%) in similar study by Shebib ZA *et al*. in Banghdad hospital. It was higher than from our finding because of they used bloody diarrhea cases (32, 33).

Regarding antimicrobial susceptibility tests, from nine (9) pathogenic isolates, all *Salmonella* spp. (100%) of the isolates were resistant for Ampicilin that is in agreement with Beyene *et al*. and Aklilu *et al* findings (40,42)but, the resistance rate was higher than Ampicilin(80.7%) was resistance in Mamuye Y *et al*. study(41),60% in study by Mengistu *et al* (44) Besides, 1(25%) of the isolates were resistant for Co-trimoxazole in our result and it was 16.7% in Aklilu *et al* and 37.5% by Mengistu *et al(*42,44*).* this might be due to antibiotic overuse, leading to development of resistance strain

In this finding, 3(75%) of *Salmonella* spp. were sensitive for Co-trimoxazole. Antibacterial agents like nalidixic acid, ciprofloxacin and ceftriaxone are still active drugs for all isolates of *salmonella* spp. which were similar findings by Shibib ZA *et al*. nalidixic acid and ciprofloxacin were sensitive for all isolates(32), in Beyene *et al*. ceftriaxone and ciprofloxacin were susceptible(36) and in Mamuye Y *et al*. ciprofloxacin 100% sensitive(37) also it agrees with Same A *et al*. ceftriaxone was sensitive(39) and also in Aklilu *et al*, all isolates were sensitive for ciprofloxacin(42), all isolates were susceptible to cefteriaxone in study by Mengistu *et al*.(44).

Shigella flexenerii was resistance to ampicilin and co-trimoxazole like study in Jimma, South West Ethiopia by Beyene *et al*. shown that 100% resistance for all isolates of S*higella* spp (40), 95.7% of the isolates were resistance in Mamuye Y *et al*. Study (41). But, nalidixic acid, ciprofloxacin and ceftriaxone were active in our findings which were similar in studies Shibib ZA *et al*., Beyene *et al* and Mulatu *et al*., 2014 there was no resistance rate observed against for nalidixic acid, ciprofloxacin and co-trimoxazole(32,40,43). And in Mamuye Y *et al*. the isolates were sensitive for ciprofloxacin (91.3%) and ceftriaxone (91.4%) (41). But, co-trimoxazole (76.5%) and ampicillin (47.1%) were resistance in study conducted by Mengistu *et al*. 2014(40).

Ciprofloxacin and nalidixic acid were 100%active against for *E.coli*O157 isolates but, ampicillin was 100% resistance for all isolates in similar study by Shebib ZA *et al*. (32) whereas in study by Adwan K.*et a*l, ampicillin was resistance in 83% of the isolates (36). But, ampicillin was susceptible for all isolates in findings of Olorunshola ID *et al*. in Lagos,Nigeria(37).

In our findings, all *E.coli* O157 strains were sensitive to naldixic acid which was also reported in Olorunshola ID *et al*. in Lagos, Nigeria and 50% of the isolates were resistance for co-trimoxazole but, it was 100% resistance in Olorunshola ID *et al* and Fard AHM *et al* .researches (35, 37).

8. Strength and Limitation of the study

8.1 Strength of this study

This study has tried to show the effect of de-worming on other gastro intestinal infections caused by bacteria.

8.2 Limitation

Our study has some limitation like not isolating campylobacter spp., viruses and the non *E.coliO157 E.coli* because of unavailability of equipment and reagents. Unequal sample size between de-wormed and non de-wormed participants, however, maximum effort has been made to include all non-dewormed in the three schools and also the design of the study was cross sectional and conveniently sampling technique was used. The study was also unable to use monovalent antiserum for pathogenic bacteria.

9. Conclusion and Recommendation

9.1 Conclusion

In this finding, the dominant bacterial isolates were *Salmonella* spp. and *E.coli*O157 from pathogenic bacteria and the least one was *Shigella* spp. The burden of the common enteric pathogens among de-wormed and non de-wormed school children was relatively similar**.** Antibiotics like Nalidixic acid, Ciprofloxacin, Ceftriaxone are active drug for those isolated pathogenic bacteria.

9.2 Recommendation

Even if mass de-worming did not expose to other gastrointestinal infection especially enteric bacteria, a broad surveillance is needed to see the effect of mass de-worming weather it increases or susceptible to other enteric bacterial infections.

Isolation and identification of *E.coli* O157 should be routinely performed in microbiology laboratory together with other pathogenic bacteria.

Measures like continuous monitoring of microbiological tests and appropriate use of antibiotics were needed.

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11. Annex

Annex I: English version of the information sheet

Addis Ababa University Post graduate School

Principal Investigator: ABIYOT MESFIN

Name of Organization: AAU, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Sciences

Information sheet for parents/guardian of the children/ the students

Title: Magnitude of common enteric bacteria and their Antimicrobial susceptibility pattern among governmental school children in a deworming setting at Sululta, North Shewa, Oromia, Ethiopia. **Aim:** To assess the magnitude burden of common enteric bacteria and their Antimicrobial susceptibility tests in a situation of deworming strategy among school children in Sululta, Oromia Ethiopia.

Duration: You will spend 20-40 minutes until the specimen is collected, the questionnaire is filled and the consent form is signed.

Procedures to be carried on: For this study to be successful we need your participation. If you are voluntary to participate in this study, you are expected to understand and sign the informed consent. Then, socio demographic statues are important for this study and will be taken. Stool sample will be taken. Collected samples will be transported to EPHI Bacteriology laboratory as soon as possible and will be analyzed for the presence of intestinal parasites and pathogenic bacteria by using standard operating procedures (SOPs).

Risk: There is no risk associated with the specimen collection except your time.

Expected benefits: As a participant of this study your child is expected to give 1-2gm of stool specimen and get check to assess the burden of common enteric bacteria and their Antimicrobial susceptibility pattern. You need to know that the results might be discussed with appropriate individuals. But your name or your child's name, address and phone number will not be disclosed to anyone and to be more precise, identification code will be used in such conditions.

Confidentiality: All information that you give and the results from your child's specimen will be used for this study only. Only limited number of professionals will have access to the information. All the information will be encoded in a computer and will be password protected.

Payment: No payment will be provided by participating in this study.

Right: Participation in the study is voluntary, and refusal to participate involves no penalty or loss of benefits to which you are otherwise entitled. You have a right to with hold information, decline to cooperate in the study and refuse provision of specimens.

Approval: This research project has got ethical clearance from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University, College of Health Sciences School of Allied Health Sciences, Department of Medical Laboratory Science, Sulutta woreda Education Health Office and your school officers.

Whom to contact: If you have any question or description about this study, you can communicate on the following address:

1. Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, Department of Medical Laboratory Sciences

Tel: +251-112-75-51-70

Fax: +251-112-75-46-69

E-mail: SMLT@ethionet.et

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2. Principal Investigator: Abiyot mesfin

Tel. +251-921219599

E-mail: abiyot2012@gmail.com

OROMIFA VERSION

Guca walii galtee hirmaattota qorannoo

Maqaa hirmataa qorannoo _____

Lakkoofsa iccitii_____

Ani maqaan koo kan armaan olitti heerame dhukkuboota ,faca'insa hirina dhigaa fi haala guddina ijoolle akkasumas baaketeroota garaa kessatii argaman ijoolle umuri wagga kudha shanii gaddii mana barnoota keenyaa irratti geessisu keessatii taasifamu irratii akkan hirmaadhu gaafatmen jira. Qoorannon kun dhiigaa fi bobbaa irrati kan taasifamu ta'uu isaa naaf ibsameera. Adeemasa kana keessa miidha ana irraa ga'uu akka hin jirre hubadheera .Kanafuu, qooranno kana irratii kanan hiramadhu fedhii kootin fi barbaachisuma qoorannichaa siirriti ergan hubadhee ta'uu mirkaneeffadheera. Dhuma qorannoo kanaa irrattis bu'aan isaa akka naaf ibasamuu fi dhukkubootni kun yoo narratti argaman yaala barbachisu akkan argadhu haallii akka mijaa'uus naaf ibsameera . kanaafu qooranno kana irrati hiramachuu fedhii koo akka ta'e ibsaa, kanaafis mallatoo kiyyaanan mirkaneessa.

Mallatoo hiramaata _____.Guyya_____

Mallatoo qoorata _____.Guyya_____

Annex II- information sheet (for participants, Amharic version)

አዲስ አበባ ዩኒቨርሲቲ,የጤና ሣይንስ ኮሌጅ,የአላይድ ጤና ሣይንስ ት/ቤት,የሕክምና ላቦራቶሪ ሣይንስ ክፍል

እድሜያቸው ከ 14 አመት በታች ከሆኑ ህጻናት ላይ የሠገራ ናሙና ተወስዶ ለሚሰራው በጸረ-ትላትል በወሰዱ ተማሪወች ላይ በተለመዱ የዐንጀት ላይ ለሚገኙ ባክቲሪያወች ለመጠቃት ያላቸዉ ዕድል ታስቦ ለተሳታፊዎች የተዘጋጀ መረጃ

በአዲስ አበባ ዩኒቨርሲቲ, የጤና ሣይንስ ኮሌጅ ,የአላይድ ጤና ሣይንስ ት/ቤት ,የሕክምና ላቦራቶሪ ሣይንስ ክፍል በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል የማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እንድትሳተፍ/እዲሳተፍ ተጋብዘዋል፡፡ እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነዉን /ኑትን ማንኛዉም ሃሳብ ይጠይቁ፡፡

መግቢያ

የጥናቱ ርዕስ፡ በልጅነት የዕድሜ ክልል በሚገኙ ህጻናት ላይ ጸረ-ትላትል ከወሰዱ በሁላ በተለመዱ የዐንጀት ላይ ለሚገኙ ባክቲሪያወች ለመጠቃት ያላቸዉ ዕድል " የሚል ሲሆን እርስዎ በዚህ ጥናት ላይ የሚኖሮት ተሳትፎ ሙሉ በሙሉ በበጎፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኋላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ ምንም የሚደርስቦት ችግር የለም፡፡በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል ፡፡ከፊለጉ ይህንን መረጃ አንድ ቅጅ ለራስዎ ሊያስቀሩ ይችላሉ፡፡

የጥናቱ ተሳታፊ በመሆኖ የሚጠበቅቦት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ከልጅዎ የሠገራ ናሙና ለመስጠት እንዲሁም ልጅዎት በተለመዱ በባክቲሪያዉች የመጠቃት ሁኒታ ለማረጋገጥ መስማማት ይጠበቅብዎታል፡፡ ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎንም ሆነ የልጅዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤ አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎን አይጨምርም፡፡ ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉልና ለማወቅ የሚያስችል መለያ

ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡ በተጨማሪም ስለልጅዎና ቤተሰቦት አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይጠበቅብዎታል፡፡

በዚህ ጥናት መሳተፍ ምን ያህል ጊዜ ይፈጃል?

የተዘጋጀዉን መጠይቅ ለመሙላት፤ የስምምነት ቅጹ ላይ ለመፈረምና ናሙና ለመስጠት ከ20-40 ደቂቃ ያስፈልጋል፡፡

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ችግሮች ምንድን ናቸዉ?

ልጅዎ ምንም አይነት ችግር አያጋጥመውም፡፡

የእኔ የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

የሰጡት ማንኛውም መረጃና ከተወሰደው ናሙና ላይ የተገኘው የላቦራቶሪ ውጤት የሚውለው ለጥናቱ አላማ ብቻ ነው፡፡ ይህንን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰራተኞች ብቻ ናቸው፡፡ ከዚህም በላይ ስለእርሶ ያለውን ማንኛውም መረጃ የተለየ የይለፍ ቃል ባላው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረገል፡፡

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድንናቸው ?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ ዕሁፍ እንደመሆኑ መጠን ለተሳታፊዎች ገንዘብ አይሰጥም፡፡

የዚህ ጥናት ተሳታፊ መብቱ ምንድን ነው ?

ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለዎት፡፡ የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ፡፡

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

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

የህክምና ላባራቶሪ ሳይንስ ት/ክፍል የአላይድ ጤና ሳይንስ ት/ቤት የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

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አብዩት መስፍን 0921219599

ለመሳተፍ ይስማማሉ?

እስማማለሁ □ **አልስማማም** □¨

Annex III Consent Form (for participants, English version)

Code number----------

Name of the participant----------

I have been informed about the study which is aimed at studying the burden of common enteric bacteria and their Antimicrobial susceptibility tests among school children who are under de-worming strategy. For this study stool samples are required and test will be carried out. The aims of the study and possible risks were explained to me as well.

I am also informed that all the information contained within the questionnaire is to be kept confidential. Moreover I have been well informed of my right to keep hold of information, decline to cooperate and make withdrawal from the study.

It is therefore with full understanding of the situation that I gave the informed consent voluntarily to the researcher to use my child's stool sample for the investigation. In addition, I have had the opportunity to ask questions about it and received clarification to my satisfaction. I have also been informed that the benefit of participation is to get the results of analysis from my child's sample measured for free via the health personnel.

Participant's signature /finger print ----------

Name of Data collectors ---------- signature---------- Date----------

Please direct any questions or problems you may encounter during this study to:

Addis Ababa University College of health Sciences School of Allied health sciences Department of medical laboratory science

Abiyot Mesfin 0921219599

For additional information, please contact Addis Ababa University, College of Health Science institutional review board (IRB) office at:

Tell. +251-11-8-96-13-96

Fax +251-11-5-51-1-51-30-99

P.O.Box. 9086, Addis Ababa, Ethiopia

Annex-IV - Consent form (for participants, Amharic Version)

<u>የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ</u>

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ በልጅነት የዕድሜ ክልል በሚገኙ ህጻናት ላይ ጸረ-ትላትል ከወሰዱ በሁላ በተለመዱ የዐንጀት ላይ ለሚገኙ ባክቲሪያወች ለመጠቃት ያላቸዉ ዕድል ስለሚደረገው ጥናት በቂ ገለጻ ተደርጎልኛል፡፡ ለጥናቱም ከእኔ የተወሰደ የሰገራ ናሙና እንደሚያስፈልግ ተገልጸልኛል፡፡ የጥናቱንም አላማዎች በሚገባ ተረድቻለሁ፡፡

በመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡ በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጸልኛል፡፡

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

የተሳታፊዉ ፊርማ /የጣት አሻራ ----------

የምስክር ሙሉ ስም ፊርማ

1. ---------- ----------
2. ---------- ----------
3. ---------- ----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የመረጃ ሰብሳቢው ስም ---------- ፊርማ ----------ቀን----------

--

ጥናቱን የሚያካሂደዉ ሰዉ ማረጋገጫ

ይህን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካሎዎት በሚከተለው አድራሻ ይጠቁሙን፡፡

አብዩት መስፍን 0921219599

ለተጨማሪ መረጃዎች የአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ ኢንስቲትዩሽናል ሪቪው ቦርድ ይጠይቁ፡፡

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Annex V: English version of the assent form for the students

Code number ……

Name of study participant: _____

I have been requested to participate about this study, which plans to determine to assess the burden of common enteric bacteria and their Antimicrobial susceptibility pattern in a situation of de-worming strategy among school children in Sululta, Oromia Ethiopia in our school. I have been informed this study which involves collecting of Stool specimen. During collection of the specimen I have been told that there is no risk except little time waste and I have also read the information sheet or it has been read to me. I have been also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and drop out of the study if I want and that none of my actions will have any bearing at all on my overall health care. It is therefore with full understanding of the situations that I agreed to give the informed assent provided my parents give their consent.

It is therefore with full understanding of the situations that I agreed to give my assent voluntarily to the researcher to use the specimen taken from me for the investigation

I was also told that results would be reported timely to the responsible body for the appropriate treatment. I agree that I am contributing to the treatment of my fellows by participating in this project. I have asked some questions and clarification has been given to me. I have given my assent freely to participate in the study, and I---------- hereby approve my agreement with my signature provided my parents/guardians give their consent.

Participants' signature: _____Date_____

Principal Investigator's signature: _____Date _____

Annex VI: Assent form (for participants, Amharic Version)

<u>የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ እድሜአቸው ከ 12-14 ለሆኑ ልጆች</u>

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ በልጅነት የዕድሜ ክልል በሚገኙ ህጻናት ላይ ጸረ-ትላትል ከወሰዱ በሁላ በተለመዱ የዐንጀት ላይ ለሚገኙ ባክቲሪያወች ለመጠቃት ያላቸዉ ዕድል ስለሚደረገው ጥናት በቂ ገለጻ ተደርጎልኛል፡፡ ለጥናቱም ከእኔ የተወሰደ የሰገራ ናሙና እንደሚያስፈልግ ተገልጸልኛል፡፡ የጥናቱንም አላማዎች በሚገባ ተረድቻለሁ፡፡

በመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡ በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጸልኛል፡፡

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡፡ የምሰጠውም ናሙና ለምርምር ብቻ እንደሚውልም ተረድቻለው፡፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ፡፡ የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡፡ ወላጆቼ እስከፈቀዱ ድረስ በዚህ ጥናት ለመሳተፍ ተስማምቻለሁ፡፡

የተሳታፊዉ ፊርማ /የጣት አሻራ ----------

የምስክር ሙሉ ስም ፊርማ

1---------- ----------

2---------- ----------

3---------- ----------

ጥናቱን የሚያካሂደዉ ሰዉ ማረጋገጫ

ይህን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካሎዎት በሚከተለው አድራሻ ይጠቁሙን፡፡

አብዩት መስፍን 0921219599

ለተጨማሪ መረጃዎች የአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ ኢንስቲትዩሽናል ሪቪው ቦርድ ይጠይቁ፡፡

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Annex VII. English and Amharic version of the questionnaire

The title of this study is the magnitude of common enteric bacteria and their Antimicrobial susceptibility tests among governmental school children in de-worming strategy at Sululta , North Shewa Zone of Oromia Ethiopia

Interview

We thank gratefully for your agreement to participate in this study. Now we are going to undertake interview with you and the interview is about general socio demographic characteristics and clinical data. All of the answers you provide in this study will be kept confidential. The information you give us is very essential for this study. Therefore, we politely ask you to give us the right response.

Section 1.Back ground information

Code No. _____ Age _____Sex M ☐ F ☐ grade_____

ID No_____ Address: Urban ☐ Rural ☐ Date___/___/____

1.1 Maternal occupation i) Office worker/gov't

ii). Merchant

iii). Farmers

iv) Self-employed

v) House wife

1.2 Maternal Educational status

I) Illiterate II) read and write only III) primary IV) high school V) higher education

1.3 Paternal occupation i) Office worker/gov't

ii). Merchant

iii). Farmers

iv. Self-employed

1.4. Paternal Educational Status

I) Illiterate II) read and write III) primary IV) high school V) higher education

Section **2.House characterization**

2.1. What type of toilet facility do you use? 1. Flush toilet 2. Ventilâted improved pit 3. Traditionnel pit toilet 4. None/bush/field

2.2. Main source of drinking water. i).piped water ii).well or spring water IV). River, dam water

Section3.Child characterization

3.1. Hand washing practice after toilet using soaps I. Always II).Sometimes III) None

3.2. Hand washing practice before meal using soaps I). Always II).Sometimes

3.3. De-worming status I) once de-wormed ii) none de-wormed

3.4. Finger nails trimming I) Trimmed II) not trimmed

3.5. Raw milk consumption I) yes ii) No

3.6. Raw meat consumption I) Yes ii) No

Questionnaire – Amharic version

አሁን ስልጅዎ ቀጣይ ስምናደርገው ምርመራ ይረዳን ዘንድ የሚከተሉትን ጥያቄወች ስስምንጠይቀወት ባክዎን ባስፉት □□ያቶች ውስጥ ያጋጠሙወትን ከዚህ ጋር ተያያዝነት ያሳቸውን ችግሮች በማስታ□ስ ትክክለኛ የሆነ ምሳሽ እዕንዲሰጡን በትህትና ዕንጠይቀወተስን፡፡

የሚስጥር ቁጥር----------ዕድሜ--------ጸታ-------ክፍል----------መለያ ቁጥር----------

አድራሻ፡ ከተማ ገጠር ቀን ---------/----------/----------

ክፍል 1. የህፃኑ/ኗን□እናት እና አባትን □ተመስከተ

1.1. የእናት ስራ ሁኔታ ፡ 1. የመንግስት ሰራተኛ 2.ነጋዴ

3.አርሶአደር 4.የቤት እመቤት

1.2. የእናት የትምህርት ሁኒታ፡ 1. ያልተማረች 2. ማንበብ እና መጻፍ ብቻ

3. የመጀመርያ ደረጃ 4.የሁለተኛ ደረጃ 5.የከፍተ ኛ ትምህርት ወይም ኮሊጅ

1.3. የአባት የስራ ሁኔታ፡ 1. የመንግስት ሰራተኛ 2. ነጋዴ 3.አርሶአደር 4.የግል

1.4. የአባት የትምህርት ሁኒታ፡ 1. ያልተማረ 2. ማንበብ እና መጻፍ ብቻ 3. የመጀመርያ ደረጃ 4. የሁለተኛ ደረጃ 5. የከፍተ ኛ ትምህርት ወይም ኮሊጅ

ክፍል 2. ቤትዎን የተመስከተ

2.1. □መ□□ □ሃ በ□ነኛነት የሚያገኙት ከየት ነው?

1. በግቢው ውስጥ ከሚገኝ ቧንቧ **2.** ከጉድጓድ ወይም ምንጭ **3.** ከወንዝ፣ ከኩሬ

2.2. የህፃኑ/ኗን ቤተስቡ የሚገለገልበት መፀዳጀ ቤት ምን አይነት ነው?

1. በ□ሃ □ሚሰራ ሽንት ቤት **2.** ሽታአልባ መ□□□ **3.** የተለመደ ዓይነት የሽንት ቤት □□ጃ□ **4.** ሜ□ ላ□ □□ም □ ካ

□□ል 3 ህፃኑን/ኗን የተመስከተ

3.1 የህፃኑ/ኗ ሽንት ቤት ከተጠቀመ/ች በኃላ እጅን የምታጠብ ልምድ

1. **ሁልግዜ** 2.**አንዳንዴ** 3.**አይታጠብም/አትታጠብም**

3.2 የህፃኑ/ኗ ምግብ ከመብላቱ/ቷ በፊት እጅን የምታጠብ ልምድ

1.**ሁልግዜ** 2.**አንዳንዴ** 3. **አይታጠብም/አትታጠብም**

3.3 **ህፃኑ/ኗ ባለፉት ስድስት ወራት ለሆድ ትላትል መከላከያ መድሃኒት ወስዶአል?**

1.**አዎ** 2.**አልወሰደም/ችም**

3.4 ህፃኑ/ናየጣት ጥፍር ሁኔታ

1.**የተቆረጠ** 2.**ያልተቆጠ**

3.5 **ህፃኑ/ና ጥሬ ስጋ ይመገባል/ትመገባለች** **1.አዎ** **2.አይመገብም/አትመገብም**

3.6 ህፃኑ/ና ጥሬ ወተት ትጠጣለች/ይጠጣል **1.አዎ** **2.አትጠጣም/አይጠጣም**

Annex VIII standard operating procedures (SOP)

STOOL CULTURES

Principle: Acute infectious diarrhea can be caused by a variety of different etiological agents: bacteria, viruses and protozoa .The common bacterial agents include *Salmonella* spp., *Shigella* spp., *Vibrio cholerae, Campylobacter* spp., enterotoxogenic and enterohemorrhagic *Escherichia coli, Aeromonas* spp., and *Plesiomonas shigelloides*. This SOP describes the procedure for the isolation of *Salmonella, Shigella, Campylobacter*, and *V. cholerae.Salmonella* and *Shigella* are screened by using differential and selective direct plating media, which are based on carbohydrate fermentation (eg. lactose and xylose) and H_2S production. Since some non-pathogenic, Gram-negative bacilli found in normal feces may produce the same reactions as enteric pathogens, biochemical screening and agglutination tests are necessary for identification.

Materials: Culture media: Blood agar (BAP), MacConkey agar (MAC), Xylose lysine deoxycholate agar (XLD), Campylobacter agar (CAMPY), Thiosulfate citrate bile salts sucrose agar (TCBS), Selenite F broth (SEL F), and Alkaline Peptone Water (APW)

Reagents: Oxidase, Indole, String test, Hippurate test, Antisera for serotyping of *Salmonella, Shigella, Vibrio cholerae*

Specimen: Stool or rectal swab transported within 2 hours after collection. If there is a delay in transport, refrigerate the specimen or place in appropriate transport medium.

Do not process the following specimens: 1) specimens from patients hospitalized for >3-4 days, except infants and toddlers; 2) duplicate specimens collected on the same day; 3) more than 3 stools from the same patient in a period of 3 weeks; 4) formed stools; and 5) dried (rectal) swabs.

Quality Control (QC): Process the specimen as soon after receipt as possible. If there is a delay in processing place the specimen in the refrigerator.

Verify that the patient name and identifiers on the specimen match those on the accompanying requisition.

Ensure that all media and supplies used have passed the required QC and are used before their expiration date.

Safety Precautions: Standard safety precautions for handling of patient specimens must be used when processing these specimens:

Procedure

Primary Inoculation: Inoculate the following primary plating media: BAP, MAC, XLD, TCBS, CAMPY, SEL F, and APW

Incubate MAC, XLD, and TCBS overnight at 35 ± 2^{o}C.

Incubate BAP for 48 hours at 35 ± 2^{o}C

Incubate CAMPY plate for 48 hours at 42 ± 2^{o}C in microaerophilic atmosphere.

Incubate SEL F overnight at 35 ± 2^{o}C with cap loosened.

Incubate APW at 35 ± 2^{o}C for 6 – 8 hours with cap loosened.

Subculture APW: Subculture one to two loopfuls of APW to a TCBS plate after 6-8 hours of incubation. The loopfuls of APW should be obtained from the surface and topmost portion of the broth, because Vibrios preferentially grow in this area. Do not shake or mix the tube before sub culturing. Incubate inoculated plate overnight at 35 ± 2^{o}C.

If the broth cannot be sub cultured after 6 – 8 hours of incubation, subculture a loopful of the broth at 18 hours to a fresh tube of APW; this second tube of APW should then be subcultured to a TCBS plate after 6- 8 hours of incubation.

Subculture SEL: Subculture one to two loopfuls of SEL F broth to an XLD plate after overnight incubation. Incubate inoculated plate overnight at 35 ± 2^{o}C.

Culture Examination: Examine primary plates (MAC, XLD, TCBS, and CAMPY) after overnight incubation.

MAC: Look for transparent or colorless colonies (NLF); select one of each type of NLF and inoculate biochemical screening media (TSI, SIM, LIA, Urea slant). Incubate inoculated tubes overnight at 35 ± 2ºC with caps loosened.

XLD: Look for red colonies (NLF - with or without black centers); select one of each type of NLF and inoculate biochemical screening media (TSI, SIM, LIA, Urea slant). Incubate inoculated tubes overnight at 35 ± 2ºC with caps loosened

BAP: *Vibrio* spp. and *Aeromonas* spp. are beta-hemolytic with greenish colonial pigment. Perform spot oxidase test on beta-hemolytic colonies. Subculture oxidase positive colonies to BAP and MAC; incubate overnight. Set up biochemical screening media on oxidase positive colonies (TSI, SIM, LIA, Urea slant).

TCBS: Look for yellow Ccolonies (sucrose fermenter); select one of each type of sucrose fermenting colony and inoculate biochemical screening media (TSI, SIM, LIA, Urea slant). Incubate inoculated tubes overnight at 35 ± 2ºC with caps loosened.

CAMPY: Observe for gray or colorless colonies on CAMPY plate; perform Gram stain and oxidase testing on suspicious colonies. Reincubate plate for one more day if negative.

Confirm identification of *Salmonella*, *Shigella*, and *Vibrio cholera* with typing sera.

Interpretation: Identification of *Salmonella, Shigella, and Vibrio*: Refer to table of Most Frequently Encountered Reactions in Screening Biochemicals. Confirm identification with serotyping.

Identification of *Campylobacter* species: Gram stain examination of the suspected colony should be performed along with an oxidase test. Oxidase positive colonies exhibiting a characteristic Gram stain appearance isolated from a CAMPY plate incubated at 42°C under microaerophilic conditions can be reliably reported as *Campylobacter* spp.

Strains isolated on a CAMPY plate that grow at 42°C, are oxidase positive, demonstrate characteristic microscopic morphology, and produce a positive result with hippurate hydrolysis should be reported as *Campylobacter jejuni*.

Colonial characteristics: Growth at 42^{o}C, gray, flat, irregular spreading colonies. Spreading along the streak line is commonly seen, particularly on freshly prepared media. As the moisture content decreases, the organisms may form round, convex, and glistening colonies with little spreading observed. Proper storage of media to ensure correct moisture content is important for optimal isolation and recognition of *Campylobacter* spp.

Gram stain morphology: *Campylobacter* spp. is Gram-negative curved to S-shaped rods. The bacilli are not easily visualized with the safranin counterstain and are somewhat thinner that other enteric Gram-negative bacteria; carbol fuchsin or 0.1% basic fuchsin may be used as the counter stain.

Hydrolysis of sodium hippurate is the major test for distinguishing *C. jejuni* from other Campylobacter species.

Gram stain, oxidase, and hippurate hydrolysis testing may be performed on colonies from a CAMPY plate.

Reporting: For negative cultures, report as: "No *Salmonella*, *Shigella*, *Campylobacter,* or *V. cholerae* isolated."

For positive cultures, report as: "*Salmonella* (indicate *serotype*) isolated."

"*Shigella* (*indicate species*) isolated."

"*V. cholerae* (indicate serotype) isolated."

"*Campylobacter* species/*jejuni* isolated."

Procedural Notes

Salmonella-Shigella (SS) agar should not be used as primary plating medium as it frequently inhibits the growth of *Salmonella dysenteriae* type 1.

If selective or diffferential media are incorrectly prepared the reaction of organism on those media can be affected.

There is no enrichment medium for *Shigella* that consistently provides a greater recovery rate than use of direct plating alone.

Media of high selectivity such as XLD requires more overlapping when streaking than media of low selectivity.

When inoculating a specimen onto a plate and streaking for isolation it is important to use the entire plate to increase the chances of obtaining well isolated colonies.

References

A. Manual of Clinical Microbiology, American Society for Microbiology (ASM), Washington D.C. USA. 10th edition, 2011.

B. Manual for the laboratory identification and antimicrobial Susceptibility testing of bacterial pathogens of public health importance in the developing world.U.S. Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, U.S.A, and World Health Organization (WHO) Geneva Switzerland. 2003

C. Clinical Microbiology Procedures Handbook. American Society for Microbiology. Washington D.C., USA, 3rd edition, 2010.

Urea Medium

Principle: Urea medium is used for the differentiation of organisms, especially the E*nterobacteriaceae*, on the basis of urease production. When organisms utilize urea, ammonia is formed during incubation, which makes the reaction of the medium alkaline, producing a red/ pink color due to phenol red indicator.

Materials

a. Urea agar dehydrated powder, 40% sterile urea solution
b. Weighing balance, weighing boats, spatula, flask, hot plate and stirrers, screw-cap tubes, autoclave, autoclave tape, water bath
c. Distilled or deionized water

Procedure

a. Weigh the urea agar base according to instructions given on the label of the dehydrated powder. Suspend the powder in distilled or deionized water. If necessary, heat with frequent agitation and boil for I minute to completely dissolve the powder.
b. Autoclave media then cool to 50^{o}C in a water bath. Add appropriate amount of sterile 40% urea solution.
c. Dispense into sterile screw-cap tubes then allow to cool. For Christensen's agar, allow to cool in a slanted position for use as slants.
d. Tighten tube caps. Label tubes with media name and preparation date.
e. Place tubes in a carton box. Label media box with media name, preparation date, expiry date, and storage temperature.
f. Record media preparation on appropriate form.
g. Perform media quality control (QC)

Storage

Store at 4°C for up to 6 month. Make sure caps are tightly closed to prevent evaporation.

Quality Control

a. Test prepared media for sterility, ability to support growth, and ability to produce appropriate biochemical reactions. Record results on QC Form.
b. If QC results are acceptable, label all media boxes with QC labels.

c. Do not use media with unacceptable QC results. Document QC failures and corrective action taken. Inform tech-in-charge of all QC failures.

Inoculation Procedure: Using a heavy inoculums (2 loopfuls) of growth from an 18- to 24-hour pure culture (TSI Agar or other suitable medium), inoculate the broth or agar (streaking back and forth over the entire slant surface). Do not stab the butt since it serves as a color control. For broth, shake tubes gently to suspend the bacteria. Incubate tubes with loosened caps at 35^{o}C in an incubator. Observe reactions after 2, 4, 6, 18, 24 and 48 hours.

Interpretation of Results

a. The production of urease is indicated by an intense pink-red (red-violet) color on the slant or throughout the broth. The color may penetrate into the agar (butt); the extent of the color indicates the rate of urea hydrolysis.
b. A negative reaction is no color change. The agar medium remains pale yellow to buff; the broth remains yellowish orange.
c. Variations in the size of the inoculum can affect the time required to reach positive results.
d. Urea Agar detects rapid urease activity of only the urease positive *Proteus* species. For results to be valid for the detection of *Proteus*, the results must be read within the first 2-6 hours after incubation. Urease-positive *Enterobacter*, *Citrobacter* or *Klebsiella*, in contrast, hydrolyze urea much more slowly, showing only slight penetration of the alkaline reaction into the butt of the medium in 6 hours and requiring 3-5 days to change the reaction of the entire butt.
e. For a listing of urease-positive organisms, consult appropriate texts.

References

a. Difco and BBL Manual for Microbiological Culture Media. Maryland, U.S.A., Becton, Dickinson and Company. 2003.
b. Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World. U.S. Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, U.S.A, and World Health Organization (WHO) Geneva Switzerland. 2003.

Triple Sugar Iron Agar

Principle: Triple Sugar Iron Agar (TSI) is used for the differentiation of gram negative enteric organisms based on carbohydrates fermentation and production of hydrogen sulfide gas.TSI Agar contains three sugars (dextrose, lactose and sucrose), phenol red for detecting carbohydrate fermentation and ferrous ammonium sulfate for detection of hydrogen sulfide production (indicated by blackening in the butt of the tube).

Carbohydrate fermentation is indicated by the production of gas and a change in the color of the pH indicator from red to yellow. To facilitate the detection of organisms that only ferment dextrose, the dextrose concentration is one-tenth the concentration of lactose or sucrose. The small amount of acid produced in the slant of the tube during dextrose fermentation oxidizes rapidly, causing the medium to remain red or revert to an alkaline pH. In contrast, the acid reaction (yellow) is maintained in the butt of the tube because it is under lower oxygen tension. After depletion of the limited dextrose, organisms able to do so will begin to utilize the lactose or sucrose. To enhance the alkaline condition of the slant, free exchange of air must be permitted by closing the tube cap loosely. If the tube is tightly closed, an acid reaction (caused solely by dextrose fermentation) will also involve the slant.

Materials

a. TSI dehydrated powder
b. Weighing balance, weighing boats, spatula, flask, hot plate and stirrers, screw-cap tubes, autoclave, autoclave tape
c. Distilled or deionized water

Procedure

1. Weigh the TSI base according to the manufacturer guidelines, as indicated on the media bottle. Suspend the powder in distilled or deionized water. Heat with frequent agitation and boil for I minute to completely dissolve the powder.
2. Dispense into tubes and autoclave with caps loosened.
3. Cool in a slanted position so that deep butts are formed.
4. Tighten caps. Label tubes with media name and batch number.
5. Place tubes in a carton box. Label media box with media name, lot # and batch #, preparation date, expiry date, and storage temperature.
6. Record media preparation on appropriate form.
7. Perform media quality control (QC).

Storage

Store tubes at 2^{o} to 8^{o}C for up to 6 months.

Prepared media that have been refrigerated should be removed from refrigeration and equilibrated to room temperature prior to inoculation avoid temperature shock to the inoculum.

Quality Control

a. Test prepared media for sterility, ability to support growth, and ability to produce appropriate biochemical reactions. Record results on QC Form.
b. If QC results are acceptable, label all media bags with QC colored stickers.
c. Do not use media with unacceptable QC results. Document QC failures and corrective action taken. Inform tech-in-charge of all QC failures.

Inoculation Procedures

To inoculate, carefully touch only the center of an isolated colony on an enteric plated medium with a cool, sterile needle. Do not take the whole colony or go through the colony because this process could result in picking up contaminants that may be present on the surface of the agar.

Stab into the medium in the butt of the tube, and then streak back and forth along the surface of the slant.

Incubate with caps loosened at 35°C and examine after 18-24 hours for carbohydrate fermentation, gas production and hydrogen sulfide production. Any combination of these reactions may be observed. Do not incubate longer than 24 hours because the acid reaction in the slant of lactose and sucrose fermenters may revert to an alkaline reaction.

Interpretation of Results

a. Carbohydrate fermentation is indicated by a yellow coloration of the medium. If the medium in the butt of the tube becomes yellow (acidic), but the medium in the slant becomes red (alkaline), the organism being tested only ferments dextrose (glucose).
b. A yellow (acidic) color in the slant and butt indicates that the organism being tested ferments dextrose, lactose and/or sucrose.
c. A red (alkaline) color in the slant and butt indicates that the organism being tested is a nonfermenter.
d. Hydrogen sulfide production results in a black precipitate in the butt of the tube.
e. Gas production is indicated by splitting and cracking of the medium.

f. Hydrogen sulfide production may be evident on Kligler Iron Agar but negative on Triple Sugar Iron Agar. Studies showed that the utilization of sucrose could suppress the enzymatic mechanisms responsible for H2S production. Padron and Dockstader found that not all H2S-positive *Salmonella* are positive on TSI.

g. Sucrose is added to TSI to eliminate some sucrose-fermenting lactose-nonfermenters such as *Proteus* and *Citrobacter* spp.

h. Further biochemical tests and serological typing must be performed for definite identification and confirmation of organisms.

References

a. Difco and BBL Manual for Microbiological Culture Media. Maryland, U.S.A., Becton, Dickinson and Company. 2003.

b. Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Improtance in the Developing World. U.S. Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, U.S.A, and World Health Organization (WHO) Geneva Switzerland. 2003

Simmon’s Citrate Agar

Principle: Simmon’s citrate agar used for the differentiation of Gram-negative bacteria based on citrate utilization. The citrate utilization test determines the ability of an organism to utilize sodium citrate as its only carbon source and inorganic ammonium salts as its only nitrogen source. Bacteria that can grow on this medium turn the bromthymol blue indicator from green (neutral) to blue (alkaline).

Materials

d. Simmon’s citrate dehydrated powder

e. Weighing balance, weigh boats, spatula, flask, hot plate with stirrers, screw-cap tubes, autoclave, and autoclave tape.

f. Distilled or deionized water

Procedure

a. Weigh the citrate agar according to the instructions given on the label of the dehydrated powder. Suspend the powder in distilled or deionized water. If required, heat with frequent agitation and boil for one minute to completely dissolve the powder.

b. Dispense into tubes and autoclave with screw caps loosened.

c. Allow to cool in a slanted position for use as slants.

d. Tighten tube caps. Label tubes with media name and preparation date.

e. Place tubes in a carton box. Label media box with media name, preparation date, expiry date, and storage temperature.

f. Record media preparation on appropriate form.

g. Perform media quality control (QC).

Quality Control

d. Test prepared media for sterility, ability to support growth, and ability to produce appropriate biochemical reactions. Record results on QC Form.

e. If QC results are acceptable, label all media boxes with QC labels.

f. Do not use media with unacceptable QC results. Document QC failures and corrective action taken. Inform tech-in-charge of all QC failures.

Inoculation Procedure

Inoculate the medium lightly on the slant by touching the tip of the needle to a colony. There is no need to stab into the butt of the medium. Do not inoculate from a broth culture, because the inoculums will be too heavy. Incubate tubes for up to 4 days at 35 ± 2^{o} C in an aerobic atmosphere. Read reaction daily and observe for development of blue color, denoting alkalinization.

Interpretation of Results

f. Growth with an intense blue color on the slant.

g. Negative: No growth to trace of growth with no change in the color (medium remains dark green)

References

a. Difco and BBL Manual for Microbiological Culture Media. Maryland, U.S.A., Becton, Dickinson and Company. 2003.

b. Bailey and Scott's Diagnostic Microbiology. Mosby, Inc. St. Louis, Missouri, USA. 13th ed. 2013.

Sulfide-Indole-Motility Medium(SIM)

Principle: Sulfide Indole Motility (SIM) Medium is used to differentiate enteric bacilli on the basis of sulfide production, indole formation and motility in a single tube. Hydrogen sulfide production, indole formation and motility are distinguishing characteristics which aid in the identification of the *Enterobacteriaceae*, especially *Salmonella* and *Shigella*. SIM Medium, therefore, is useful in the process of identification of enteric pathogens.

Sodium thiosulphate and ferrous ammonium sulphate are indicators of hydrogen sulfide (H_2S) production in the medium. The ferrous ammonium sulfate reacts with H_2S gas to produce ferrous sulfide, a black precipitate.

Casein peptone is rich in tryptophan which is attacked by certain micro organisms resulting in the production of indole. The indole is detected by the addition of Kovacs reagent following the incubation period or by the spot indole test.

Motility detection is possible due to semisolid media. Growth radiating out from the central stab line indicates that the test organism is motile.

Materials

a. SIM dehydrated powder
b. Weighing balance, weighing boats, spatula, flask, hot plate and stirrers, screw-cap tubes, autoclave, autoclave tape
c. Distilled or deionized water

Procedure

a. Weigh the SIM agar according to the instructions given on the label of the dehydrated media. Suspend the powder in distilled or deionized water. If necessary, heat with frequent agitation and boil for I minute to completely dissolve the powder.
b. Dispense into tubes and autoclave with screw caps loosened.
c. Allow the medium to solidify upright, forming a deep butt with no slant. When the medium is solidifed and cooled, leave caps loose until the surface of the medium has dried.
d. Tighten caps. Label tubes with media name and preparation date.
e. Place tubes in a carton box. Label media box with media name, preparation date, expiry date, and storage temperature.
f. Record media preparation on appropriate form.
g. Perform media quality control (QC).

Storage

Store at 4°C for up to 6 months making sure caps are tightly closed to prevent evaporation.

Quality Control

a. Test prepared media for sterility, ability to support growth, and ability to produce appropriate biochemical reactions. Record results on QC Form.

b. If QC results are acceptable, label all media boxes with QC labels.
c. Do not use media with unacceptable QC results. Document QC failures and corrective action taken. Inform tech-in-charge of all QC failures.

Inoculation Procedure

To inoculate, carefully touch only the center of an isolated colony on an enteric plated medium with a cool, sterile needle. Do not take the whole colony or go through the colony because this process could result in picking up contaminants that may be present on the surface of the agar.

Stab about 1-2 cm down into the medium. Incubate with caps loosened at 35°C and examine after 18-24 hours for motility and hydrogen sulfide production.

Note: Do not use an inoculating loop to inoculate a tube of SIM.

Interpretation of Results

a. Motility: Diffuse growth outward from the stab line or turbidity.
b. H_2S production: Blackening along the stab line (may diffuse into the medium)
C. Indole production: Add 2 -3 drop of kovacs reagent and observe for a formation of a red or pink line on the interface.

References

a. Difco and BBL Manual for Microbiological Culture Media. Maryland, U.S.A., Becton, Dickinson and Company. 2003.
b. Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World. U.S. Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, U.S.A, and World Health Organization (WHO) Geneva Switzerland. 2003.

Annex IX: Declaration

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

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Date of submission_____/_____/_____

This proposal has been submitted with my approval as university advisor.

Name of advisors: Mr. Kassu Desta(MSc, PhD fellow) Signature _____

Aster Tsegaye (MSc, PhD) Signature _____

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Date of submission_____/_____/_____