# Prevalence of *Salmonella* and *Shigella* Species among Under-five Children and Antibiotic Resistance Patterns at Jimma University Medical Center and Serbo Health Center, Southwest Ethiopia


By:

Ephrem Awulachew

Research Thesis Submitted to Jimma University, Institutes of Health, School of Medical Laboratory Sciences for Partial Fulfillment of Requirement of Master Degree in Medical Microbiology.

FEBRUARY, 2018

JIMMA, ETHIOPIA

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FEBRUARY, 2018

JIMMA, ETHIOPIA

**JIMMA UNIVERSITY**

**POSTGRADUATE PROGRAM DIRECTORATE**

As Thesis Research advisor, we hereby certify that we have read and evaluated this thesis prepared, under our guidance, by Ephrem Awulachew, entitled 'Prevalence of *Salmonella* and *Shigella* Species among Under-five Children and Antibiotic Resistance Patterns at Jimma University Medical Center and Serbo Health Center, Southwest Ethiopia'. We recommend that it can be submitted as fulfilling of the thesis requirement.

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Signature Date

As member of the Board of Examiners of the MSc Thesis Open Defense Examination, we certify that we have read, evaluated the thesis prepared by Ephrem Awulachew and examined the candidate. We recommended that the thesis to be accepted as fulfilling the thesis requirement for the Degree of Master of Science in Medical Microbiology.

_____ _____ _____

**Chairperson** Signature Date

_____ _____ _____

**Internal Examiner** Signature Date

_____ _____ _____

**External Examiner** Signature Date

## Declaration Sheet

I, the undersigned, declare that this thesis is my own work. I have followed all ethical and technical principles of research in the preparation, data collection, data analysis and compilation of this thesis. Any scholarly matter that is included in the thesis has been given recognition through citation.

This thesis is submitted in partial fulfillment of the requirements for M.Sc. degree at Jimma University. I confidently declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

**Principal investigator**

Ephrem Awulachew signature:_____ date_____

**Author's contribution**

**Ephrem Awulachew:** Conceptualized and designed the study, performed the laboratory work, carried out the initial analyses and interpretation of the data, drafted the initial manuscript and approved the final manuscript as submitted.

**Dr. Alemseged Abdissa:** Designed the study, supervised the data collection and laboratory analysis, revised the manuscript, and approved the final manuscript submitted.

**Mr. Getenet Tesfaw:** Designed the study, supervised the data collection and laboratory analysis, and approved the final manuscript as submitted.

**Dr. Øystein H Johansen:** Designed the study, supervised the data collection and laboratory analysis, revised the manuscript, and approved the final manuscript as submitted.

**Dr. Zeleke Mekonnen:** Designed the study, revised the manuscript, and approved the final manuscript as submitted.

**Mr. Wakjira Kebede:** Designed the study, revised the manuscript, and approved the final manuscript as submitted.

**ABSTRACT**

**Introduction**: *Salmonella* and *Shigella species* are common causes of bacterial diarrhea from mild to severe forms of intestinal tract infection. Worldwide, an estimated 21 million cases of gastroenteritis are due to *Salmonella*, resulting in 200,000 deaths each year where 80% of deaths occur among children under-five years of age. *Shigella* species is the leading pathogen among the top six attributable pathogens causing childhood diarrhoea.

**Objective:** To determine the prevalence of *Salmonella* and *Shigella* Species among under-five children and antibiotic resistance patterns at Jimma University Medical Center and Serbo Health Center, Southwest Ethiopia from February 17 to June 30 15/2017.

**Methods:** Cross-sectional study design was used to collect data. The stool samples were inoculated on MacConkey agar, xylose lysine dextrose agar and incubated aerobically at 37$^{o}$C for 18 to 24 hrs. The same samples were plated onto Selenite F broth for enrichment of *Salmonella* species. All positive stool cultures were identified and characterized on the basis of morphology, cultural characters and biochemical tests. The antibiotic susceptibility testing using Kirby-Bauer disk diffusion against commonly used antibiotics was done on Mueller Hinton agar. The results of the susceptibility tests reported as susceptible, intermediate or resistant according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guideline.

**Results:** From 348 stool samples screened, 39 samples were positive for bacterial growth. The overall prevalence of *Salmonella* and *Shigella* species was 5.2 % and 6.0% respectively. Frequently isolated *Salmonella* species was *Salmonella typhi* (44.5%) while presumptive identification of *Shigella* species showed 57.1% was *Shigella flexneri*. About 76.2% of *Shigella* species and 66.7% of *Salmonella* isolates were multidrug resistant. *Shigella* and *Salmonella* species showed highest frequency of drug resistance against ampicillin (100%, 88.9%), cefuroxime (85.7%, 72.2%) respectively.

**Conclusion:** *Salmonella* and *Shigella* species remain significant causes of bacterial diarrhea. Higher level of drug resistance observed in the present study. Fluoroquinolones and ceftriaxone are still treatment of option for *Salmonella* and *Shigella* species.

**Recommendation:** This study recommends health facilities to regularly update the treatment guideline based on national and local antimicrobial susceptibility patterns of organisms and it also recommends adopting of stool culture with antimicrobial susceptibility testing prior to treatment.

**Key words:** Antibiotic resistance, Diarrhea, *Salmonella*, *Shigella*, Ethiopia

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# ABBREVIATIONS

AGE - Acute gastroenteritis

ATCC- American Type Culture Collection

BSAC -British Society for Antimicrobial Chemotherapy

CLSI -Clinical and Laboratory Standards Institute

EBR -Ethiopian Birr

EUCAST- European Committee on Antimicrobial Susceptibility Testing

GEMS-Global Enteric Multicenter Study

MAC- MacConkey

MIU- Motility Indole Urea

MSD-Moderate to Severe Diarrhea

OPD –Out Patients Department

ORS- Oral Rehydration Salts

SS -*Salmonella Shigella* Medium

XLD -Xylose lysine deoxycholate agar

WHO –World Health Organization

UNICEF –United Nation International Children's Emergency Fund

## OPERATIONAL DEFINITION

**Gastroenteritis** is defined as a decrease in the consistency of stool (loose or liquid) and/or an increase in the frequency of excretion (typically 3 or more in 24 hours), with or without fever or vomiting.

**Diarrhea:** is either the passage of 3 or more loose or watery stools (i.e. stool taking the shape of a container), or 1 or more loose stools with visible blood, in the last 24 hours period

**Persistent diarrhea:** diarrhea for 14 days or more

**Chronic diarrhea:** diarrhea for 28 days or more

**Bloody diarrhea:** presence of blood in the stools; also called dysentery

**Susceptible (S):** The "susceptible" category implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the dosage recommended to treat the site of infection is used according to Clinical and Laboratory Standards Institute (CLSI) guideline.

**Resistant (R):** The "resistant" category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate minimum inhibition concentrations (MICs) or zone diameters that fall in the range where specific microbial resistance mechanisms (e.g., β-lactamases) are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies according to CLSI guideline.

**Multidrug resistance:** Is defined as resistance to more than three families of drugs and above.

# 1. INTRODUCTION

## 1.1. Background

*Salmonella* and *Shigella* species are common causes an inflammation of the intestinal mucosa (1, 2). Gastroenteritis caused by *Salmonella* and *Shigella* species is usually manifested by diarrhea (3, 4). Bacterial gastroenteritis; commonly referred to as bacterial diarrhea can be caused by a number of different agents including *Salmonella* species, *Shigella* species, *Vibrio, Campylobacter, Plesiomonas,* and *Escherichia coli* (5-7). World Health Organization (WHO) and United Nation International Children's Emergency Fund (UNICEF) reported that, there are about two billion cases of diarrheal disease worldwide each year, and 1.9 million children younger than 5 years of ages are dying of diarrhea each year, mostly in developing countries (8).

Microbial agents are responsible for death due to diarrhea, of which *Salmonella* and *Shigella* species are commonly isolated bacteria (9, 10). *Salmonella* and *Shigella* species are microorganisms which have the potential of causing disease in the intestinal tract. *Salmonella* and *Shigella* cause mild to severe forms of intestinal tract infection. *Salmonella* species causes usually self-limited gastro-enteritis and sometimes its severe forms systemic typhoid fever (11). *Salmonella* has a broad distribution throughout the natural world and a widespread occurrence in animals, especially in poultry and swine (12, 13).

Gastroenteritis due to *Shigella* species is an invasive enteric infection which is clinically manifested by diarrhea that is frequently bloody and it is also known to cause watery diarrhea according to Global Enteric Multicenter Study (GEMS) (14). Shigellosis is endemic in many developing countries and also occurs in epidemics forms causing considerable morbidity and mortality in under-five children (15). *Shigella* affects all children but high among under-five, since they are most susceptible age group as a result of poor personal hygiene, low immunity and lack of previous exposures. The main route of transmission of enteric bacteria is fecal-oral through the ingestion of contaminated food or fluids or by direct person-to-person contact. The factors that increase the transmission of enteropathogens in developing countries

include contaminated water and food, poor sanitation and hygiene, lack of and/or improper use of breastfeeding, malnutrition, deficiencies in micronutrients like zinc or vitamin A, crowded living environment, and living close to domestic animals (16, 17).

Diarrheal diseases due to *Salmonella* and *Shigella* species and its complications remain a major cause of morbidity and mortality in children, especially in developing countries (18, 19). In Ethiopia *Salmonella* and *Shigella* infections are common due to contamination of water, food, and poor sewage disposal system. The children's, elderly, and immunocompromised individuals are particularly more susceptible to *Salmonella* and *Shigella* infections at a lower infective dose than healthy adults (20).

Public health interventions rely on estimates of pathogen specific burden for prioritization (21, 22). Previous estimates of the infectious causes of diarrhea have been derived from studies that used varying approaches for pathogen detection. Management of a child presenting with diarrhea include Low-osmolality oral rehydration solution (ORS), or intravenous electrolyte solution in cases of severe dehydration and drug therapy (22). Choice of appropriate drug is a major problem because of the rapid spread of antimicrobial resistance. Stool culture with antimicrobial susceptibility testing is very important in identifying enteric bacteria in the management of diarrhea (22, 23).

Hence, one way to control and decrease the burden diarrhea caused by *Salmonella* and *Shigella* species in under-five children can be achieved through improving hygiene and sanitation, clearly identifying commonly isolated species of *Salmonella* and *Shigella* species and choice of correct antibiotic for treatment.

## 1.2. Statements of the problem

Globally 21% of all deaths in under-five children are estimated to be due to infectious diarrhea (24, 25). Infectious diarrhoea is the second most common cause of death in children under 5 years old in developing countries (26, 27).

Infectious diarrhoea is the leading causes of death in developing countries with the highest under-five mortality in the world being in West and Central Africa accounting for 10% of all under-five deaths, in Eastern and Southern Africa 10%, and South Asia all of under-five deaths 9% (12, 28). In Ethiopia, 15-19% of deaths among children aged 0-59 months attributable to diarrhoea in 2015 (28).

Worldwide, an estimated 21 million cases of gastroenteritis are due to *Salmonella* species, resulting in 200,000 deaths each year where 80% of deaths occur among children under-five years of age (1).

*Shigella* cause inflammatory diarrhea and dysentery, thus presenting a serious challenge to public-health authorities (8, 29). Shigellosis is endemic in most developing countries and is the most important cause of bloody diarrhea worldwide and it is also known to cause watery diarrhea. Shigella species are a leading cause of childhood diarrhoea with case-fatality rates of up to 28% in under-five children (30). About 113 million episodes of diarrhea due to *Shigella* occurred each year among under-5-yearolds in the developing world (31). *Shigella* species cause at least 80 million cases of bloody diarrhea and 700,000 deaths in the world although >90% occur in developing countries. 60% of deaths of shigellosis occur in the under-five age group (1). In 2013 alone, about 28,000 to 48,000 deaths occur amongst under-five children in developing countries due to diarrhea caused by *Shigella* species (14, 32).

In 2016, Global Enteric Multicenter Study (GEMS) identified an increased burden of Shigellosis and reported it as the leading pathogen among the top six attributable pathogens causing childhood diarrhoea. GEMS data also indicate that moderate severe diarrhea MSD among children living in these resources limited areas remains associated with a significantly increased risk of morbidity and mortality (14). This is because children living in poor or remote communities are most at risk and evidence shows children are dying from this preventable disease because effective interventions are not provided equitably across all communities (33).

In Ethiopia, diarrhea caused by *Salmonella* species and *Shigella* species are common among under-five children. *Shigella* and *Salmonella* affects all under-five children as they are most susceptible age group as a result of poor personal hygiene, low immunity and lack of previous exposures (29). In Ethiopia studies have shown that the prevalence of diarrhea both in urban and in rural areas was 13% among all age groups where prevalence of diarrhea was relatively high among children aged from 0 to 59 months. In southern Ethiopia, about 25.5% of children had diarrhea, where three-fourths occurred among rural children (29, 34). The prevalence of *Salmonella* and *Shigella* species among diarrheic patients in Ethiopia ranges from 1.5% to 12% and 2.5% to 20%, respectively (1, 35). The proportion of children with diarrhea for whom treatment was sought from a health care provider increased from 13% in 2000 to 22% in 2005 and 32% in 2011 which is very indicative for empirical treatment is increasing from year to year in country like Ethiopia where antibiotic susceptibility is not part of routine laboratory test (21)**.**

The emergence of antibiotic resistant *Salmonella* and *Shigella* species is becoming major medical and public health problem. Median prevalence of resistance to chloramphenicol in enterobacteriacae, isolated from patients ranges between 31.0% and 94.2%, while median prevalence of resistance to third-generation cephalosporin ranged between 0.0% and 46.5% (36). So, emergence of increased antimicrobial resistance of *Shigella* species is a global challenge, particularly in developing countries where increased misuse of antimicrobial agents occurs. Extensive and poorly controlled use of antibiotics has led to the emergence of multi drug resistant *Shigella* strains (37). Empirical prescription of antibiotics by treating physicians is common in Ethiopia due to the lack of microbiological laboratory facilities to test antimicrobial susceptibilities (35).

Hence, one way to assess the burden caused by *Salmonella* and *Shigella s*pecies and to support management decision is to use diagnostic stool culture for identifying *Salmonella* and *Shigella* species and to determine and report antibiotic susceptibility of the isolates (38). Due to increasing prevalence of infectious diarrhea and the emergence bacteria that are resistant to common antibiotics, it is recommended to identify *Salmonella* and *Shigella* species. Thus, in order to effectively manage bacterial diarrhea due to *Salmonella* and *Shigella* species, it is imperative to identify commonly circulating species of these bacteria, their antibiotic susceptibility pattern and associated factors in a given community. Therefore, this study

aimed to determine prevalence of *Salmonella* and *Shigella* species among under-five children and to test antibiotic resistance of the isolates in the study area.

## 1.3. Significance of the study

The finding of this study will provide information about prevalence of *Salmonella* and *Shigella* species and their antibiotic sensitivity profile. Despite high prevalence of diarrheal disease among children under-five years, antibiotic susceptibility test is not part of routine child care in study area. Hence, this study is in part attempted to determine the occurrence of antimicrobial resistance for *Salmonella* and *Shigella* species isolated from childhood diarrhea in Jimma referral hospital and Serbo Health Center, Southwest Ethiopia. Knowledge about the prevalence of *Salmonella* and *Shigella* species will guide clinicians for management of cases and data obtained from drug sensitivity test will help and guiding health care workers to select the best antibiotics for treatment, helps to minimize inappropriate use of antibiotics. It will also serve as source of information for other researches to be conducted in the future in the same area.

# 2. OBJECTIVES

## 2.1. General objective

- To determine prevalence and antibiotic resistance patterns of *Salmonella* and *Shigella* species among under-five children with diarrhea attending pediatrics ward in Jimma University Medical Center and Serbo Health Center, Southwest of Ethiopia from February 17 to June 30/2017 EC

## 2.2. Specific objectives

- To determine prevalence of *Salmonella* and *Shigella* species among under-five children
- To determine the antibiotic resistance pattern of the *Salmonella* and *Shigella* species
- To identify risk factors of bacterial diarrhea among under-five children

# 3. LITERATURE REVIEW

## 3.1. Prevalence of *salmonella* and *Shigella* species

Globally, prevalence of bacterial diarrhea ranges between 17-26% of which 4.6% is due to *Shigella* species and 1.9% is due to *Salmonella* (36). Study conducted in developing countries indicated that 50-60% diarrheal cases are of bacterial origin of which 1.9% and 1.7% is due to *Salmonella* species and *Shigella* species respectively (38).

A hospital based Cross-sectional study conducted on prevalence and etiology of diarrhea among under-five children in Vietnam, the bacterial genera *Salmonella, Shigella,* and *Campylobacter* were isolated from 57 (4.0%), 48 (3.4%), and 31 (2.2%) cases, respectively (39). Other study in Vetnam showed the prevalence of *Shigella* species among under-five children was 4.7% (40). Study conducted in Jakarta, Indonesia on 612 children aged less than 12 years of age, prevalence of *Shigella* species were 9.3% where *Shigella flexneri* is most frequent species isolated (63.2%) (41). Similar study done in India out of the 71 *Salmonella* isolates, 54 (76%) *non-typhoidal Salmonella* (NTS) were isolated from diarrheal patients, of which, *Typhimurium* accounts (21%). From isolated *Shigella, S. flexneri* was the most common isolate (88%), followed *by S. sonnei* (10%), *S. dysenteriae* type 1(1%) and *S. boydi* (1%), (42).

Another study conducted in Nigeria, reported that the frequency of diarrhoeagenic bacteria isolated showed that 39.06% were *Salmonella,* and *Klebsiella* were 13.02% and other bacteria 6.25% (43). Another study conducted in Tanzania showed that the overall prevalence of *Salmonella* and *Shigella* species was 2.5% and 5.4% respectively (44). A study conducted in Khartoum, Sudan in 2015 stated that prevalence of *Shigella* species were 36 (8 %), while 17 (4 %) were *Salmonella specie,*(45). Similar study in Kenya shows that out of the 651 patients screened, 115 (17.7%) cases were culture positive among which *Salmonella* was 3.5% and *Shigella* was 2% (46).

Study conducted in Gaborone, Botswana on children under-five years with diarrhoea showed the prevalence of *Shigella* and *Salmonella* isolated from rectal swabs was 43/221 (21%), 8/221 (3%) respectively. In that particular study *S. boydi* (13%) was the most prevalent *Shigella* species followed by *S.flexineri* (6%) and *S. sonnei* (2%) while *Salmonella* species identified were *S. typhimurium* and *S. paratyphi* A (47).

In Ethiopia, study conducted in Hawassa Adare Hospital and Millennium Health Center showed that the most isolated bacteria were *Campylobacter* species, 12.7%, *Shigella* species, 7.0%, and *Salmonella* species, 2.5% (48). Similar study conducted in Addis Ababa, showed that from a total of 190 enteropathogens isolated, 24.1% was *E. coli*, while 9.1% and 3.95% was *Shigella* and *Salmonella* species respectively (35).

Study conducted in Butajira showed 10.5% *Salmonella* and 4.5% *Shigella* species were isolated from 382 patients. The *Salmonella* strains isolated were 6 (15%) group A, 5 (12.5%) group B and 3 (7.5%) group C while 16 (40%) could not be typed with the available antisera. In the same study among 17 *Shigella* species 35.3% was *S. sonnei* while 29.5%, 17.6%, (17.6%) was *S. flexneri*, *S. dysenteriae* and *S. boydi* respectively. Another Study conducted in Jimma in 2014, showed 6.2% prevalence of *salmonella* species and 2.5% prevalence of *Shigella* species (49).

## 3.2. Associated factors

Study conducted in Tehran showed that about 55.4% and 40.5% children below five years with *Shigella* infection had vomiting and fever respectively while about 66.7% and 22.9% of children with *Salmonella* infection had vomiting and fever respectively. In same study most *Shigella* and *Salmonella* species were isolated from mucoid diarrhea 43.3% and 36% respectively (50).

Study in Kenya showed that occupation of the study participant's parents, absence of hands washing before eating and after visiting the toilet were factors that are associated with diarrhoea. While sex of the study participants, drinking water from the river, absence of use of toilet regularly and absence of hands washing after eating were less likely to have caused diarrhoea in the study participant (16).

According to study in Burkina Faso fever was occurred in 64% of under-five children with diarrhea, most commonly it was common in children with shigellosis, salmonellosis or *Rotavirus* infection. Vomiting was reported for 61% and dehydration for 42% of under-five children with diarrhea (51).

Study conducted in Hawassa showed that about 50% of under-five children with *Salmonella* infection had fever and abdominal cramp while vomiting and fever was recognized in about

36% of *Shigella* infection and majority of under-five children for whom *Salmonella* and *Shigella* species isolated were visited hospital within 1-5 days of duration of diarrhea (48).

According to study conducted in Harar, Ethiopia the most common appearance of diarrhea was mucoid for both *Salmonella* (42.8%) and *Shigella* species (52.9%) (52).

### 3.3. Antimicrobial susceptibility patterns of *Salmonella* and *Shigella* species

Antibiotic treatment can be a life-saving intervention, but the emergence of antibiotic resistance limits its clinical efficacy. Data on the burden and risk factors for antibiotic resistance in enteric pathogens are needed to inform diarrhea management recommendations and resistance control interventions. Assessment of antimicrobial susceptibility patterns of clinical bacterial isolates is important to know gradual microbial drug susceptibility (53).

Studies conducted in different parts of the world have detecting resistant strain of *salmonella* and *Shigella* for example according to study in Nepal, 10% of the *Salmonella* isolates was resistant to chloramphenicol while 70.0% were resistant to ampicillin and about 70.0% of *Salmonella* species were multi drug resistant (29). In Bangladesh, none of *Salmonella* isolates were resistant to ciprofloxacin, while 75% and 20% were resistant to amoxicillin and chloramphenicol respectively (47).

Study conducted in Mozambique among under-five children with diarrhea showed, *Shigella* isolates had high levels of resistance to sulfamethoxazole (84%), ampicillin (56%), and chloramphenicol (52%) while 25%, 18% and 15% of *Salmonella* species was resistant to ampicillin, sulfamethoxazole and chloramphenicol respectively (54).

According to study conducted in Gaborone, Botswana on children under-five years with diarrhea Antibiograms of the predominant isolates showed that most *Shigella* species were resistant to ampicillin and the *Salmonella* species were susceptible to chloramphenicol, cotrimoxazole, and ampicillin (47).

In in Khartoum, Sudan, 17 % of *Shigella* were resistant to chloramphenicol (45). Similar study conducted in Kenya showed, *Shigella* isolates level of resistance ranged from 80% to 100% for ampicillin and trimethoprim-sulphamethoxazole (46).

Study conducted in Burkina Faso showed 20% of *Salmonella* and 25% of *Shigella* species were resistant to chloramphenicol, 28% of *Salmonella* and 50% of *Shigella* was resistant to ampicillin, 24% of *Salmonella* and 63% of *Shigella* species was resistant to streptomycin and 20% of *Salmonella* and 81% of *Shigella* species were resistant to trimethoprim-sulfamethoxazole (51).

In study conducted in Addis Ababa Ethiopia reported that the overall resistance rates of isolated *Shigella* and *Salmonella* species were high for ampicillin (95.7%, 80.0%). More than 87% of *Shigella* species were multiple resistant, whereas multi drug resistance was 70.0% for *Salmonella* species (35).

Study conducted in Mekele Hospital showed that *Shigella* isolates were 100 % resistant to amoxicillin while resistance to cotrimoxazole accounted 66.6 %. Low resistance was observed to ciprofloxacin and norfloxacin (6.7 % each) (55).

According to study in Hawassa, about 75% of *Salmonella* species were resistant to ceftriaxone. *Shigella* species showed high resistance against ampicillin (63.6%). However, low resistance rate was observed against chloramphenicol (9.1%) and there was no resistance rate observed against ciprofloxacin and cotrimoxazole (48).

Moreover, according to study conducted by Wogi Tosisa. the highest resistance among the total entrophatogenic bacteria was observed against ampicillin (95.8%) followed by cotrimoxazole (58.3%), chloramphenicol (41.7%), and cefotaxime (4.7%)(56).

Study conducted in Butajira reported the resistance *Shigella* and *Salmonella* isolates to cotrimoxazole was 76.5%, and 37.5% respectively. It also reported the resistance *Shigella* and *Salmonella* isolates to ampicillin was 47.1% and 60% respectively. While all isolates were sensitive to ceftriaxone with exception of 6 intermediate level *Salmonella* isolates. Fifty three percent of *Shigella* isolates were Multi-Drug Resistant (MDR) (≥ 3 drugs) and from *Salmonella* isolates 27.5% were multi-drug Resistant (49).

# 4. METHODS AND MATERIALS

## 4.1. Study area

The Jimma University medical center is located in Jimma Town south west of Ethiopia. It is located 354km far from Addis Ababa and Serbo Health Center is found 18Km to the east of Jimma town. The Hospital is the Referral Hospital in Jimma zone, south west of Ethiopia. Pediatrics ward have 108 beds and 78 health professionals at pediatrics ward (12 pediatricians, 21 residents and 45 nurses). Services in pediatrics ward includes management of a child presenting with diarrhea include Low-osmolality oral rehydration solution (ORS), or intravenous electrolyte solution in cases of severe dehydration and drug therapy.

## 4.2. Study period

The study was conducted in Jimma University Medical Center and Serbo Health Center from February 17/2017 to June 30/2017.

## 4.3. Study design

Cross-sectional study design was used to collect clinical data and stool sample to determine prevalence and antibiotic resistance of *Salmonella* and *Shigella* species.

## 4.4. Population

### 4.4.1. Source population

All under-five children who have been presented to the pediatric ward or the paediatric outpatients department (OPD) of Jimma University Medical Center and Serbo Health Center

### 4.4.2. Study population

Under-five children who had diarrhea during the study period were the study population**.**

## 4.5. Inclusion and exclusion criteria

### 4.5.1. Inclusion

As this study was part of CRYPTO-POC project the inclusion criteria were the children who aged between 0-59 months with diarrhea and not enrolled as a case in previous 60 days prior

to data collection, had not been inpatient for longer than 24 hours and children whose parents gave written consent were included.

#### 4.5.2. Exclusion

Under-five children with diarrhea who started treatment at a time of data collection were excluded.

## 4.6. Sample size determination

The sample size for this study was calculated using the formula for estimation of single population proportion

$$n=Z^2pq/d^2$$

Where: P= was taken from prevalence of *Shigella* species from study conducted in Ambo hospital is 29% (57),

Z=value is 95% confidence level which is 1.96; and

q= the proportion who do not have the feature (1-p)

n=desired sample size,

d=degree of accuracy desired (0.05)

So, n= (1.96)2x 0.29(1-0.29)/ (0.05)2

n= (3.8416) x 0.29(0.71)/ (0.0025)

n= 316.3≈ 316

The total sample size is **n (**including 10% non-response rate**) ≈ 348**

## 4.7. Sampling technique

Convenient sampling technique was used to select study participants. All under-five children with diarrhea presented to the pediatric ward or the paediatric outpatients department (OPD) with diarrheal cases were recruited consecutively until the required sample size was reached.

## 4.8. Data collection instrument and techniques

Interviewer administered questionnaire was developed through critical thinking and review of literatures and then translated to Amharic and Afan Oromo language. Demographic information, clinical and data on risk factors were collected using structured, interviewer administered questionnaire among the participating under-five children's parents or guardian. Face to face interview of the parents of children were conducted by trained data collectors.

## 4.9. Screening and Identification of bacteria

### 4.9.1. Stool sample collection

About 2-5 ml fresh diarrheic stool sample, (if present, those parts that contain blood and/or mucus) or 1-2g of loose stool was collected in clean tight fitting container. It was transported to Jimma University Microbiology Laboratory within 2 hours for isolation and identification. If delay was unavoidable it was transported using cold box at 4'C within 8hrs for isolation and identification of *Salmonella* and *Shigella* species in Jimma University microbiology research laboratory.

### 4.9.2. Stool sample rejection criteria:

- Formed stool, specimen contaminated with urine, residual soap, or disinfectants.
- Specimens received in grossly leaking transport containers; diapers; dry specimens; specimens submitted in fixative or additives;
- Samples which have been exposed to direct sun light for prolonged time,
- Stools not in transport medium received >2hrs after collection and
- Samples which were submitted with inappropriate preservatives (e.g. formalin or PVA) were not tested.

### 4.9.3. Inoculation and Incubation

The stool samples were inoculated on MacConkey agar (Oxoid) and Xylose Lysine Dextrose agar (Oxoid), and incubated aerobically at $37^0$C for 18 to 24 hrs. The same samples were plated onto Selenite F broth (Mast Diagnostics DM 210, Mast Diagnostics, and UK) and incubated at $37^o$C for 18 to 24 hrs. Aseptic technique was maintained. Purity plates (negative growth controls) was incubated after each new batch of produced media plates. Growth conditions were controlled by parallel incubation of the following control strains *Shigella*

*flexneri ATCC 12022* and *Salmonella typhimurium ATCC 14028* obtained from Ethiopian public health institutes in every batch of media preparation.

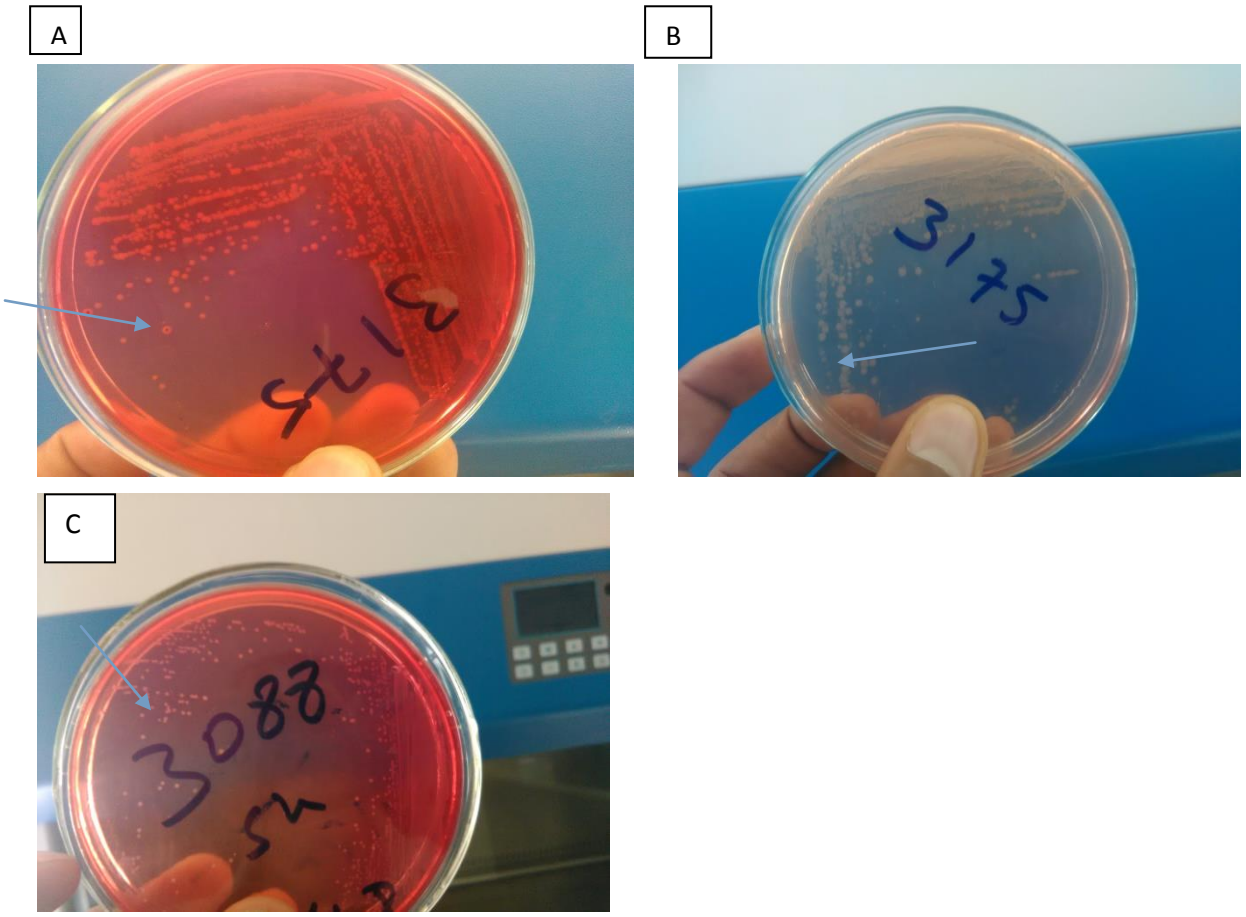



**Figure 1: (A)** *Salmonella* species pink colonies with black center on XLDs, (**B**) colorless colonies of *Salmonella* species on MAC, (C) pink colonies of *Shigella* species on XLD

#### 4.9.4. Isolation and Identification

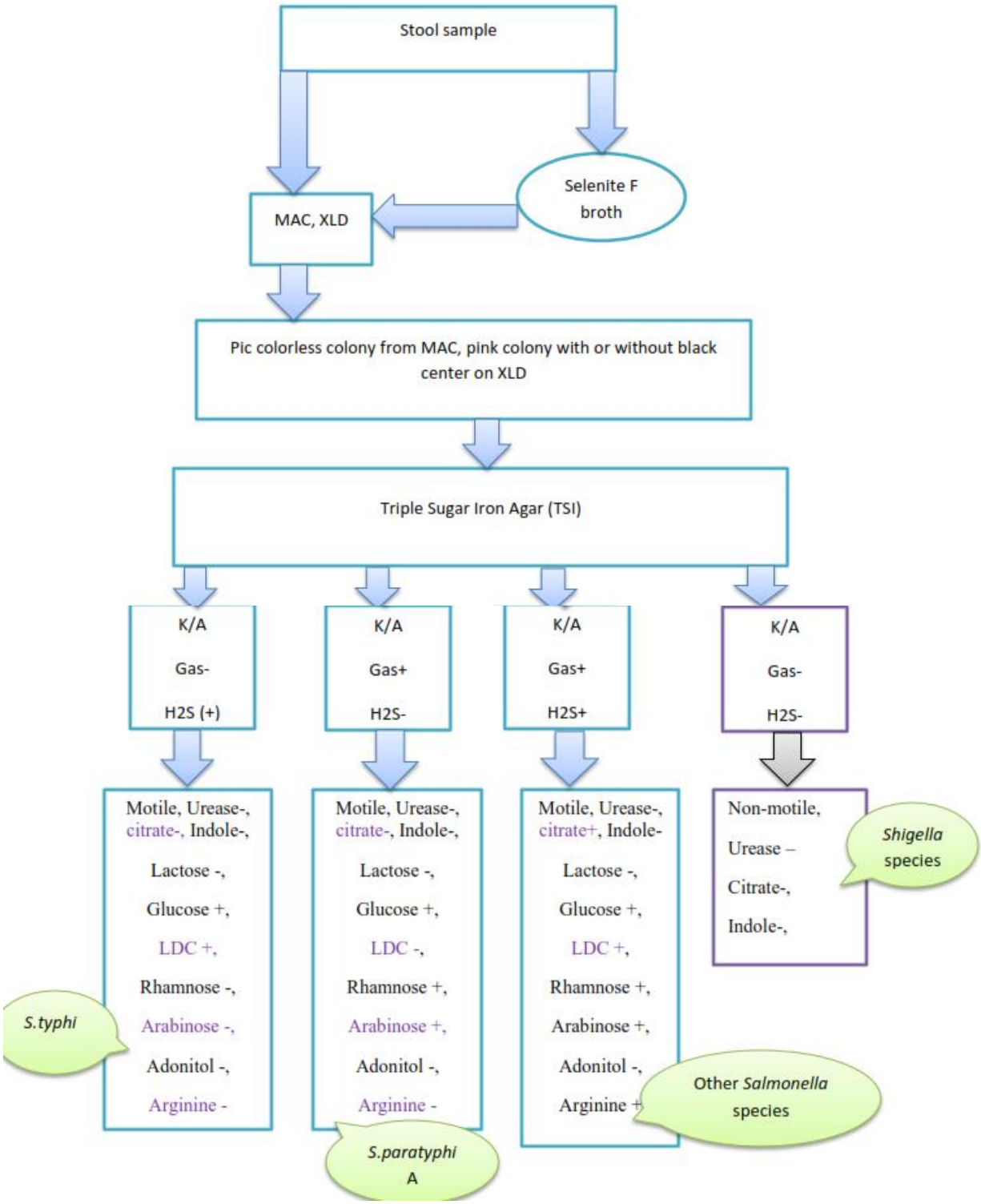
After overnight incubation, culture plates were examined for *Salmonella* and/or *Shigella*-like colonies:

- ✓ Colorless and lactose non-fermenting colonies (2-4 mm) on MAC
- ✓ Clear to light pink colonies with distinct black centers, and clear to white / pale-red colonies on XLD
- ✓ Was picked for sub-culturing to non-selective media.

All positive stool cultures were identified and characterized on the basis of morphology, cultural characteristics, testing using standard procedures (58).

For biochemical tests, pure colonies obtained by sub-culturing on Nutrient agar and morphologically identical 2-3 colonies of the suspected strains were taken from the agar plates and suspended in nutrient broth. Then the suspensions were inoculated to biochemical testing media and were incubated aerobically at 37°C for overnight.

There after isolates were characterized by series of biochemical tests such as Kligler Iron slant agar (KIA) (Oxoid), urease test and Oxidase test (Oxoid) and Sulfide-Indole-Motility (SIM), carbohydrate fermentation (glucose, lactose, sucrose, Rhamnose, Arabinose, Adonitol), and decarboxylation of amino acid (lysine, Arginine) (58). The reaction pattern was then used in an identification algorithm allowing the identification of *Salmonella spp* and *Shigella species* (38, 59).

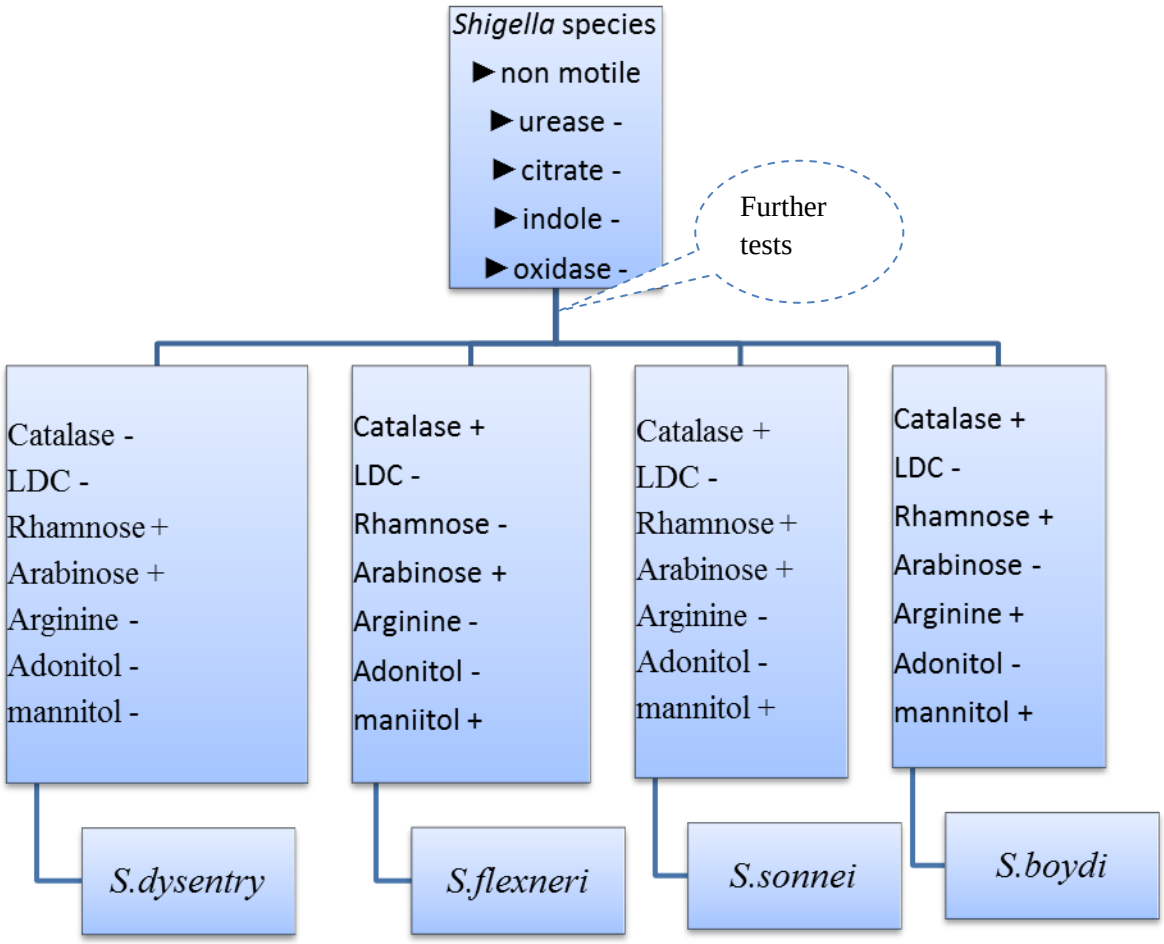



**Source (59).**

**Notes:** 1. K/A=alkaline/acid, Gas-=gas non former, Gas+=gas producer, H2S (+) =small amount of H2S produced, H2S-=no H2S produced, H2S+= presence of H2S

2. *S.typhi=Salmonella typhi*, *S.paratyphi* A= *Salmonella paratyphi* A,

**Figure 2:** Flow chart for identification of *Salmonella* and *Shigella* species from stool sample




**Notes:** *S.dysentry= Shigella dysentery, S.flexineri= Shigella flexneri, S.sonnei= Shigella sonnei, S.boydi= Shigella boydi*

**Figure 3:** Presumptive identification of *Shigella* species

#### 4.9.5. Antibiotic sensitivity test

The antibiotic susceptibility patterns of *Salmonella* and *Shigella* species isolated from the stool specimen against commonly used antibiotics was done on Mueller Hinton agar (MHA) (Oxoid) and incubated at 37°c; aerobically for 24 hrs. Inoculum of direct colony suspension, visually equivalent to 0.5 McFarland standards was used to adjust the turbidity of the inoculum for the susceptibility testing. The standard disk diffusion technique of modified Kirby-Bauer method was used as recommended by European Committee on Antimicrobial Susceptibility Testing (EUCAST) (60). Following overnight incubation at 37 °C, clear zones produced by antimicrobial inhibition of bacterial growth were measured in mm using a straight-line ruler. and the results were recorded as sensitive (s) or resistance (R) based on EUCAST guidelines (60). For the susceptibility testing the following ten antimicrobial drugs and concentrations were used: chloramphenicol (30 µg), ampicillin (10 µg), ciprofloxacin (5 µg), cefotaxime (5 µg), ceftazidime (10 µg), cefuroxime 30 µg, ceftriaxone (30 µg), norfloxacin (10 µg), and trimethoprim-sulfamethoxazole (1.25 / 23.75) µg, and amoxicillin-clavulanic acid (20/10 µg). Multidrug resistance was defined as résistance to ≥ 3 of the antimicrobial agents tested (61, 62)**.** The criteria for selection of those antimicrobial agents tested were their activity against gram negative bacteria and the fact that six of the listed agents, ampicillin, chloramphenicol, cotrimoxazole, norfloxacin, ciprofloxacin and ceftriaxone are used in the treatment of severe diarrhoea in Ethiopia (63)**.**

## 4.10. Study variables

#### 4.10.1. Dependent/outcome variables

Prevalence of *Salmonella* and *Shigella* and

Patterns of drug resistance

#### 4.10.2. Independent/explanatory variables

Socio-demographic variables like age and sex of child

Clinical data like previous history of diarrhea and treatment

Hand washing before feeding children,

Type of water used for drinking,

Previous antibiotic treatments in the last 90 days, and contact with patients with diarrhea, practice of water treatment

### 4.11. Data quality assurance

Training was given to laboratory assistant and data collectors for two days on the procedures of data collection and handling of collected data. Pre-testing was done and the collected data were checked for completeness at the end of data collection. During laboratory analysis of stool cultures, standard operating procedures were followed. Culture media was prepared and sterilized based on the manufactures instruction. Then the sterility of culture media was checked by parallel incubation of un inoculated plate in every newly prepared culture plates and observed for bacterial growth if there is any. Finally, those media which showed any sign of growth was discarded. Control strains *Shigella flexneri ATCC 12022* and *Salmonella typhimurium ATCC 14028* obtained from Ethiopian Public Health Institute was used as a quality control during stool cultures, biochemical tests and antimicrobial susceptibility testing.

### 4.12. Data analysis

The Collected data were checked for completeness, and coded, entered and cleaned using Epi-Data version 3.02. Analysis of data were done using SPSS version 20. Descriptive statistics such as frequency, percentage and cross tabulation was used to present the findings. Logistic Regression and Chi-square was performed to evaluate whether variables were significantly associated with the outcomes of interest at 95% confidence limits or 5% level of significance.

### 4.13. Ethical consideration

Institutional ethical clearance was obtained from Jimma University Health Research Ethics Review Committee. During data collection each participant's parent/legal guardians were informed about the aim of the study and written consent was obtained before the start of data collection. The parent /guardian of the participating children were informed that they have full right to allow their child to participate or not. Samples with positive culture result were communicated to physicians in order for patients to get treatment according to drug susceptibility results of isolates.

## 4.14. Result dissemination

The result of the study will be presented on scientific workshops and publication to scientific journal to be used by researchers. The findings of the study will be presented and submitted to Jimma University College of health science postgraduate studies as partial fulfillment of master's degree in Medical Microbiology. Culture results and antimicrobial susceptibility results will be communicated to the Hospital and Health centers.

# 5. RESULTS

## 5.1. Socio-demographic information

A total of 348 stool samples were collected from under-five children with diarrhea. The mean age of children enrolled in the study was 15 months with standard deviation ±12 months. Majority of patients (62.4%) were aged between 7-36 months and 188 (54%) of them were female. Majority 251 (72.1%) were from urban area. About 47(18.7%) of urban resident have domestic animal in their home. On the other hand, 88 (90.7%) children from rural area have domestic animals in their home and 56 (41.8%) of domestic animal were cattle. Majority of the domestic animals in urban areas were dogs (53%).

**Table 1:** Socio demographic status of under-five children attending Jimma University Medical Center and Serbo Health Center, Southwest Ethiopia

| **Socio-demographic information** | | **Number (%)** |
|---|---|---|
| **Age** | 0-6 months | 116(33.3%) |
| | 7-36 months | 214(61.5%) |
| | 37-59 months | 18(5.2%) |
| **Total** | | **348(100%)** |
| **Sex** | Male | 160(46.0%) |
| | Female | 188(54.0%) |
| **Total** | | **348(100%)** |
| **Residence** | Urban | 251(72.1%) |
| | Rural | 97(27.9%) |
| **Total** | | 348(100%) |
| **Presence of domestic animals** | Yes | 135(38.8%) |
| | No | 213(61.2%) |
| **Total** | | **348(100%)** |
| **Types of domestic animal** | Cattle | 56(41.5%) |
| | Sheep and goat | 9(6.7%) |
| | Dog | 46(43.1%) |
| | Cat | 24(17.8%) |
| **Total** | | **135(100%)** |

### 5.2. Environmental Factors

The main sources of drinking water for the participants were tap water 274 (78.7%) while 70 (20.1%) source of water were stream water particularly for 67% of rural resident (Table 2). Only about 55 (15.8%) of the participant's parent treated drinking water in the house. Majority, 315 (90.5%) of parents/guardian of the children washed their hands before feeding them. About 269 (77.3%) of the respondents had private latrine. Practice of hand washing before feeding was significantly associated with detection *Salmonella* and *Shigella* species (p=0.001).

**Table 2:** Environmental factors associated to diarrhea in under-five children in Jimma University Medical Center and Serbo Health Center, Southwest of Ethiopia

| **Environmental factors** | | **Number (%)** |
|---|---|---|
| **Water source** | Tap water | 274(78.7%) |
| | Stream water | 70(20.1%) |
| | well water | 4(1.1%) |
| **Total** | | **348(100%)** |
| **Private Latrine** | Yes | 269(77.3%) |
| | No | 81(22.7%) |
| **Total** | | **348(100%)** |
| **Water treatment** | Yes | 55(15.8%) |
| | No | 293(84.2%) |
| **Total** | | **348(100%)** |
| **Hand washing before feeding** | Yes | 315(90.5%) |
| | No | 33(9.5%) |
| **Total** | | **348(100%)** |
| **Means of hand washing** | Ash & water | 11(3.2%) |
| | Water only | 217(62.4%) |
| | Soap and water | 120(34.5%) |
| **Total** | | **348(100%)** |

### 5.3. Clinical Data

Abdominal cramp was prominent sign and symptom in all children with acute diarrhea as claimed by the parents and older children while 201 (57.8%) and 187(53.7%) of the patients had sign and symptoms of fever and vomiting respectively. Of the total of 116 children below six months, 99(86.1%) were exclusively breastfed. About 90 (25.9%) of children had history of previous treatment by antibiotics within the last three months and about 67(19.3%) of the patients had history of previous contact with patients with diarrhea (Table 3).

**Table 3:** Clinical data of under-five children with diarrhea in Jimma University Medical Center and Serbo Health Center, Southwest of Ethiopia

| Clinical data | | Number (%) |
|---|---|---|
| **Fever** | Yes | 201(57.8%) |
| | No | 147(42.2%) |
| **Abdominal cramps** | Yes | 348(100%) |
| **Vomiting** | Yes | 187(53.7%) |
| | No | 161(46.3%) |
| **History of previous treatment** | Yes | 90(25.9%) |
| | No | 258(74.1%) |
| **History of previous contact with patients with diarrhea** | Yes | 67(19.3%) |
| | No | 281(80.7%) |
| **Exclusive breast feeding** | Yes | 99(86.1%) |
| | No | 16(13.9%) |

Out of 348 stool samples collected the consistency of 194 (55.7%) of stool was watery diarrhea, while 31 (8.9%), 29(8.3%) was bloody and mucoid diarrhea respectively.

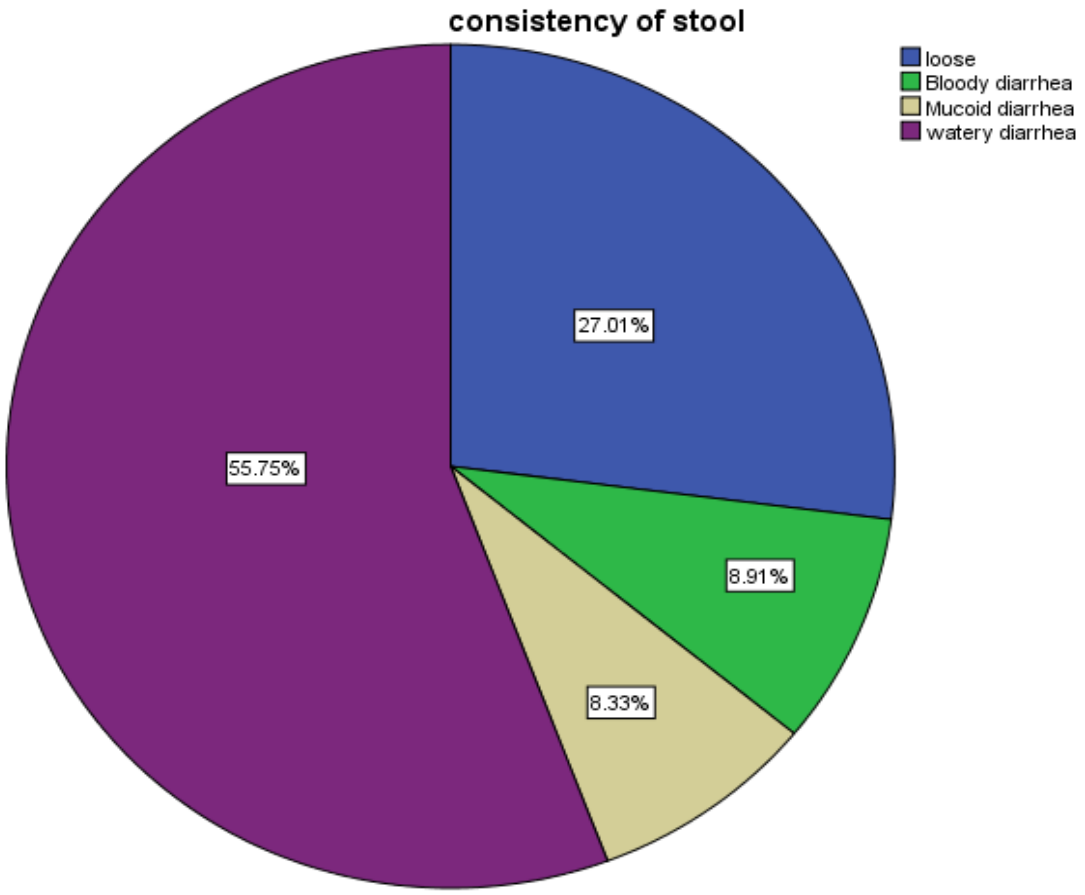



**Figure 4:** Consistency of stool collected for cultures from under-five children in Jimma University Medical Center and Serbo Health Center, Southwest of Ethiopia

Majority, 268 (77%) of children with diarrhea were admitted for medical care within 1-5 days of duration of diarrhea.

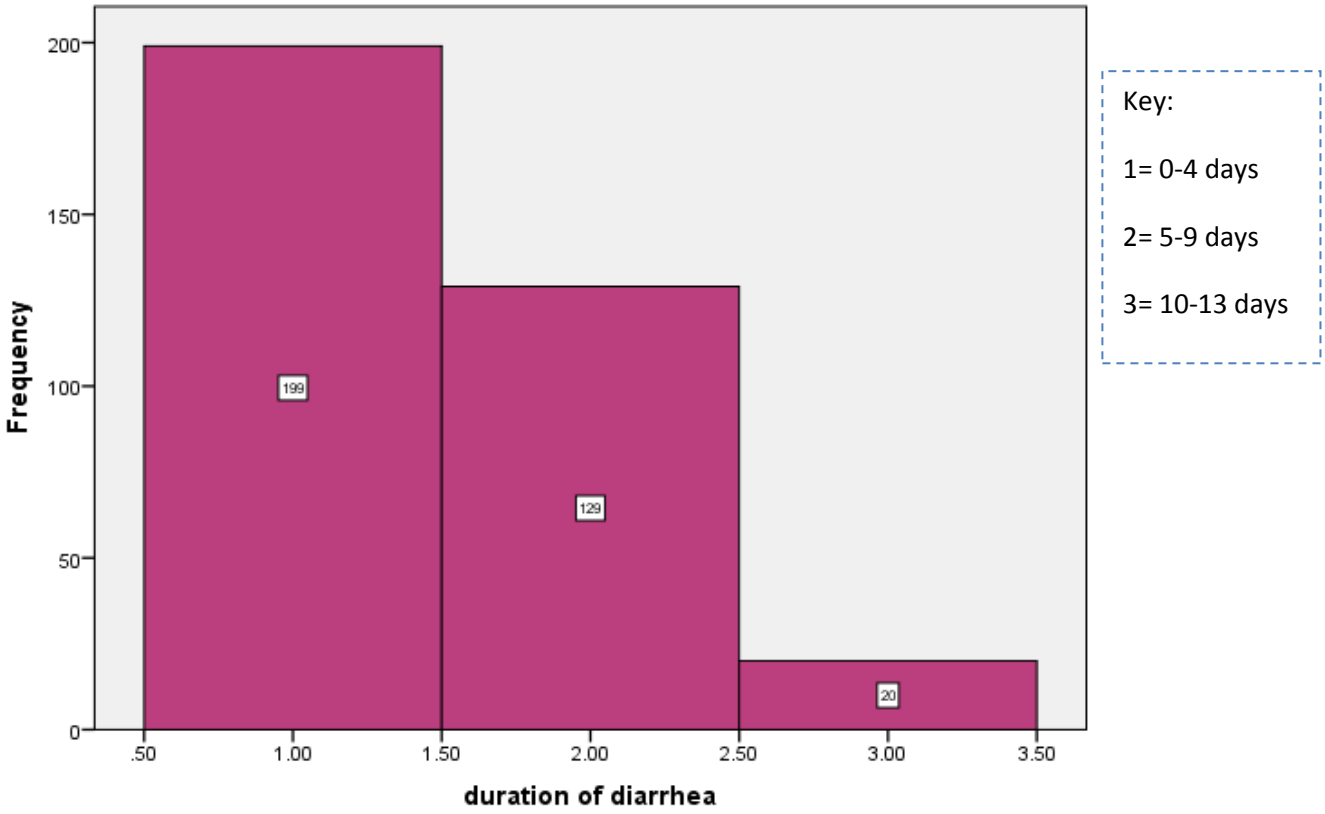



Figure: 2 Duration of diarrhea of under-five children at a time of data collection in Jimma University Medical Center and Serbo Health Center, Southwest of Ethiopia

### 5.4. Prevalence of *Salmonella and Shigella* species

From the total of 348 samples examined, 39 samples were positive for bacterial growth and the highest incidence occurred in the age group of 7-36 months (61.5%). The overall prevalence of *Salmonella* species was 18 (5.2%). Frequently isolated *Salmonella* species was *S .typhi* 44.5% (8/18) followed by *S. paratyphi* A 33.3% (6/18) and other *Salmonella* species 22.2% (4/18).

The overall prevalence of *Shigella* species was 21 (6.0%) and presumptive identification of *Shigella* species showed *S. flexneri* was the most frequent species which accounted for 57.1% (12/21). Majority of *Shigella* species 61.9% (13/21) were isolated from mucoid diarrhea followed by bloody diarrhea from which 19.0% (4/21) *Shigella* species isolated, while least numbers of *Shigella* species were isolated from watery diarrhea and loose stool which was (2/21) each.

**Table 4**: Presumptive identification of Salmonella and Shigella species isolated from diarrhea

| **Species isolated** | | **Frequency** | **Percentage** |
|---|---|---|---|
| *Salmonella* species | *S. typhi* | 8 | 44.5% |
| | *S. Paratyphi* A | 4 | 22.2% |
| | Other *Salmonella* species | 6 | 33.3% |
| *Shigella* species | *S. dysentery* | 6 | 28.6% |
| | *S. flexneri* | 12 | 57.1% |
| | *S. boydi* | 1 | 4.8% |
| | *S. sonnei* | 2 | 9.5% |

*Shigella* species were not directly associated with types of water sources while *Salmonella* species did. Previous history of contact with patients with diarrhea was significantly associated with infection of *Salmonella* and *Shigella* species (p=0.001). *Shigella* species were not identified in exclusively breastfeed children aged fewer than six months. Hand washing before feeding children is significantly associated with presence of *Salmonella* i.e. hand washing is protective (p=001; OR =16.88 (CI= (6.0-46.3))

**Table 5:** Description of under-five children for who stool culture was conducted for isolation of *Salmonella* and *Shigella* species in Jimma University Medical Center and Serbo Health Center

| **Demographic factors** | | ***Salmonella* species isolated** | | | ***Shigella* species isolated No (%)** |
|---|---|---|---|---|---|
| | | ***S.typhi* No (%)** | ***S.paratyphi* A No (%)** | **Other *Salmonella* spp. No (%)** | |
| Age | 0-6 months | 0(0) | 0(0) | 0(0) | 8(38.1%) |
| | 7-36 months | 8(44.4%) | 4(22.2%) | 6(33.3%) | 6(28.6%)) |
| | 37-59months | 0(0) | 0(0) | 0(0) | 7(33.3) |
| **Total** | | **8(44.4%)** | **4(22.2%)** | **6(33.3%)** | **21(100%)** |
| Sex | Male | 6(33.3%) | 3(16.7%) | 1(5.5%) | 3(14.3%) |
| | Female | 2(11.1%) | 1(5.5%) | 5(27.8%) | 18(85.7%) |
| **Total** | | **8(44.4%)** | **4(22.2%)** | **6(33.3%)** | **21(100%)** |
| **Clinical sign and symptoms** | | | | | |
| Fever | | 2(11.1%) | 1(5.5%) | 5(27.8%) | 7(33.3%) |
| Abdominal cramps | | 8(44.4%) | 4(22.2%) | 6(33.3%) | 21(100%) |
| Vomiting | | 8(44.4%) | 4(22.2%) | 6(33.3%) | 14(66.7%) |
| **Total** | | **18(75%)** | **9(75%)** | **17(94%)** | **42((66.6%)** |
| **Stool consistency** | | | | | |
| Loose | | 6(33.3%) | 3(16.7%) | 1(5.5%) | 2(9.5%) |
| Bloody diarrhea | | 2(11.1%) | 0(0) | 3(16.7%) | 4(19.1%) |
| Mucoid diarrhea | | 0(0) | 1(5.5%) | 0(0) | 13(61.9%) |
| watery diarrhea | | 0(0) | 0(0) | 2(11.1%) | 2(9.5%) |
| **Total** | | **8(44.4%)** | **4(22.2%)** | **6(33.3%)** | **21(100%)** |

All (100%) non-typhoidal *Salmonella* species were isolated from under-five children came from households with domestic animals. Cattles are significantly associated with detection of non-typhoidal *Salmonella* species (p=0.012). (Table 4&5)

**Table 6:** Risk factors of diarrhea in under-five children at Jimma University Medical Center and Serbo Health Center

| **Associated factors** | | ***Salmonella* species (No)** | | **P-value** |
|---|---|---|---|---|
| | | Yes | No | |
| Water source | Tap water | 6 | 268 | |
| | Stream water | 12 | 58 | 0.010 |
| | well water | 0 | 4 | 0.001 |
| Private Latrine | Yes | 8 | 261 | |
| | No | 10 | 69 | 0.012 |
| Water treatment | Yes | 0 | 55 | |
| | No | 18 | 275 | 0.001 |
| Hand washing before feeding | Yes | 8 | 307 | |
| | No | 10 | 23 | 0.021 |
| Means of hand washing | Ash & water | 0 | 11 | |
| | Water only | 18 | 199 | 0.003 |
| | Soap and water | 0 | 120 | |
| History of previous contact with patients with diarrhea | Yes | 13 | 54 | 0.001 |
| | No | 5 | 276 | |

**Table 7:** Risk factors of diarrhea in under-five children at Jimma University Medical Center and Serbo Health Center

| **Associated factors** | | ***Shigella* species (No)** | | **P-value** |
|---|---|---|---|---|
| | | Yes | No | |
| Water source | Tap water | 15 | 259 | |
| | Stream water | 6 | 64 | |
| | well water | 0 | 4 | |
| Private Latrine | Yes | 15 | 256 | |
| | No | 6 | 71 | |
| Water treatment | Yes | 0 | 55 | |
| | No | 21 | 272 | 0.024 |
| Hand washing before feeding | Yes | 0 | 294 | |
| | No | 21 | 33 | 0.021 |
| Means of hand washing | Ash & water | 0 | 11 | |
| | Water only | 13 | 204 | 0.001 |
| | Soap and water | 8 | 112 | |
| History of previous contact with patients with diarrhea | Yes | 11 | 56 | 0.031 |
| | No | 10 | 271 | |

### 5.5. Antimicrobial Resistance pattern

Antimicrobial susceptibility tests showed that 16 (88%) of the *Salmonella* isolates were resistant to ampicillin and 13 (73%) isolates were resistant to chloramphenicol. Resistance to ciprofloxacin and ceftriaxone was observed in 17% (3/18), and 11% (2/18) respectively. *Salmonella* species also showed 38.9% resistance to trimethoprim-sulfamethoxazole to commonly used drug in pediatrics patients.

All *Shigella* isolates showed 100% (21/21) resistance to ampicillin and 86% (18/21) isolates were resistant to cefuroxime while 14% (3/21) and 5% (1/21) of the isolates showed resistance to norfloxacin and ciprofloxacin respectively. 52% of *Shigella* species were resistant to trimethoprim-sulfamethoxazole and about 62% of *Shigella* species were also resistant to ceftazidime (Table 5).

**Table 8:** Antibiotic resistance of *Salmonella* and *Shigella* species isolated from stool samples in under-five children in Jimma University Medical Center and Serbo Health Center

| Antibiotics | Resistance of *Salmonella* species | | | Resistance of *Shigella* species |
|---|---|---|---|---|
| | *S.typhi* | *S.paratyphi* A | Other *Salmonella* spp. | |
| Ampicillin. 10 µg | 7 (38.9%) | 4 (22.2%) | 5 (27.8%) | 21 (100%) |
| Chloramphenicol. 30 µg | 5 (27.8%) | 3 (16.6%) | 5 (27.8%) | 12 (57%) |
| Ciprofloxacin. 5µg | 1 (5.6%) | 1 (5.6%) | 2 (11.1%) | 1 (4.8%) |
| Norfloxacin. 10 µg | 2 (11.1%) | 2 (11.1%) | 3 (16.6%) | 3 (14.3%) |
| Cefuroxime. 30 µg | 5 (27.8%) | 3 (16.6%) | 5 (27.8%) | 18 (85.7%) |
| Cefotaxime. 5 µg | 2 (11.1%) | 0 (0) | 0 (0) | 3 (14.3%) |
| Ceftazidime. 10 µg | 5 (27.8%) | 1 (5.6%) | 3 (16.6%) | 13 (61.9%) |
| Ceftriaxone. 30 µg | 1 (5.6%) | 1 (5.6%) | 0 (0) | 0 (0) |
| Trimthoprim-sulfamethoxazole 1.25 / 23.75 µg | 3 (16.6%) | 2 (11.1%) | 2 (11.1%) | 11 (52.4%) |
| Amoxacillin-Clavulanic acid 20-10 µg | 6 (33.3%) | 3 (16.6%) | 3 (16.6%) | 20 (95.2%) |

Multidrug resistance was considered when the isolate was resistant to three and more classes of drugs. Multidrug resistant strains of *Salmonella* and *Shigella* species have been detected. All *Shigella* species were at least resistant to two classes of drugs. Four species of *Shigella* isolates developed resistant to four classes of drugs. Out of 21 isolates of *Shigella* species 16 (76.2%) were multidrug resistant. From a total of 18 isolates of *Salmonella* species only one isolate was found to be susceptible to all drugs while 12 (66.7%) of *Salmonella* isolates were multidrug resistant. According to presumptive identification *S. flexneri* was frequently isolated multi-drug resistant species of *Shigella* species.

**Table 9:** Patterns of multidrug resistance *Salmonella* and *Shigella* species isolated from stool samples among under-five children in Jimma University Medical Center and Serbo Health Center

| No. of Antibiotics | Antibiotic resistance patterns | Salmonella species NO. (%) | Shigella species NO. (%) |
|---|---|---|---|
| **Two drugs** | AMP/CEFU | 0(0) | 1(4.8) |
| | AMP/CEFTA | 1(5.6%) | 2(9.6%) |
| | CIP,NOR,CEFTA/CRO | 1(5.6%) | 0(0) |
| | AMOX-CLAV,AMP/CEFTA,CEFU | 1(5.6%) | 2(9.6%) |
| **Total** | | **3(16.8%)** | **5(24%)** |
| **Three drugs** | AMP/CEFO,CEFTA,CEFU/SXT | 0(0) | 3(14.3%) |
| | AMOX-CLAV,AMP/C/CEFU | 1(5.6%) | 3(14.3%) |
| | AMOX-CLAV,AMP/CEFTA,CEFU/SXT | 1(5.6%) | 4(19%) |
| | AMOX-CLAV,AMP/C/CEFTA,CEFU | 3(16.6%) | 2(9.6%) |
| | AMOX-CLAV,AMP/C/NOR,CEFO,CEFTA,CEFU | 2(11.1%) | 0(0) |
| **Total** | | **7(39%)** | **12(57%)** |
| **Four and above drugs** | AMOX-CLAV,AMP/C/NOR,CEFU,/SXT | 2(11.1%) | 3(14.3%) |
| | AMOX-CLAV, AMP/C/CIP,NOR,CEFU,/SXT | 2(11.1%) | 0(0) |
| | AMOX-CLAV, AMP/C/CIP,CEFTA,CEFU,/SXT | 0(0) | 1(4.8%) |
| | AMOX-CLAV,AMP/C/CIP,CEFU,CEFTA/SXT/CRO | 1(5.6%) | 0(0) |
| **Total** | | **5(27.8%)** | **4(19%)** |

**Notes:** 1. patterns seen as drug family of; Penicillin /fluoroquinolones/3$^{rd}$ generation cephalosporin/ 4$^{th}$ generation cephalosporin

2. AMOX= Amoxicillin, 2. C= Chloramphenicol, 3. CIP= Ciprofloxacin 4. NOR= Norfloxacin 5. CEFO=Cefotaxime 6. CEFU=Cefuroxime 7. CEFTA= Ceftazidime 8. CRO=Ceftriaxone 9. SXT=Trimethoprim sulfamethoxazole 10. AMOX-CLAV= Amoxicillin clavulanic acid.

# 6. DISCUSSION

Infectious diarrhoea is the most common cause of morbidity and mortality in under-five children in developing countries. A total of 348 fresh stool samples were collected from under-five children with acute diarrhea for isolation of *Salmonella* and *Shigella* species.

In this study *Salmonella* and *Shigella* species was isolated in 11.2% of diarrhea in under-five children in the study area. The highest prevalence occurred in the age group of 7 – 36 months (61.5%). Prevalence of *Salmonella* and *Shigella* species in this study was lower than a study in Sudan which showed 47.37% of diarrheas caused by *Salmonella* and *Shigella* species. This could be due to geographic difference and low isolation rate of *Salmonella* and *Shigella* in our study relative to the previous study might be due to improved awareness of the community about personal and environmental hygiene from the continuous interventions made Ethiopian ministry of health (64).

The overall prevalence of *Salmonella* species in under-five children in this study was 5.2 % which is higher than prevalence of *Salmonella* species observed in study conducted among under-five children in Vietnam (4%) (40) and in Kenya 3.5% (46). These variations might be due to method difference where this study used biochemical tests alone while they include PCR.

Prevalence of *Salmonella* species in this study was higher than prevalence reported in Hawassa (2.5%) and Addis Ababa (3.95%) (35). On the other hand prevalence of *Salmonella* species was comparable with prevalence reported in Jimma in 2014 in children less than 15 years of age where prevalence of *Salmonella* was 6.2% (65).

In this study frequently isolated *S.* species were *S. typhi* (44.5%) followed by *S. paratyphi* (33.3%) which is in line with study conducted in Khartoum, Sudan among under-five children where frequently isolated *Salmonella* species were *S. typhi* (76%) followed by *S. paratyphi* (24%) (45).

In this study the overall prevalence of *Shigella* species was 6.0%. The over prevalence of *Shigella* species in this study was higher than prevalence reported in Vetnam (3.4%) and Kenya (2.3%) (40, 46). These variations might be due to difference in method of identification where this study used culture alone while they include PCR.

Prevalence of *Shigella* species in this study is lower than various study conducted in Africa, including; 8% reported from Sudan and 21% reported from Botswana (45, 47). This might be due to differences in awareness of the people about personal and environmental hygiene in the prevention of diarrhea caused by *Shigella* and *Salmonella* species across different countries. Prevalence of *Shigella* species is comparable with prevalence reported in Hawassa Adare Hospital and Millennium Health Center (7%) (48). But it is lower than prevalence of *Shigella* species among under-five children (9.1%) in Addis Ababa (35). This might indicate variation in distribution of shigellosis across the country.

From presumptive identification frequently isolated *Shigella* species of this study is in line with study conducted in India (42), Sudan (45), in Addis Ababa (35) and in Hawassa (66) where 88%, 56%, 54% and 100% isolated *Shigella* species were *S. flexneri* respectively.

Most *Shigella* species were isolated from mucoid diarrhea followed by bloody diarrhea this can be supported by study conducted in Tehran and Harar, Ethiopia where 43.3% and 52.9% about *Shigella* species were isolated from mucoid diarrhea (50, 52).

About 50% of patients with *Salmonella* infection had fever. This is in line with study conducted in Hawassa where about 50% of under-five children with *Salmonella* infection had fever as well as in line to the present study majority of under-five children for whom *Salmonella* and *Shigella* species isolated were visited hospital within 1-5 days of duration of diarrhea (48). Fever in children with *Salmonella* infection in the present study is higher than study conducted in Tehran where about 22.9% of children with *Salmonella* infection had fever (50). This variation could be due to difference in patient selection.

Presence of domestic animals in the house is significantly associated with *Salmonella* infection ($p=0.001$) and this might be due to animals are source of non typhoidal *Salmonella* infection. Practice of hand washing before feeding is important in reducing diarrhea caused by *Salmonella* and *Shigella* species ($p=0.001$) and this is likely because *Salmonella-Shigella* species are transmitted through fecal-oral routes.

Of the total of 116 children below six months, 99(86.1%) were exclusively breastfed. This is higher than national figure of exclusive breast feeding practice (32-52%). This might be due to efforts made by health beaurou of Jimma Zone and health extension worker in the study area as well as developmental team training program and community based training program

of Jimma University may contribute to this (21). *Salmonella* species were not isolated from those who have washed their hands with soap and water and ash and water.

Antibiotic treatment can be a life-saving intervention, but the emergence of antibiotic resistance limits its clinical efficacy.

In this study 88% of *Salmonella* isolates were resistant to ampicillin and 72.2% to chloramphenicol which showed higher rate of resistance than study in Gonder (67). But it is comparable to study in Addis Ababa where 95.7% of isolated *Salmonella* species were resistant for ampicillin. In line to this study, in Addis Ababa low resistance to ciprofloxacin (4.3%), and ceftriaxone (4.3%) were reported (35).

Drug resistance results of this study was in line with several study conducted in different countries and as well as in Ethiopia. Study conducted in Nepal, 70.0% of *Salmonella* species were MDR which is in line to our study (29).

In this study 66.7% of *Salmonella* isolates were multidrug resistant which is comparable with study done in Addis Ababa where more than 70.0% of *Salmonella* species were multiple resistant (35).

Antimicrobial susceptibility tests of this study showed that 76.2% of *Shigella* species were multidrug resistant which is lower than study done in Addis Ababa where more than 87% of *Shigella* species were multiple drug resistant(35). This difference might be due to the fact that in this study multidrug resistance was considered when the isolate was resistant to three and more drugs unlike in Addis Ababa which considered resistance to more than one drugs. All *Shigella* isolates (100%) in this study were resistant to ampicillin which can be supported with similar studies conducted in Hawassa and Mekele where all isolates were resistant to ampicillin (48, 55). All *Shigella* isolates were susceptible to ceftriaxone which is in line with study done in Nepal (29), Addis Ababa (35), Hawassa (48) and Gonder (67).

In Sudan, 83% *Shigella* species showed high rate resistance against Chloramphenicol which is higher than this study (45), and higher rate of drug resistance also observed in Kenya where *Shigella* isolate levels of resistance ranged from 80% to 100% for ampicillin and trimethoprim-sulfamethoxazole (46). In this study *Shigella* species showed 4.8% resistance to ciprofloxacin, this is supported by study done in Mekele where 6.7% resistance was observed for ciprofloxacin (55).

We acknowledge that our study had some limitations. One of this was the study design (cross-sectional) which cannot rule out cause and effect relationship of associated factors which indeed require control groups. Presumptive identification of the isolated strain was made with series of biochemical reaction, carbohydrate fermentation tests and amino acid decarboxylation but has not been serotyped. Despite these limitations, the findings of our study came up with the prevalence *Salmonella* and *Shigella* species and appearance of *Salmonella* and *Shigella* strains with the high level of resistance to Penicillin and third generation cephalosporins in our study area. These results could help the physicians to employ the best choices to treat diarrhea caused by *Salmonella* and *Shigella* species in the region.

# 7. CONCLUSION AND RECOMMENDATION

## 7.1. Conclusion

In the present study, *Salmonella* and *Shigella* species were isolated from 11.2% of under-five children with diarrhea where the highest prevalence occurred in the age group of 6-36 months.

Practice of hand washing before feeding was significantly associated with detection *Salmonella* and *Shigella* species. Shigella species were not identified in exclusively breastfeed children aged fewer than six months.

The results of the present study showed that high frequency of multi drug resistance *Shigella* and *Salmonella* species circulating in the studied groups. About 76.2% of *Shigella* species and 66.7% of *Salmonella* species were multidrug resistant. All *Shigella* species were resistant to ampicillin while more than half of both *Salmonella* and *Shigella* species were resistant to chloramphenicol, ceftazidime and cefuroxime. None of *Shigella* species were resistant to ceftriaxone while low resistance rate observed against ciprofloxacin and norfloxacin.

In the present study the results of antimicrobial susceptibility tests showed that fluoroquinolones and ceftriaxone are still treatment of option for diarrhea that might be caused by *Salmonella* and *Shigella* species in the area.

## 7.2. Recommendation

This study indicated the presence of multi drug resistance *Salmonella* and *Shigella* species in the study area. According to this study, ampicillin is no longer effective for the treatment of diarrhea that might be caused by *Salmonella* and *Shigella* Species at least in the study area and its surroundings. Further epidemiological studies which include control group and serotyping need to be conducted.

This study also recommends the adoption of stool culture for routine laboratory test as the test is easy and materials required are reasonable in cost. Being part of routine laboratory test it reduces empirical treatment of the patients without antibiotic susceptibility profile of the isolate.

This study also recommends health facilities to regularly update the treatment guideline based on national and local antimicrobial susceptibility patterns of organisms.

This study also suggests that Ministry of Health to have strict regulation on rational antibiotic use and supervision of local pharmaceuticals for drug vending without physician prescription to reduce misuse of antibiotics.

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# 9. ANNEXES I: INFORMED CONSENT

Name of Principle Investigator: Ephrem Awulachew

## PART 1: Information Sheet

### Purpose of the Research

Improvement in the treatment and follow-up of children with diarrhea is important. Most diarrhoea is caused by infections with tiny organisms. The main purpose of this study is to find an easy way to identify some of these organisms that cause diarrhoea. If we can find a practical way to identify the cause of the child's diarrhoea, we can choose the best medication to treat the infection. The results will be used to improve the management and follow-up of other children with the same infection.

### Voluntary Participation

The child's participation in this research study is entirely for you to decide. On the child's behalf, you have the choice of whether to participate or not. If your change your mind later, you can stop participating even if you agreed earlier. Your decision will not affect the treatment of your child at the hospital or at the Health Centre in any way. We will describe the study and go through this information sheet with you. If you agree to participate, we will ask you to sign a consent form.

You will not receive any direct payments and there is no payment for giving samples to our study. If you agree on the participation of your own or custody child, we will interview you and do some measurements of the child as described below. Your child will receive treatment for diarrhoea according to [Asendabo Health Centre / Jimma University Hospital] protocol.

### You

Questionnaire: You will be asked questions about your family situation, disease history, breastfeeding and dietary intake, and care of your child.

### Your child

1) Stool samples

We will collect a small stool sample from your child to test for infection. The sample will be collected at the Health Centre or the outpatient department of the hospital.

**What will happen to the samples?**

We will take the samples to our laboratory at Jimma University microbiology research laboratory where we will look for microscopic organisms in the stool sample, and where we will culture the sample for bacterial isolation. We will conduct the most important tests on the samples immediately. This will include a test of the stool sample for an infection that can cause diarrhoea. Tests might take weeks and we are asking permission to store the samples in Jimma University microbiology center.

**What are the possible risks of taking part?**

The study will not cause any harm to your child in any way. In the event of any problem, hesitation or concern, the interviewer will ask you whether you would like to continue to be asked further questions.

**What are the possible benefits of taking part?**

If we find anything in the stool or blood samples of relevance for the treatment of your child the doctors will be informed, and the appropriate treatment given. We might not find anything abnormal and taking part in this study might therefore not benefit your child directly, but we hope that it will benefit other children that are sick with diarrhoea.

**Will taking part in this study be kept confidential?**

All the information that we collect will be strictly confidential. Information of relevance to your child's treatment will be told only to you and the doctors.

All other information collected about you and your child during the research study will be stored safely and no one but the investigators will be able to see it. Any information about your child will have a number instead of a name. Only the investigators will know that number and they will keep that information secure. It will

not be shared with or given to anyone except the investigators. The identification information will only be known to the study team during the study period and will be removed and destroyed before starting any data analysis.

**What will happen to the results of the research study?**

The results will be prepared for publication in scientific journals and presentation at international meetings. We can provide you with a copy of the articles after publication if you wish. The names of you and your child will not appear in any report or publication. The identity of you and your child will be safeguarded at all times.

**Further information and contact details.**

If you have any further questions, please do not hesitate to contact the main research nurse in Jimma Hospital (name_____+phone number_____), at Asendabo Health Centre ( name_____+phone number_____) or the investigators Ephrem Awulachew who will be in Jimma University during working hours or it can be contacted by the following phone numbers (phone 0949181161) . They will be able to explain in greater detail about our study. You should also contact any of these persons if you would like to withdraw from the study, or if you have any worries regarding your participation in this study.

## PART 2: Certificate of Consent

**Patient study ID number: _____**

I have been fully informed about this study and been given written information, and understand that its aim is to measure infection parameters that can cause or contribute to diarrhoea and also body weight and length/height, during assessment or admission in Jimma Hospital or Asendabo Health Centre. I also understand that the results will be helpful to improve the management of children with diarrhoea.

I have had the opportunity to ask questions and they have been answered to my satisfaction. I have been informed that the study does not cause any harm, but may be associated with minimal discomfort.

I agree on the behalf of my own or custody child to the participation in the study, and to the examinations as described in the information sheet. I permit stool samples to be collected from my child or custody child.

I am aware that should I not wish for my own or custody child to continue participating in this study, I can withdraw my consent at any time, and it will not affect his or her treatment. Equally, I understand that by participating I and my own or custody child will not be entitled to any special services or payment or gifts.

This authorization is only valid for this study.

I hereby consent voluntary to participate on behalf of my own child or custody child.

Name _____Signature of participant_____Date_____

## ANNEXES 2: QUESTIONNAIRES

### Collage of Health Science

### Master of Medical Microbiology Program

**Thesis research to be conducted on child health for fulfillment master's degree in Medical Microbiology interview Questionnaires**

| | | |
|---|---|---|
| **I) SOCIO-DEMOGRAPHIC DATA** | | |
| 1 | Age | _____ |
| 2 | Sex | 1. Male 2. Female |
| 3 | Residence of the child | 1. Urban 2. Rural |
| 4 | Domestic animal in the house? | 1. Yes 2. No |
| 5 | Types of domestic animal in the house | 1.Cattle 2. Goat & Sheep 4. Dog 5. cat |
| **II) CLINICAL DATA** | | |
| 6 | Presence of fever | 1. Yes 2. No |
| 7 | Presence of abdominal cramps | 1. Yes 2. No |
| 8 | Presence of vomiting | 1. Yes 2. No |
| 9 | Type of diarrhea | 1. Watery diarrhea 2. Bloody diarrhea 3. Mucoid diarrhea |
| 10 | Duration of diarrhea | 1. 1-5 days 2. 5-10 days 3. 11-15 days 4. >16 |
| 11 | History of contact with diarrheic patients | 1. Yes 2. No |
| 12 | History of previous antibiotic treatment | 1. Yes 2. No |
| 13 | Did your child have previous history of diarrhea? | 1. Yes 2. N0 |
| 14 | Practice of exclusive breast feeding for children <6 months | 1. Yes 2. No |
| **Environmental factors** | | |
| 15 | What is the type of water source your child use for drinking? | 1. Tap water 2. Pond water 3. |

| | | |
|---|---|---|
| | | Stream water 4. Well water |
| 16 | Is your water treated in in any way to make it safer before drinking? | **A.** yes **B.** no |
| 17 | Do you have a private latrine in your home? | 1. Yes 2. No |
| 18 | Do you wash your hands before feeding your child? | 1. Yes 2. No |
| 19 | What do you normally use to wash your hands? | **A.** Ash & water **B.** only water **C.** soap & water |

ጅማ ዩኒቨርሲቲ

የጤና ሳይንስ ኢንስቲትዩት

የሜዲካል ማይክሮ ባዮሎጂ ዲፓርትመንት

ለድህረ ምረቃ ማሟያ በህጻናት ጤና ለይ ለሚሰራው ምርምር የህጻናት ጤና መረጃ መሰበሰቢያ ቃለ መጠይቅ

የታካሚው ህጻን ስም_____መለያ ቁጥር_____

| | |
|---|---|
| 1) እድሜ | _____ |
| 2) ጾታ | 1) ወንድ 2) ሴት |
| 3) የመኖሪያ አከባቢ | 1) ከተማ 2) ገጠር |
| 4) የቤት እንሰሳት በቤት ውስጥ ስለመኖራቸው | 1) አሉ 2) የሉም |
| 5) ቤት ውስጥ ያሉ የቤት እንሰሳት አይነቶች | 1) ከብት 2) በግና ፍየል 3) ውሻ 4) ድመት |
| **የጤና ሁኔታ** | |
| 6) ትኩሳት | 1) አለ 2) የለም |
| 7) ሆድ ቁርጠት | 1) አለ 2) የለም |
| 8) ማስመለስ | 1) አለ 2) የለም |
| 9) የተቅማጡ አይነት ከሚከተሉት የቱ ነው? | 1) ውሃ ተቅማጥ 2) ደም የቀላቀለ ተቅማጥ 3) ንፍት መሰል ተቅማጥ |
| 10) ለምን ያህል ግዜ ሲያስቀምጥ የቆየው? | 1) 1_5 ቀናት 2) 6_10 ቀናት 3) 11_15 ቀናት 4 ) 〉 16 ቀናት |
| 11) ከዚህ በፊት በተቅማጥ ከተጡቁ ሰዎች ጋር ንኪኪ ነበረ ወይ? | 1) አለ 2) የለም |
| 12) ከዚህ በፊት አስቀምጦት (ጧት) ያውቅ ነበር? | 1) አለ 2) የለም |
| 13) ለተቅማጥ መድሃኒት ውስዶ (ወስዳ) ያውቃል ( ለች? | 1) አለ 2) የለም |
| 14) ከሰደሰተ ወር በታች ላሉ ህጻናት ጡት ብቻ እመግባሉ? | 1) አዎ 2) የለም |
| **የአከባቢ ሁኔታ** | |

| | |
|---|---|
| 15）የመጠጥ ውሃ ምንጭዎ ምንድን ነው？ | 1）ቧንቧ 2）የኩሬ ውሃ 3）የምንጭ ውሃ 4）የጉድጓድ ውሃ |
| 16）የውሃ ህክምና ትጠቀማላችሁ？ | 1）አለ 2）የለም |
| 17）የግል ሽንት ቤት አለዎት？ | 1）አለ 2）የለም |
| 18）ልጆትን ከመመገቦ በፊት እጅን ይታጠባሉ？ | 1）አለ 2）የለም |
| 19）እጅ በምንድን ነው የምታጠቡት？ | 1）በአመድና ውሃ 2）በውሃ ብቻ 3）በሳሙናና በውሃ |

# JIMMA UNIVERSITY INSTITIYUTII FAYYAA

# KUTAA BARNOOTAA MEDICAL LABRATOORII

Gaaffilee qo'aannaa fayyaa daa'immanii irratti mata duree "Prevalence of Salmonella and Shigella Species among Under-five Children and Antibiotic Resistance Patterns in Jimma University Medical Center and Serbo Health Center, Southwest Ethiopia" jedhamuuf ragaa sassaabuuf garagaaru.

| | | |
|---|---|---|
| 1 | Uumrii | _____ |
| 2 | Saala | 1) Dhiira 2) dhalaa |
| 3 | Iddoo jireenyaa | 1) Magaalaa 2) Badiyyaa |
| 4 | Beeyladni mana kessaa jiru | 1) Jira 2) hin jiru |
| 5 | Gosti beeylada mana keessa jiru | 1) Horii 2) Hoolaa FI Re'ee 3)saree 4) Adurree |
| 6 | Bowwoo mataa | 1) Jira 2) hin jiru |
| 7 | Garaa Kaasaa | 1) Jira 2) hin jiru |
| 8 | Ol deebisuu | 1) Jira 2) hin jiru |
| 9 | Baasaa | 1) Jira 2) hin jiru |
| 10 | Turtii baasaa dhukkubsataa irratti ture | 1) Guyyaa 1-5 2) Guyyaa 5-10 3) Guyyaa 11-15 4. >16 |
| 11 | Warra dhukuba baasaa qaban waliin tutuqaa qabaa? | 1) Jira 2) Hin jiru |
| 12 | kana dura Dawaa fudhatanii beekuu? | 1)Jira 2) Hin jiru |
| 13 | Kana dura dhukkuba baasaatiin dhukkubsatanii beekuu? | 1)Jira 2) Hin jiru |
| 14 | Daa'imman ji'a 6 gad jiraniif harma haadhaa qofa kennuu irratti hoo? | 1)Jira 2) Hin jiru |
| 15 | Maddi bishaan dhugaatii maali? | 1) Bishaan boombaa 2) bishaan boollaa 3) burqaa 4) haroo |
| 16 | Bishaan mana kessatti in wal'aanuuntu? | 1)Jira 2) Hin jiru |
| 17 | Mana fincaanii dhuunfaa qabduu? | 1)Jira 2) Hin jiru |
| 18 | Daa'imman nyaachusuun dura in dhiqattuu? | 1)Jira 2) Hin jiru |

| | | |
|---|---|---|
| 19 | Harka maaliin dhiqattu? | 1) Saamunaa fi bishaan 2) daaraa fi bishaaniin 3) bishaaniin qofa |

# ANNEXES 3: LABORATORY PROCEDURES

## Specimen collection

1) Instructing the parents or the guardian of children to collect stool sample the specimen if present, those parts that contain blood, mucus or pus.
2) Once the specimen has been placed in the specimen container, the lid should be sealed.
3) Then they should be told to transfer the stool to clean containers without disinfectant or detergent residue and with tight-fitting, leak-proof lids.
4) Stool is transported by using cold chain to Jimma University microbiology research lab for analysis.
5) Prepare a faecal suspension by suspending approximately 1g of the stool sample in a tube containing 1 ml of sterile saline. If the stool sample is liquid, saline does not need to be added.
6) Inoculate on MAC and XLD, and add three or more loopful of faecal suspension to the enrichment broth (selenite F broth).
7) Subculture selenite F broth on selective plating media (MAC, XLD).
8) Incubate the plates for the isolation of *Salmonella* and *Shigella* at 37 $^{o}$C for 24 hrs.
9) Pick salmonella-Shigella suspected colony and subculture on non-selective media
10) Identified and characterized by standard biochemical testing flow chart

Laboratory format

| ID | TSI | H2 S | Gas | motility | Urease | Citrate | Indole | Oxidase | Catalase | lactose | LD C | Rhamnose | Arabinose | Arginin e | Adonitol | strain |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | k/A | **(+)** | - | + | - | - | - | - | | - | + | - | - | - | - | *S.typhi* |
| 2 | k/A | - | + | + | - | - | - | - | | - | - | + | + | - | - | *S.paratyphi A* |
| 3 | k/A | + | + | + | - | + | - | - | | - | + | + | + | + | - | *Other Salm.sp.* |
| | | | | | | | | | | | | | | | | |
| **Presumptive identification of *Shigella* species** | | | | | | | | | | | | | | | | |
| | k/A | - | - | - | - | - | - | - | - | - | - | + | + | - | - | *S.dysentry* |
| | k/A | - | - | - | - | - | - | - | + | - | - | - | + | - | - | *S.flexineri* |
| | k/A | - | - | - | - | - | - | - | + | - | - | + | + | - | - | *S.sonnei* |
| | k/A | - | - | - | - | - | - | - | + | - | - | + | - | + | - | *S.boydi* |
| | | | | | | | | | | | | | | | | |

**NB:** [a]for KIA: K = alkaline (red); A = acid (yellow); G = gas production; + = black $H_2S$ produced (weak); – = no H2S

[b] for LIA: K= alkaline (purple); A= acid (yellow); G = gas production; + = black H2S produced (weak);– = no H2S

[c] this reaction occurs 97% of the time.

**McFarland 0.5 turbidity standards preparation**

1. Add 0.5 ml of a 1.175% (wt/vol) barium chloride dehydrates (BaCl2.2HO) solution to 99.5 ml of 1% (vol/vol) sulfuric acid.

2. The turbidity standard is then aliquoted into test tubes identical to those used to prepare the inoculum suspension.

3. Seal the McFarland standard tubes with wax, Para film, or some other means to prevent evaporation.

4. McFarland standards may be stored for up to 6 months in the dark at room temperature (22° to 25°C).

5. Before each use, shake well, mixing the fine white precipitate of barium sulfate in the tube.

**Antibiotic susceptibility test**

1. Pick Colony on TSI and make suspension in nutrient broth or normal saline
2. Check turbidity against 0.5 McFarland standards
3. Streak on MHA by sterile swab to get confluent growth
4. After drying it place antibiotic disk carefully
5. Incubate at $37^{o}$c for 24 hrs.
6. Look for inhibition zone around the disk after overnight incubation period
7. Measure the diameter using straight ruler
8. Report as resistant or sensitive according to EUCAST guideline.

**Drug susceptibility**

| Isotates | chloramohenical | ampicillin | ciprofloxacillin | cefotaxime | ceftazidime | ceftriaxone | meropeneme | Trime-sulf | Amo-clav |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |