Jimma University

College of Natural Sciences

Department of Biology

Prevalence of *Salmonella* and *Shigella* in Out Patients of Jimma University Specialized Hospital, Jimma zone, south west Ethiopia

By:-Tesfahun Lamboro

A Thesis Submitted to the Department of Biology, College of Natural Sciences, Jimma University, In Partial Fulfillment of the Requirement for the Degree of Master of Science in Biology (Applied Microbiology)

June, 2014

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DECLARATION

I declare that the information presented here in my thesis is my original work, has not been presented in this or other university and that all sources of material used for the thesis have been duly acknowledged.

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List of Acronyms

ANOVA	Analysis of Variance
BPW	Buffered Peptone Water
CDC	Center for disease control and prevention
DCA	Deoxycholate citrate agar
ETEC	Entero-toxigenic E. coli
HUS	Hemolytic uremic syndrome
JUSH	Jimma University Specialized Hospital
MDR	Multidrug resistance
MASL	Meter above sea level
NTS	Non typhoidal *Salmonella*
OPD	Out-patient Department
PCR	Polymerase chain reaction
SIM	Sulfide indole motility
TSI	Triple sugar iron agar
WHO	World Health Organization
XLD	Xylulose Lysine Dexycholate agar

Abstract

Food borne diseases related to unhygienic food handling practices remain a major public health problem across the globe. The problem is severe in developing countries due to limitations in securing optimal hygienic food handling practices. Data shows that an estimated 70% of cases of diarrheal diseases are associated with the consumption of foods contaminated with pathogenic microorganisms.*Among these microorganisms Salmonella and Shigella are the major ones. Thus, this study was designed to investigate the prevalence of Salmonella and Shigella in Out-patients in Jimma University Specialized Hospital. A cross-sectional study was conducted from January 2014 to March 2014. A total of 176 stool specimens of both adult and pediatric out-patients were collected, over night enrichment with selenite F broth, cultured in to XLD agar media. After 24hr incubation the media were examined for the presence of Salmonella and Shigella colonies. Then the isolates were confirmed by biochemical test. The drug resistance patterns of the isolates were evaluated using galleries of nine commonly used antibiotics. The growth potential of Salmonella and Shigella isolates in selected traditional foods was assessed following standard methods. In the current study, 19(10.8%) Salmonella and 2(1.1%) Shigellas were isolated. The prevalence of Salmonella and Shigella are higher in children aged less than 10 years and youth aged between 20-24.In the current drug susceptibility test ,Salmonella spp showed resistance to ampicillin (100%) followed by tetracycline (47.4%) and nalidixic acid (26.3%).On the other hand Shigella spp were highly resistant (100%) to ampicillin and tetracycline. Multidrug resistance towards four drugs was observed in both pathogens. In the challenge study the pathogens grow to their infective dose in both gruel and firfir within 24hr (6.2 and 7.5log cfu/g).There was relatively greater fluctuation in pH of gruel within 24 hr, 6.2 at 0 time and ends below pH 5 whereas steady increment in pH of firfir was observed and reach to5.28 at the end of 24hr. In conclusion, this study showed that, these pathogens are still public health problems. Therefore, there needs to be frequent monitory and evaluation system so as to plan intervention strategies for at risk population in the area of problems regarding water sanitation and hygienic food handling practice to minimize the burden posed by the diseases associated with Salmonellosis and Shigellosis.*

1. Introduction

Food borne diseases remain a major public health problem across the globe. The problem is severe in developing countries due to difficulties in securing optimal hygienic food handling practices. In developing countries, up to an estimated 70% of cases of diarrheal diseases are associated with the consumption of contaminated food (Zeru and Kumie, 2007).Transmission of enteropathogenic bacteria is affected directly or indirectly through objects contaminated with faeces. These include food, water, nails, and fingers, indicating the importance of faecal-oral human-to-human transmission (Gashaw *et al*., 2008).

Diarrheal diseases are of great concern throughout the world as they are responsible for considerable mortality and morbidity in developing countries particularly in sub-sahran African countries including Ethiopia (Jaffari *et al*., 2008).Diarrheal illness causes six million deaths per year and clearly linked with poor hygiene and contamination of water and food. Diarrheal disease and enteric infections are responsible for over a quarter of all childhood deaths in the developing world. Poor environmental sanitation, malnutrition, inadequate water supply, poverty and limited education are the major factors implicated in the occurrence, spread and severity of diarrheal diseases (Khan *et al*., 2004).

Infections of *Salmonella* and *Shigella* are major global public health problems. More than one billion cases of diarrhea result worldwide due to nontyphoidal *Salmonella* every year leading to 3 million deaths (Goburn *et al*., 2007). Ninety-nine percent of the 200 million cases and more than 650,000 deaths per year that result from infection with *Shigella* commonly occur in developing countries, primarily among children and young adults (Kasper *et al*., 2005). *Salmonella* and *Shigella* cause mild to severe forms of intestinal tract infection. *Salmonella* cause self-limited gastro-enteritis and the more severe forms of systemic typhoid fever (Kasper *et al*., 2005; Goburn *et al*., 2007).

Salmonella is a leading cause of foodborne illness worldwide and can cause enterocolitis (salmonellosis), enteric fever (typhoid fever), and septicemia. A characteristic feature of *Salmonella* is its wide host range including mammals, birds, and cold-blooded animals in addition to humans. It primarily inhabits the gastrointestinal tracts of animals. The organism almost always enters via the oral route, usually with contaminated food or

drink. Infections of humans can be acquired through direct contact with carrier, domestic or wild animals or through the consumption of contaminated foods or water. Infections with nontyphoidal *Salmonella*, also called salmonellosis, are one of the most commonly recorded causes of gastroenteritis in humans. The general symptoms of human salmonellosis are fever, diarrhea, abdominal cramps, nausea, vomiting, chills, and prostration. Usually the disease lasts a few days and is self-limited. Occasionally the infection can be more serious, with loss of fluid and electrolytes, and can be fatal, especially to the sick, infants, and the elderly (Arslan and Ayala, 2010).

Studies on human Salmonellosis in Ethiopia began in the 1970s. However, the number of studies so far carried out is small and do not include all segments of the population and geographic regions of the country. Most studies were carried out in Addis Ababa and its surroundings (Tadesse, 2014). Epidemiological investigation in the countries like Ethiopia is difficult because of the very limited scope of the studies and lack of coordinated surveillance systems (Beyene and Tasew, 2014).

Shigella species are limited to the intestinal tract of humans and cause bacillary dysentery leading to watery or bloody diarrhea. They are transmitted through ingestion of contaminated food and water. These infections are prevalent in developing countries where lack of clean water supply, lack of proper sewage disposal system and flies aggravate the spread of the diseases (Kasper *et al*., 2005). Shigellosis is an acute inflammatory reaction of intestinal tract caused by the genus *Shigella.* It is a highly infectious disease worldwide and its prevalence is highest in tropical and subtropical regions of the world where living standard is very low and access to safe and adequate drinking water supply and proper excreta disposal system are often very limited or even absent(Arora,2008). Ethiopia, as developing and tropical country is frequently subjected to shigellosis (Sebhat *et al*., 2007; Tiruneh, 2009).Humans appear to be the only normal host reservoir for *Shigella* and they become infected by ingestion of contaminated food and water (Arora,2008).

Shigellosis is primarily a pediatric disease with more than half of all infections occurring in children between six months to ten years of age, although it can affect susceptible

individuals at any age who are subjected to poor sanitation. The disease is transmitted by feaco oral route and the rate of transmission is high because of the low inoculums of bacteria necessary to cause illness in humans (Qureishi *et al*., 2008).Shigellosis prevalence is extensively underestimated because *Shigella* are very fragile and sometimes in low number in faeces, which necessitates inoculation of media within four hours after stool emission and the use of enrichment medium. In the current study, this disadvantage was taken into account. Although molecular techniques are rapid and sensitive in the diagnosis of *Shigella* infection, they cannot be put into practice on a routine basis. *Shigella* remains a burden in developing countries; sensitization of the population and practitioners to this lethal infection would contribute to limiting its severity and prevalence (Arora, 2008).

Rapid detection methods are required for the purpose of diagnosis as well as for the prevention of food contamination and food borne outbreaks (Ng *et al*., 1996). Until now, conventional culture methods have traditionally been considered the "gold standards" for the isolation and identification of food borne pathogens. However, culture methods are labor-intensive and time-consuming which are not suitable for routine testing of large numbers of samples (Bohaychuk *et al*., 2007). Therefore, the rapid, cost-effective and automated diagnosis of food borne pathogens throughout the food chain continues to be a major concern for industry and public health authorities. Because of these requirements, the Polymerase Chain Reaction (PCR) has become a powerful tool in microbiological diagnostics during the last decade (Myint *et al*., 2006).

Growth potential is defined as the difference between the population of a microorganism at the end of shelf-life of specific food and its initial population. *Salmonella and Shigella* spp. are the most common pathogenic bacteria associated with a variety of foods (Harris *et al*., 2003). In a microbiological challenge study, the levels of live challenge microorganisms are enumerated at each sampling point. The media used during enumeration depends on the type of pathogen. If the product does not have a substantial background microflora, non-selective media can also be used for direct enumeration.

In general, food borne diseases related to unhygienic food handling practices remain a major public health problem across the globe. The problem is severe in developing countries due to difficulties in securing optimal hygienic food handling practices. Among these food borne pathogens *Salmonella* and *Shigella* relatively takes greater morbidity and mortality rate than others.Since the problem is more pronounced in developing world, Africa including Ethiopia are in challenge of this problem. Evidently, there were several studies on prevalence of *Salmonella* or *Shigella*, restricted to health centers or includes some age groups such as pediatric or adults in the country (Awole *et al*. 2002; Reda *et al*., 2011; Beyene and Tasew, 2014; Mengistu *et al*., 2014). Moreover, periodic epidemiological surveillance in the area among humans is of vital importance to detect outbreaks and control the diseases caused by these pathogens. Though, there were some reports in Ethiopia and particularly in Jimma on the prevalence of *Salmonella* and *Shigella* involving only children or adults, to date there was no report on Out-patients involving children and adults. Thus, this study was aimed to determine the prevalence of *Salmonella* and *Shigella* in Out-patients in Jimma University Specialized Hospital.

2. Objectives

2.1. General objective

- To assess the prevalence of *Salmonella* and *Shigella* in Out-patients of Jimma University Specialized Hospital Jimma Town, South west Ethiopia.

2.2. Specific Objectives

- To determine the prevalence of *Salmonella* and *Shigella* in out -patients seeking medication in Jimma University Specialized Hospital.
- To evaluate the growth potential of *Salmonella* and *Shigella* isolated from Out-patients in some home- made foods (Gruel and Firfir).
- To determine antimicrobial susceptibility of *Salmonella* and *Shigella* isolated from diarrheal Out-patients in Jimma University Specialized Hospital

3. Literature review

3.1. Salmonellosis

American veterinarian Daniel Elmer Salmon, who first isolated *Salmonella enterica* serotype Choleraesuis from pigs in 1885 (Rabsch *et al.,* 2003).Humans infections of *Salmonella* are divided into typhoid fever, caused by *Salmonella typhi* and *Salmonella paratyphi*, and also other clinical syndromes, including diarrhoeal disease, caused by a large number of *non-typhoidal salmonella* serovars (NTS)(Gordon, 2008). *Salmonella* species are Gram-negative, flagellated facultative anaerobic rods characterized by O, H, and VI antigens. According to (Foley and Lynne, 2008), there are over 2,500 identified serotypes of *Salmonella*.

3.1.1. Epidemiology

Typhoid cases are stable with low numbers in developed countries, but nontyphoidal *salmonellosis* has increased worldwide. Typhoid fever usually causes mortality in 5 to 30% of typhoid-infected individual in the developing world. The World Health Organization (WHO) estimates 16 to 17 million cases occur annually, resulting in about 600,000 deaths. The mortality rates differ from region to region, but can be as high as 5 to 7% despite the use of appropriate antibiotic treatment. On the other hand, nontyphoidal cases account for 1.3 billion cases with 3 million deaths. In the United States, approximately 2 to 4 million cases of *Salmonella* gastroenteritis occur with about 500 deaths per year. A more accurate figure of *salmonellosis* is difficult to determine because normally only large outbreaks are investigated whereas sporadic cases are under-reported. Data on salmonellosis are scarce in many countries of Asia, Africa and South and Central America where only 1 to 10% of cases are reported (Hanes, 2003; Hu and Kopecko, 2003).

Typhoid fever is endemic throughout Africa and Asia as well as persists in the Middle East, some eastern and southern European countries and central and South America. In the US and most of Europe, typhoid is predominantly a disease of the returning traveler. Typhoid incidence in endemic areas is typically low in the first few years of life, peaking in school-aged children and young adults and then falling in middle age. Most infections

occur in childhood especially in Mekong Delta region of Vietnam and are recognizable although often mild (Wray and Davies, 2003). The most famous outbreak of enteric fever is Typhoid Mary. Mary Mallon, a New York City hired household cook, transmitted typhoid fever to at least 22 individuals causing 3 deaths between 1900 and 1907. After being apprehended by public health officials in 1907, she was isolated for 3 years. Even though she was released with the stipulation that she never cook again, she broke the promise and consequently caused at least 25 more cases of typhoid fever at Manhattan maternity hospital when she was employed as a cook in 1915. She was finally isolated until her death in 1938 (Scherer and Miller, 2001; Parry, 2006)

In studies conducted in Jordan on 283 food handlers for potential pathogens in their stool, the rate of isolation of *Salmonella* was 6% (Al-Lahham *et al*., 1990). Another study showed in two hospitals in Winchester, Southern England that Faecal screening of asymptomatic catering staff demonstrated 12.3% *Salmonella* (Dryden *et al*., 1994). Prevalence of chronic typhoidal *Salmonellae* carriers among food vendors in Kumasi, Ghana showed that Typhoidal *Salmonellae* were isolated from six people out of 258, giving a carriage rate of 2.3% and three of the *Salmonellae* isolated were *S. typhi***,** and the other three were non-typhoidal *Salmonellae* (Feglo *et al*., 2004). Another study done in Nigeria showed that *Salmonella spp.* (three *S. typhi* [5.7%], three *S. enteritidi*s [5.7%] and one *S. choleraesuis* (1.9%) were recovered from seven (13. 2%) of the 53 stool samples processed.

Studies indicated the widespread occurrence and distribution of *Salmonella* in Ethiopia. In recent years the number of out breaks of *Salmonella* in humans has increased considerably in the country. Much more is known now about the extent of food borne illness and how severe it can be, not just in terms of acute illness, but also in terms of long term consequences. Study indicated various percentages of *Salmonella* isolates in towns of Ethiopia (Abera *et al*., 2010). Moreover, high percentages of *S. typhi* isolates have been found to be resistant for antimicrobial agents. In addition, the very young, elderly and immunocompromized individuals are particularly more susceptible to *Salmonella* infections at a lower infective dose than healthy adults. This is more

important in developing countries such as Ethiopia where HIV/AIDS is highly prevalent and *Salmonella* is an important opportunistic infection in HIV/AIDS patients

In the study conducted by Ashenafi and Gedebou (1985), 45 *Salmonella* were isolated from 1000 adult diarrheal out-patients with the prevalence rate of 4.5%. Mache (1995) reported a 6.4% prevalence rate of *Salmonella* which showed some increment from the previous prevalence rate. Another study conducted by (Andualem and Geyid, 2005) indicated a 10.7% prevalence rate of salmonellosis. A study conducted in Gonder town showed that *Salmonella* spp was not detected in stool sample of food handlers. (Gashaw *et al*., 2008). On the other hand Prevalence study in Bahirdar town showed that six (1.6%) food handlers were found positive for *S. typhi* (Abera *et al*., 2010).Reda *et al.* (2011) in Harer reported a prevalence rate of 11.5%. A report from Gonder indicated a low prevalence rate 1.3% as compared to the previous studies in Ethiopia (Dagnew, 2013).A very recent report from Jimma indicated a prevalence rate of 6.2% (Beyene and Tasew, 2014).

Although primarily intestinal bacteria, *Salmonella* are widespread in the environment and commonly found in farm effluents, human sewage, and in any material subject to fecal contamination. Salmonellosis has been recognized in all countries but appears to be most prevalent in areas of intensive animal husbandry, especially poultry and swine production (Wray and Davies, 2003). Risk factors for salmonellosis include gastric hypoacidity, recent use of antibiotics, extremes of age, and immunosuppressive conditions (Crum-Cianflone, 2008).

3.1.2. Pathogenesis and Pathology

Pathogenic *Salmonellae* ingested in food can survive passage through the gastric acid barrier and invade the mucosa of the small and large intestine and produce toxins (Scherer and Miller, 2001). Invasion of epithelial cells stimulates the release of pro-inflammatory cytokines which induce an inflammatory reaction. The acute inflammatory response causes diarrhea and may lead to ulceration and destruction of the mucosa. The bacteria can disseminate from the intestines to cause systemic disease

3.1.3. Clinical features and complications

The clinical pattern of salmonellosis in human can be divided into four disease patterns namely enteric fever, gastroenteritis, bacteremia and other complications of nontyphoidal Salmonellosis as well as chronic carrier state.

3.1.3.1. Enteric fever

The symptoms for *Salmonella*, which is the cause of enteric fever, are a gradually increasing fever, a non-productive cough, frontal headaches, constipation, and occasionally diarrhea. Infections of Salmonellosis occur due to ingestion of food or water contaminated with human waste. In recent years, antibiotic-resistant strains have been isolated in most endemic areas, particularly Southeast Asia, India, Pakistan and Middle East (Scherer and Miller, 2001).Roughly 10% of patients may relapse, die or encounter serious complications such as typhoid encephalopathy, gastrointestinal bleeding and intestinal perforation. Relapse is the most common occurrence probably due to persisting organisms within reticuloendothelial system (RES).Typhoid encephalopathy, often accompanied by shock, is associated with high mortality. Slight gastrointestinal bleeding can be resolved without blood transfusion but in 1 to 2% of cases can be fatal if a large vessel is involved (Hu and Kopecko, 2003; Parry, 2006).

3.1.3.2. Gastroenteritis

Gastroenteritis is the most common result of *Salmonella* infection: in this case, the initial symptoms may be nausea and vomiting. Afterwards, symptoms such as abdominal pain, mild to severe diarrhea, temperatures ranging from 100.4 to 102.2°F (38 to 39 °C), and bloody stools appear, though the leukocyte count in such individuals is generally found to be normal Nontyphoidal salmonellosis or enterocolitis is caused by at least 150 *Salmonella* serotypes with *Salmonella Typhimurium* and *Salmonella Enteritidis* being the most common serotypes in the United States. Infection always occurs via ingestion of water or food contaminated with animal waste rather than human waste. The emergence of multidrug-resistant *S. Typhimurium* DT104 has been associated with outbreaks related to beef contamination and resulted in hospitalization rates twice than that of other foodborne salmonellosis (Yousef and Carlstrom, 2003). Antibiotic treatment is usually

not advised except for rare cases because it can prolong the presence of bacteria in the stool

3.1.3.3. Bacteremia

If untreated, 8% of the cases of salmonellosis result in bacteremia. Bacteremia is a serious condition in which bacteria enter the bloodstream. It has been associated with highly invasive serotypes like Cholearaesuis or Dublin. Bacteremia caused by *Salmonella* should be taken into account in cases of fever of unknown origin. Patients with such complications should be treated with antibiotics (Scherer and Miller, 2001; Hanes, 2003).

3.1.3.4. Chronic carrier state

Salmonellosis can be spread by chronic carriers who potentially infect many individuals, especially those who work in food-related industries. Factors contributing to the chronic carrier state have not been fully explained. On average, nontyphoidal serotypes persist in the gastrointestinal tract from 6 weeks to 3 months, depending on the serotypes. Only about 0.1% of nontyphoidal *Salmonella* cases are shed in stool samples for periods exceeding 1 year. About 2 to 5% of untreated typhoid infections result in a chronic carrier state. Up to 10% of untreated convalescent typhoid cases will excrete *S. Typhi* in feces for 1 to 3 months and between 1 and 4% become chronic carriers excreting the microorganism for more than one year (Scherer and Miller, 2001; Parry, 2006).

3.1.4. Diagnosis

Diagnosis depends on demonstrating the pathogen in blood, bone marrow, stool or urine cultures. However, bacteriological methods are time consuming and usually require 5-11 days. Additionally, in developing countries like Pakistan sensitivity of blood culture is lowered due to irrational use of antibiotics. The widal test; a serologic test has a number of limitations including failure to diagnose *S. Paratyphi A* infection. Polymerase chain reaction (PCR), in addition to analysis of foods, has also been successfully applied to the detection and identification of pathogenic organisms in clinical and environmental samples. It has been successfully used for diagnosis of *S. Typhi* and proved superior to

conventional methods. A similar approach for diagnosis of *S. Paratyphi A* can be of great help (Ali *et al*., 2008).

3.1.5. Prevention

In many urban centers, eating and drinking in public establishments, such as Hotels, Restaurants, and Snack bars is a common practice in many countries. These establishments prepare, handle, and serve large quantities of food and drink to large groups of people within a short period of time implying a possible risk of infections if sanitary and hygienic norms are not strictly followed. The world health status review indicates that the health problem of developing nations is mainly linked to inadequate sanitation (Kumie *et al*., 2002).

Better education of food industry workers in basic food safety and restaurant inspection procedures may prevent cross-contamination. Food handling errors can lead to outbreaks. Improvements in farm animal hygiene, in slaughter plant practices, and in vegetable and fruit harvesting and packing operations may help prevent salmonellosis caused by contaminated foods. Pasteurization of milk and treatment of municipal water supplies are highly effective prevention measures that have been in place for decades. Wider use of pasteurized egg in restaurants, hospitals, and nursing homes is an important prevention measure. In the future, irradiation or other treatments may greatly reduce contamination of raw meat (CDC, 2008).

3.1.6. Treatment

Salmonellosis in humans can be treated with a number of antibiotics including ampicillin, amoxicillin, gentamicin, trimethoprim/sulfamethoxazole and fluoroquinolones. Many isolates are resistant to one or more antibiotics, and the choice of drugs should, if possible, be based on susceptibility testing. Antibiotics are used mainly for septicemia, enteric fever or focal extraintestinal infections. Focal infections may require surgery and prolonged courses of antibiotics. In the elderly, infants and immunosuppressed persons, who are prone to septicemia and complications, antibiotics may be given for gastroenteritis. However most healthy people recover spontaneously in 2 to 7 days and

may not require antibiotic treatment. Antibiotics do not usually shorten this form of the disease. They also prolong the period of bacterial shedding and increase the development of antibiotic-resistant strains. Symptomatic treatment of dehydration, nausea and vomiting may be required (Majowicz, 2010)

3.2. Shigellosis

Shigella is discovered over 100 years ago by Kiyoshi Shiga, a Japanese scientist, they are Gram-negative, non motile, facultative anaerobic, non-spore-forming rods. Its difference from the closely related *Escherichia coli* is on the basis of pathogenicity, physiology (failure to ferment lactose or decarboxylate lysine) and serology. The genus is divided into four serogroups with multiple serotypes: *Sh. dysenteriae*, 12 serotypes; *Sh. flexneri*, 6 serotypes; *Sh. boydii*, 18 serotypes; and *Sh. sonnei*, 1 serotype (Thomas and Gerald, 2000). *Shigellosis* is an acute invasive enteric infection; it is clinically manifested by diarrhea that is frequently bloody. *Shigellosis* is endemic in many developing countries and also occurs in epidemics causing considerable morbidity and mortality. Challenges to control *shigellosis* include the ease with which *Shigella* spreads from person to person and the rapidity with which it develops antimicrobial resistance (WHO, 2005).

3.2.1. Epidemiology

Annually, there are 165 million cases of shigellosis resulting in 1.1 million deaths in the developing world (Michael *et al*., 2008). The most frequently reported factor associated with the involvement of the infected worker was bare hand contact with the food followed by failure to properly wash hands, inadequate cleaning of processing or preparation equipment or utensils, cross-contamination of ready-to-eat foods by contaminated raw ingredients (Todd *et al*., 2007).During a one-year period, 283 food handlers in Irbid, Jordan were investigated for the presence of potential enteropathogens in their stools. The isolation rate of *shigella* was 1.4% (Al-Lahham *et al.,* 1990).

In the study conducted by Desta (2010) only one *Shigella* spp. (0.4%) was isolated from stool culture of food handlers. Low prevalence of *Shigella* spp. in food handlers was also reported in some studies Omdurman, Sudan (1.3%) (Saeed and Hamid, 2010) and Ethiopia (3.1%) (Gashaw *et al*., 2008). In other studies, no *Shigella* recovered from stool

specimens of food handlers' (Simsek *et al*., 2009). However, *Shigella* was the most common bacterial isolated among food handlers in a tertiary care hospital of North India (13.3%) (Khurana *et al*., 2008). Other study in Ethiopia showed that, *Shigella dysenteriae* and *Shigella flexineri* have been identified as the species that account for about 80% of *Shigella* isolates. Study done in Gonder town showed that *Shigella* species were isolated from stool samples of four food-handlers (3.1%) out of 127 food handlers (Gashaw *et al*., 2008). In the study conducted by (Reda *et al*., 2011) Twenty eight (11.5%) *Salmonella* and 17 (6.7%) *Shigellas were* isolated (Table 1).

The prevalence rate of *Shigella* were also showed in different studies conducted in Ethiopia, a report (9%) by Ashenafi (1983) and 11.7% isolation rate reported by (Asrat *et al*, 1999) at Tikur Anbessa, Ethio-Swedish children's hospital and a report 6.7% by (Reda *et al*., 2011) in Harar. In another study conducted in Jimma relatively smaller prevalence rate 2.3% (Beyene and Tasew, 2014) were observed.

Table 1:-Prevalence of *Salmonella* and *Shigella* and Appearance of diarrhea in patients in Harar, eastern Ethiopia, between Januarys to February 2007. (Reda *et al*., 2011)

Appearance of diarrhea	Organisms isolated		
	Salmonella (n, %)	*Shigella* (n, %)	Total
Bloody	7(25)	1(59)	8(17.8)
Mucoid	12(42.8)	9(52.9)	21(46.8)
Watery	0(0)	1(5.9)	1(2.2)
Mucoid and bloody	9(32.1)	3(17.6)	12(42.8)
Other	0(0)	3(17.6)	3(6.7)
Total	28(100)	17(100)	45(100)

3.2.2. Pathogenesity and pathology

Shigellosis is initiated by ingestion of the pathogen usually via fecal-oral contamination. An early symptom, diarrhea (possibly elicited by enterotoxins and/or cytotoxin), may occur as the organisms pass through the small intestine (Thomas and Gerald, 2000). The hallmarks of shigellosis are bacterial invasion of the colonic epithelium and inflammatory colitis. These are interdependent processes amplified by local release of cytokines and by the infiltration of inflammatory elements. Colitis in the rectosigmoid mucosa, with concomitant malabsorption, results in the characteristic sign of bacillary dysentery: scanty, unformed stools tinged with blood and mucus.

3.2.3. Clinical features and complications

Shigella is a pathogen that primarily infects the lower intestinal tract. Patients with *Shigella* gastroenteritis typically present with high fever, abdominal cramps, and bloody, mucoid diarrhea. The approximate prevalence of these signs and symptoms is Fever (30 to 40 %); abdominal pain (70 to 93%), mucoid diarrhea (70 to 85%), bloody diarrhea (35 to 55%), watery diarrhea (30 to 40%), vomiting (35%). The incubation period ranges from one to seven days, with an average of three days (Maurelli and Lampel, 1994). The disease typically begins with constitutional symptoms such as fever, anorexia, and malaise; diarrhea initially is watery, but subsequently may contain blood and mucus.

Frequency of stools typically is 8 to 10 per day but may increase to up to 100 per day. The stools are of small volume, so significant fluid loss typically does not occur (average approximately 30 mL/kg per day) (Acheson and Ceuchi, 1995). These findings are characteristic of diarrhea caused by infection of the colon (the major site of infection with *Shigella*) because the colon functions as a storage organ. This is in contrast to small bowel infections in which the diarrhea typically is watery; of large volume; and associated with abdominal cramping, bloating, gas, and weight loss.

The spectrum of severity of disease varies according to the serogroups of the infecting organism. *Shigella sonnei* commonly causes mild disease, which may be limited to

watery diarrhea, although *Shigella dysenteriae* 1 or *Shigella flexneri* commonly causes dysenteric symptoms (bloody diarrhea) (Keusch *et al*., 1990). The course of disease in a normal healthy host generally is self-limited, lasting no more than seven days when left untreated. The typical course of disease varies with age group. In a review of 318 infants and children hospitalized with shigellosis in Bangladesh, infants had fewer days with diarrhea (four versus six) and were more likely to have watery (as opposed to bloody) stools, hyponatremia, abdominal distension, and acidosis than were older children (Huskins *et al*., 1994). Older children were more likely to have a leukemoid reaction than were infants. The mortality rate for infants was twice that of older children. Infants who were breast fed were less frequently infected and had a milder illness than infants who were not breast fed (Stoll *et al*., 1982).

3.2.3.1. Intestinal complications

Rectal prolapse

The severe inflammation of the rectum and distal colon that is induced by invasion of the organism into the colonic mucosa.

Toxic megacolon

Toxic megacolon occurs primarily in the setting of *S. dysenteriae* 1 infection. The pathogenesis is unclear; it occurs in the setting of pancolitis and seems to be related to the intensity of inflammation rather than being mediated by Shiga toxin. The incidence of toxic megacolon in children with diarrhea is low (3%). Intestinal obstruction — severe colonic disease may result in intestinal obstruction. The incidence in one series of 1211 patients with shigellosis was 2.5% (Bennish *et al*., 1991). The patients with obstruction were more likely to be infected with *S. dysenteriae* 1 and were more severely ill, as evidenced by a significantly higher white blood cell (WBC) count and lower serum sodium concentration than patients without evidence of obstruction

Colonic perforation

This is an unusual complication of shigellosis. It occurs principally in infants or severely malnourished patients and is associated with infection caused by *S. dysenteriae* 1 or *S. flexneri* (1.7% of fatal cases) (Azad *et al*., 1986).

Bacteremia

Occurs in approximately 4% of patients with *Shigella* gastroenteritis and is associated with an increase in mortality (Struelens *et al*., 1985). Young, malnourished children are at greatest risk. Bacteremia with another gram-negative organism is seen in approximately 5% of patients who have a stool culture positive for *Shigella*.

Metabolic disturbances

The stool volume in Shigellosis is usually low. In a review of 412 patients with shigellosis, 36% had mild, 12% had moderate, and 2% had severe dehydration (Stoll *et al*., 1982). In another series, hyponatremia (defined as serum sodium below 120 meq/L) was noted in 29% of patients hospitalized with diarrhea caused by *S. dysenteriae* 1. Generally, hyponatremia is caused by the syndrome of inappropriate ADH secretion, not by volume depletion (Keusch *et al*., 1989).

Leukemoid reaction

In approximately 4% of the patients, a leukomoid reaction has been noted most commonly in children between 2 and 10 years of age (Butler *et al*., 1984).The WBC count in these patients ranged from 50,000 to 195,000/mm3 and was accompanied by an increased number of immature forms. The mortality rate also was increased (21 versus 7.4% in those without a leukemoid reaction). In contrast, an earlier study conducted in the United States found no association between disease severity and a high WBC count (Barrett-Connor and Connor, 1970).

Neurologic disease

Seizures, due to *Shigella* infection, are always associated with fever (usually greater than 39ºC) and not associated with neurologic deficits (Ashkenazi *et al*., 1987). Seizures can be seen with all serotypes of *Shigella* infection, but are least common with *S. dysenteriae*1

Hemolytic-uremic syndrome

It can occur as a complication of infection caused by *Shigella dysenteriae.* Because HUS is mediated by Shiga toxin, which is present in *S. dysenteriae* type 1, but not other species of *Shigella*, only *S. dysenteriae* type 1 can cause HUS. HUS is a potentially life-threatening illness that is characterized by a microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure.Enterohemorrhagic Ecsherichia coli (EHEC) infection accounts for about 70 percent of cases in the United States (Siegle, 1995)

3.2.4. Diagnosis

Stool with blood and abdominal pain, the absence of watery diarrhea and vomiting in patients over one year old are the signs and symptoms of shigellosis (Stoll *et al*., 1982). The four *Shigella* species (*Sh. dysenteriae, Sh. flexneri, Sh. boydii, and Sh. sonnei*) are classically identified by culture of fecal specimens on selective media and testing of isolates for agglutination in species-specific antisera (Echeverria *et al*., 1991). If it is not easily detected by routine stool culture, alternative diagnostic methods can also be used. One of these techniques, nucleic acid probe hybridization, has been used to identify *Shigella spp.* And entero invasive *Escherichia coli* (EIEC) in stool specimens through the detection of genetic material encoded by a specific large approximately 200-kbp virulence-related plasmid (Oberhelman *et al*., 1993).

3.2.5. Prevention

Prevention of dysentery caused by *Shigella* relies primarily on measures that prevent spread of the organism within the community and from person to person. These include: hand-washing with soap, ensuring the availability of safe drinking water, safely disposing of human waste, breastfeeding of infants and young children, safe handling and

processing of food, and control of flies. These measures will not only reduce the incidence of shigellosis, but of other diarrheal diseases as well. In all cases, health education and the cooperation of the community in implementing control measures are essential (WHO, 2005).

3.2.6. Treatment

Due to resistance to antibiotics; antimicrobial drug choices has changed over the years. The following antibiotics were used to treat *Shigella*

• Class: beta-lactams: ampicillin, amoxicillin, first and second generation cephalosporin's (cefixime, ceftriaxone) and Pivmecillinam;

• Class: quinolones: nalidixic acid, ciprofloxacin, norfloxacin, ofloxacin;

• Class: macrolides: azithromycin; others: sulphonamides, tetracycline, cotrimoxazole, and furazolidone.

The WHO now recommends that clinically diagnosed cases of *Shigella dysentery* be treated with ciprofloxacin as first line treatment, and pivmecillinam, ceftriaxone, or azithromycin as second line treatment and lists the others as ineffective (WHO 2005a). However, resistance to quinolones has also been observed since the late 1990s, and some authors have questioned the effectiveness of this class for *Shigella* (Talukder, 2004).

3.3. Antibiogram of *Salmonella* and *Shigella*

The increased use of antimicrobial agents in food animal production and human medicine as a means of preventing and treating diseases, as well as promoting growth, is a significant factor in the emergence of antibiotic-resistant bacteria. Therefore, the antibiotic resistance developed as a result of antibiotic use in animal agriculture can be transferred to humans through the food chain. Contamination of food with antibiotic resistant bacteria can be a major threat to public health, causing community outbreaks of infectious diseases. There is also the hazard of therapeutic failure due to the increasing incidence of antimicrobial resistance among *Salmonella* species (Tambekar, 2005).

The use of antimicrobial agents in any environment increases selective pressures that may favor the survival of antibiotic resistant strains. Emerging resistance in *Salmonella Typhi*

has been described especially in Africa and Asia and the appearance of *Salmonella Typhimurium* DT104 in the late 1980s raised main public health concern, thereby threatening the lives of infected individuals (Montville and Matthews, 2008). (Van *et al*., 2007) stated that multi-resistance occurred in *Salmonella* serotypes including Albany, Anatum, Havana, London and Typhimurium.

The resistance towards the traditional first-line antibiotics such as ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole define multidrug resistance (MDR) in *Salmonella enterica* (Crump and Mintz, 2010). This is of great concern because majority infections with MDR *Salmonella* are acquired through the consumption of contaminated foods of animal origin such as swines and chicken eggs mentioned that cephalosporin and fluoroquinone-resistant strains of *S. Choleraesuis* have been identified in swines in Taiwan and Thailand. Apart from that, antibiogram testing by (Singh *et al.*, 2010) revealed *Salmonella* isolates from chicken eggs in marketing channels and poultry farms in North India were resistant to bacitracin, colistin and polymyxin-B.

Due to the use of antibiotics for the promotion of growth and prevention of disease in food animals, there is an increase of human salmonellosis cases caused by foodborne MDR *Salmonella* nowadays (Yang *et al*., 2010). This indiscriminate and injudicious use of antibiotics in any setting especially in food animals worldwide should be monitored to reduce the transfer risk of MDR *Salmonella* to humans.

In the study conducted by (Asrat, 2008), the *Salmonella* species were resistant to ampicillin (81.2%), cephalothin (86.4%), chloramphenicol (83.7%), erythromycin (100.0%), gentamicin (75.6%), nalidixic acid (37.8%), sulfonamide (81.1%), tetracycline (94.5%) and TMP–SXT (75.7%). All strains were susceptible to norfloxacin (100.0%). Among *Salmonella* spp. a comparatively low level of resistance (20%–25%) was detected in *S. typhi* to all antimicrobial agents tested except for erythromycin. Multidrug resistance (2 or more antibiotics) was noted in 80%– 90% of both isolates. Resistance to ampicillin, cotrimoxazole, chloramphenicol and nalidixic acid was observed in 62.5, 31.3, 18.8% and 12.5 of *Salmonella* isolate respectively. All *Salmonella* isolates were resistant against Amoxicillin and susceptible to ceftriaxone, ciprofloxacin and gentamicin. On the other

hand 62.5% of *Salmonella* species were multidrug resistant, ranging from 2 to 4 drugs (Beyene and Tasew, 2014). Finally, there is a need of continuous surveillance and sharing of antimicrobial susceptibility data for *Salmonella* among countries worldwide to ensure the effectiveness of control programmes.

The increasing antimicrobial resistance of *Shigella* species is a major problem in treating shigellosis. The major route for dissemination of multiple resistances is by horizontal transfer of plasmids carrying antibiotic resistance (Rplasmids). A commonly isolated plasmid carries resistance against ampicillin, chloramphenicol, tetracycline, sulfonamides, streptomycin, and trimethoprim (Bhattacharya, 2005). Ampicillin resistance also is mediated by beta-lactamases.

High rates of antimicrobial resistance were first reported in Asia, Africa, and South America, but antimicrobial resistance has rapidly spread to developed countries. In India and Bangladesh, 20 percent or more of isolates are resistant to nalidixic acid (Bennish *et al*., 1992). Resistance to nalidixic acid also been reported in England and the United States. Antimicrobial resistance is an increasing problem in the United States. Between 1999 and 2002, the following results were reported:

- All isolated *Shigella* were susceptible to ceftriaxone and ciprofloxacin.
- 78% of isolates were resistant to ampicillin, 46% to TMP-SMX, 38% to both ampicillin and TMP-SMX, and 1 percent to nalidixic acid.

In Ethiopia, strains of *Shigella* that were resistant to many commonly used drugs have been reported in different parts of the country by several studies (Beyene and Tasew, 2014). In the aforementioned Ethiopian study reports, the strains were found to be most commonly resistant to tetracycline (>80%), ampicillin (>65%), and cotrimoxazole (>70%). Multiple drug resistance to ampicillin, chloramphenicol, tetracycline, and streptomycin was also very high in those studies. Belay and his colleagues have reported a strain that was resistant to eight drugs out of the nine antimicrobials they used

Shigella species showed hundred percent resistances to ampicillin, amoxacillin, and cotrimoxazole while all (100%) isolates were susceptible to ceftriaxone, ciprofloxacin and gentamicin. Over all three resistance patterns were seen among *Shigella* isolates. All *Shigella* species were multi-drug resistant (resistant to three or more antimicrobial drugs). About 66.6% of *Shigella* species were resistant to three (ampicillin, amoxacillin, cotrimoxazole) antibiotics (Beyene and Tasew, 2014). Antibiograms of *Shigella* species showed that most strains were resistant to ampicillin (78.7%), cephalothin (86.7%), chloramphenicol (74.7%), erythromycin (100.0%), sulfonamide (54.7%), tetracycline (97.3%) and TMP–SXT (45.3%), but susceptible to gentamicin (100%), nalidixic acid (97.3%) and norfloxacin (100.0%)(Asrat, 2008).

The highest level of resistance was detected for ampicillin and amoxicillin in which all (100%) *Salmonella* and *Shigella* isolates were found to be resistant. The highest level of was detected for gentamicin and norfloxacin, where, respectively, 92.8 (26) and 89.3% (25); and 94.1% (16) and 88.2% (15) of the *Salmonella* and *Shigella* isolates were susceptible (Reda *et al*., 2011)

Besides the temporal changes in the antibiogram of *Shigella* species, it is well known that antibiotic susceptibility patterns in *Shigella* may differ between geographical areas. Such differences are never stable and may change rapidly especially in places where antibiotics are used excessively (particularly in developing countries) (Montville and Matthews, 2008). This warrants for frequent observation on the change in the pattern of antibiogram for this organism

3.4. Growth potential of *Salmonella* and *Shigella*

Although myriad foods can serve as *Salmonella* sources, meat and meat products, poultry products, and dairy products are significant sources of foodborne pathogen infections in humans. Presence of *Salmonella spp*. in fresh raw products can vary widely (Harris *et al*., 2003). Frequency usually ranges from 1 to 10 %, depending on a range of factors including organism, farming and/or food production practices, and geographical factors (Harris *et al*., 2003). Poultry and egg products have long been recognized as an important *Salmonella* source in fact, contaminated poultry, eggs and dairy products are

probably the most common cause of human Salmonellosis worldwide (Herikstad *et al*., 2002).

Salmonella serotypes can grow and survive on a large number of foods. Their behavior in foods is controlled by a variety of environmental and ecological factors, including water activity, pH, Eh, chemical composition, the presence of natural or added antimicrobial compounds and storage temperature; as well as processing factors such as heat application and physical handling (Escartin, 1989). For example, optimum pH for growth in *Salmonella* is approximately neutral, with values > 9.0 and < 4.0 being bactericidal. Minimum growth in some serotypes can occur at pH 4.05 (with HCl and citric acids), although this minimum can occur at pH as high as 5.5, depending on the acid used to lower pH (Harris *et al*., 2003). Growth in *Salmonella* can continue at temperatures as low as 5.3 °C (*S. Heidelberg*) and 6.2 °C (*S. Typhimurium*), and temperatures near 45 °C (temperatures ≥ 45 °C are bactericidal). In addition, available moisture (aw) inhibits growth at values below 0.94 in neutral pH media, although higher aw values are required as pH declines to near the minimum growth values (Harris, *et al*., 2003).

The determination of growth potential of *Salmonella* and other food borne pathogens in ready to eat food can be very useful to determine likely threats to food safety (Sant'Ana *et al*., 2012). Erku_and Ashenafi (1998) reported that the potential of *Salmonella spp.* to grow in weaning foods was also determined on one common factory-produced infant food and one home-made infant food. In both items, *Salmonella* increased by approximately 4 log units within eight hours and reached counts as high as log 8 cfu/ml within twelve hours. Another study in Ethiopian street vended foods (Muleta and Ashenafi, 2001), *Salmonella typhimurium* reached counts > 10^8 cfu/g within 24 hours in all the food items tested (Egg sandwich, macaroni and lentil sandwich). Counts increased by about **1** log unit in the first 4 hours and showed a steady growth thereafter.

The growth and survival of *Shigella* spp. in foods is influenced by a number of factors such as temperature, pH, salt content and the presence of preservatives. For example, survival of *S. flexneri* has been shown to increase with: decreasing temperature, increasing pH, and decreasing NaCl concentration (Zaika and Phillips, 2005).

The temperature range for growth of *Shigella spp*. is 6–8 to 45–47°C . Rapid inactivation occurs at temperatures around 65°C. In contrast, under frozen (-20°C) or refrigerated (4°C) conditions *Shigella spp*. can survive for extended periods of time (Warren *et al*. 2006). *Shigella* spp. grows in a pH range of 5–9. Zaika (2001) demonstrated that *S. flexneri* is tolerant to acid and can survive at pH 4 for 5 days in broth when incubated at 28°C. *Shigella spp*. are better able to survive lower pH conditions at reduced temperatures, with *S. flexneri* and *S. sonnei* able to survive for 14 days in tomato juice (pH 3.9–4.1) and apple juice (pH 3.3–3.4) stored at 7°C (Bagamboula *et al*. 2002). *S. flexneri* is salt tolerant and is able to grow in media containing 7% NaCl at 28°C). It is sensitive to organic acids typically used to preserve food. For example, lactic acid has been demonstrated to be effective at inhibiting *S. flexneri* growth, followed in order by acetic acid, citric acid, malic acid and tartaric acid (Zaika 2002b).

Fruits and vegetables can support the growth or survival of *Shigella*. Escartin *et al*. (1989) artificially contaminated fresh cut papaya, jicama, and watermelon with *S. sonnei*, *S. flexneri*, or *S. dysenteriae* and within 6 hours at room temperature, growth was observed. Rafii and Lunsford (1997) inoculated raw cabbage, onion, and green pepper with *S. flexneri* and although counts decreased slightly at 4°C, survival was observed after 12 days on onion and green pepper, at which time sampling was terminated due to spoilage (Rafii and Lunsford, 1997). Low pH foods can support survival of *Shigella* when held at refrigerated temperatures. Bagamboula *et al*. (2002) demonstrated *S. sonnei* and *S. flexneri* survival in apple juice (pH 3.3 to 3.4) and tomato juice (pH 3.9 to 4.1) held at 7°C for 14 days.

Prepared foods can also support the survival of *Shigella*. Islam *et al*. (1993b) investigated the growth and survival of *S. flexneri* in boiled rice, lentil soup, milk, cooked beef, cooked fish, mashed potato, mashed brinjal, and raw cucumber. All food samples, except

raw cucumber, were autoclaved prior to inoculation. Ten gram or 10 ml samples of each food were inoculated with 10^5 cells of *S. flexneri*, incubated at 5, 25, or 37°C and sampled over 72 hr. All of the foods tested supported growth up to 10^8 to10^{10} cells per g or ml within 6 to 18 hr after inoculation at 25°C and 37°C (Islam *et al*., 1993b).

Growth of *Shigella flexneri* was markedly fast in the first 4 hours in “macaroni" compared to "egg sandwich" and "lentil sandwich". Final counts in the food items varied slightly and higher counts were noted in "macaroni" followed by "lentil sandwich. Growth rate in "lentil sandwich" was relatively steadier. Initial inoculum level of the test strain was much lower than that of the other test strains (Muleta and Ashenafi, 2001). *Shigella* is able to survive on produce packaged under vacuum or modified atmosphere. Satchell *et al*. (1990) investigated the survival of *S. sonnei* in shredded cabbage packaged under vacuum or in a modified atmosphere of nitrogen and carbon dioxide when stored at room temperature or under refrigeration

In a microbiological challenge study, the levels of live challenge microorganisms are enumerated at each sampling point. The type of pathogen used determines which enumeration method and media has to be used. If the product does not have a substantial background microflora, non-selective media for direct enumeration may be used. It is practical to analyze the product, including uninoculated control samples, at each or selected sampling points in the study to see how the background microflora is behaving over product shelf life. For example, if a product has a high background microflora, it may suppress the growth of the challenge inoculums. In some cases, this is useful and desirable because the product spoils before pathogens can grow (Vestergaard, 2001)

4. Materials and Methods

4.1. Description of the study area

The study was conducted in Jimma town, located at 353 km southwest of Addis Ababa, the capital city of Ethiopia (Fig. 1). According to Jimma Town Central Statistic Office Jimma town has a population of 127, 945. The town's geographical locations are 7°41'N latitude and 36°50'E longitude. The study area has an average altitude of 1, 780 MASL. It lies in the climatic zone locally known as "Woyna Daga" (1,500 - 2,400 MASL) which is considered as model for agriculture as well as human settlement. The town is generally characterized by warm weather with mean annual maximum and minimum temperatures of 30°C and 14 °C respectively (Abebe *et al.*, 2011).The annual rainfall ranges between 1138-1690 millimeter. Jimma University Specialized Hospital provides service for about 9000 in patient and 80, 000 out-patient attendants who annually come to the Hospital from catchment population of 15,000,000 including Jimma with bed capacity of 450 and a total of more than 550 staff members. The patient flow of the hospital and health centers ranges from rural and urban dwellers to clients of diverse socioeconomic and ethnic backgrounds (WWW.ju.edu.et/jimma-univesity-specialized-hospital-jush)

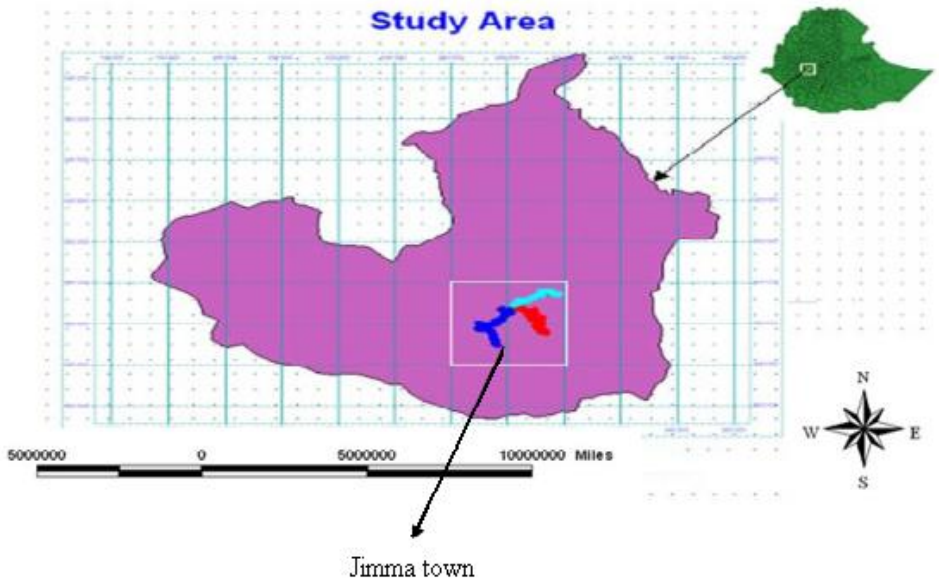

Figure 1. Map of the study site (Abebe *et al.*,2014)

4.2. Study Design

A cross sectional study design was employed.

4.3 Population

4.3.1 Source population

The source population was all attendants of the out-patient department (OPD) of Jimma University Specialized Hospital

4.3.2 Study population

The study populations were all diarrheal adult and pediatric Out-patients.

4.4 Illegibility criteria

4.4.1 Inclusion criteria

All health seeking out-patients visiting the Hospital and have symptoms of diarrhea were included in the current study.

4.4.2 Exclusion criteria

Those out-patients who have taken antibiotic one week prior to data collection period were excluded from the study.

4.5. Sampling technique

All consecutive consenting diarrheal outpatient who visited the OPD during the study period were enrolled in the study provided that inclusion criteria were full filled

4.6. Sample size determination

From the observation made during preliminary survey, the average number of Out-patients examined on daily basis for problems associated to diarrhea in Jimma University Specialized Hospital was eleven so there would be a target population of 510 outpatients

since the data was collected for 45 days. So, the sample size was determined using the statistical formula given below

(Kothari, 2004)

$$n = \frac{n_0}{1 + \frac{n_0}{N}} \qquad \text{Where } n_0 = \frac{Z^2_{\alpha/2} pq}{d^2}$$

n = sample size

d= margin of error, N = total number of diarrheal out patients, p= proportion of population, α= level of significance, q = 1-p

Where: d = 0.06 , p = 0.5, α=0.05

$$n_0 = \frac{(1.96)^2 \times 0.5 \times 0.5}{0.06^2} = 267$$

Considering the population correction factor into account the sample size should be:

$$n = \frac{267}{1 + 267/510} = 176$$

4.7. Sample Collection and Handling

A gram of stool specimen was collected using sterilized screw capped containers with transport media (9ml buffered peptone water (Oxoid, UK)) and transported to Jimma University, Research and Postgraduate Laboratory for microbial analysis. The samples were incubated for 24 hours at 37^0c.

4.8. Culture and identification

After 24 hours, 1ml of the sample was transferred into 10ml of selenite F broth (Oxoid, UK) which was used as secondary enrichment media for *Salmonella* and *Shigella* species, and incubated for 24 hours at 37°C. On the next day, a loopful was subcultured on xylose-lysine-desoxychocolate agar (XLD) (Oxoid, UK) at 37°C for 24 hours. The typical colonies were then further characterized based on colony morphology, appearance, and Gram staining. *Shigella* appears as pink to red colonies on XLD, while *Salmonella* appears as red with black center due to the production of hydrogen sulfide.

For further identification basic biochemical test were employed. The biochemical tests were done according to the procedure of Johnson and Case (2007).

4.8.1. Cell morphology

In order to assess the cell morphology of pure culture, Gram staining and wet mount were used. The morphological study includes cell shape, cell arrangement, presence or absence of endospore and motility.

4.8.2. Gram staining

Gram staining of the presumptive *Salmonella* or *Shigella* isolates was performed following the standard gram staining procedure using freshly prepared chemicals. Accordingly, smears of pure isolate were prepared on a clean slide and allowed to air dry and heat fix. The heat fixed smear was flooded with crystal violet dye for 1 minute and rinsed under tap water for 3 seconds. Then the slide was flooded with iodine solution for 1 minute and rinsed under tap water for 3 seconds. After rinsing the smear was decolorized with 95% of ethanol for 20 seconds and rinsed slides gently under tap water for 3 seconds. Then after the smear was counter stained with safranin and dried by absorbent paper. The air dried smear was observed under oil immersion objective. Gram-negative bacteria appeared pink/red and Gram positive bacteria appeared blue (Elmanama, 2009). The isolates Gram reaction was further confirmed with the rapid method proposed Gregerson (Gregerson, 1978). Here, on a microscopic slide containing 3% KOH solution, a colony aseptically picked was stirred for ten seconds to two minutes. The inoculating loop was raised slowly from the mass when the KOH solution becomes viscous; the thread of slime followed the loop for 0.5 to 2cm or more in Gram-negative bacteria. If there was no slime, but a watery suspension that do not follow the loop, the reaction was considered negative and the isolate was considered as Gram-positive bacteria (Gregerson,1978).

4.8.3. Catalase test

It was carried out by flooding young colonies with a 3% solution (H_2O_2). The formation of bubbles indicates the presence of catalase (McFadden, 1980)

4.8.4. Biochemical identification for *Salmonella* spp.

Colony from XLD was picked, activated in overnight culture and inoculated on nutrient agar media for purification purpose and incubated at 37^0 c for 24hrs. Pure colony from nutrient agar was transferred in to nutrient broth for activation purpose and incubated at 37 ^oC for 24 hrs. The culture was stabbed in butt by using sterile wire and streaked on slant tubes by using sterile loop. All biochemical test tubes were incubated at 37^0 c for 24hrs.

4.8.4.1. Triple Sugar Iron Agar

Triple Sugar Iron Agar (Oxoid) detects fermentation of glucose, sucrose and lactose as well as production of H_2S. The presence of alkaline (red) slant and acid (yellow) butt, with or without production of H_2S were considered as presumptive for *Salmonella* spp.

4.8.4.2. Lysine Iron Agar

Lysine Iron Agar (Oxoid) detects the presence of lysine decarboxylase enzyme. The production of an alkaline reaction (purple color) throughout the medium was used as presumptive for *Salmonella* spp. There was also blackening of the media due to H_2S production.

4.8.4.3. Urea Agar

Urea Agar (Oxoid) detects the hydrolysis of urea; this resulted in pinkish red color formation. No color change was considered as a positive test for *Salmonella*.

4.8.4.4. Simmons Citrate Agar

Simmons Citrate Agar (Oxoid) used to determine citrate utilization as a sole source of carbon. The presence of growth and color change from green to blue was considered as presumptive for *Salmonella spp*.

4.8.4.5. Sulfide Indole Motility (SIM) Medium

The SIM medium (Oxoid) was used to determine H_2S production, indole production and motility. Production of indole was investigated by adding Kovac's reagent. The non-utilization of indole and absence of deep red color at the surface of agar and diffused growth throughout the medium was considered as a presumptive for *Salmonella* spp.

4.8.5. Biochemical test for *Shigella spp*.

Colony from XLD was purified, activated and inoculated in to biochemical media as clearly described above for *Salmonella*

4.8.5.1. Triple sugar iron agar

After overnight incubation, the slants were observed for reactions typical of *Shigella*. An alkaline (red) slant and an acid (yellow) butt with little or no gas production were considered as a presumptive for *Shigella* spp.

4.8.5.2. Lysine Iron Agar

Lysine Iron Agar (Oxoid) detects the presence of lysine decarboxylase enzyme. Pink slant and yellow butt is a presumptive for *Shigella* spp. *Shigella* lacks lysine decarboxylase enzyme.

4.8.5.3. Sulfide indole Motility (SIM) agar

Sulfide indole Motility agar was inoculated with a strong inoculating needle, making a single stab about 1-2cm down in to the medium. It was examined after overnight incubation at 35^0c to 37^0c, absence of diffused growth (only along the stab line) was considered as a presumptive for *Shigella* species.

4.8.5.4. Urea medium

Pale yellowish appearance of the media was observed after overnight incubation that was a presumptive for *Shigella* spp. which is always urease negative.

4.8.5.5. Simmons Citrate Agar

Simmons Citrate Agar (Oxoid) used to determine citrate utilization as a sole source of carbon. The presence of growth and color change from green to blue was considered as positive test for citrate utilization. *Shigella* is citrate negative.

4.9. Antimicrobial sensitivity test

Antimicrobial susceptibility of 19 *Salmonella* spp and 2 *Shigella* spp were carried out by disc diffusion method on Mueller-Hinton agar using commercial antibiotics. The results were interpreted as per the criteria of the National Committee for Clinical Laboratory Standards (NCCLS, 2007). A standardized suspension of the bacterial isolates was prepared and the turbidity of the inoculum was matched with the turbidity standard 0.5 McFarland (Bauer *et al.,* 1966). McFarland is a barium sulphate standard against which the turbidity of the test and controlled inoculum was compared. McFarland was prepared by mixing two solutions; solution "A" and solution "B". Solution "A" is 1 % v/v solution of sulphuric acid (H_2SO_4) and solution "B" is 1 % w/v solution of barium chloride ($BaCl_2$). To get 0.5 McFarland standard, concentration equivalents to cell density of about 10^7- 10^8 $CFUg^{-1}$, the amount of 0.5 ml $BaCl_2$ of 1 % solution "A" was mixed with 99.5 ml H_2SO_4 of 1 % solution "B".

A small volume of the turbid solution was transferred to a screw-cap bottle of the same types as used for preparing test and control inoculums. Culture containing test tube with approximately equal concentration or density with 0.5 McFarland standards were used for inoculation of media. The standard was immediately used after shaking and stored in a well sealed container in a dark place at room temperature (20 - 28 ^oC). When matched with the standard, the culture was swabbed by cotton swab onto the Muller-Hinton Agar (Oxoid) and allowed to dry. Thereafter, the antibiotic discs were placed using sterile forceps on the medium and incubated at 37°C for 18 hrs and the zones of inhibition was measured manually with a transparent ruler.

The results of the antimicrobial susceptibility were interpreted based on the guidance of National Committee for Clinical Laboratory Standards (NCCLS, 2007). Finally, the isolates were classified as sensitive, intermediate, or resistant. Intermediates were considered as resistant for purpose of analysis The following standard drug discs (Oxoid) and their potency (μgml^{-1}) were used depending up on the antibacterial spectrum, toxicity, effectiveness and availability (Vlkova *et al*., 2006). Accordingly, Ampicillin (10 μgml^{-1}), Nalidixic Acid (30 μgml^{-1}), amikacin (30 μgml^{-1}), Tetracycline (30 μgml^{-1}), chloramphenicol (30 μgml^{-1}), Norfloxacin (10 μgml^{-1}), Gentamycin (10 μgml^{-1}), Ciprofloxacin (5 μgml^{-1}) and cotrimoxazole (25 μgml^{-1}) were used for *salmonella* and *Shigella* spp. A reference strain of E. Coli ATCC 25922 was used as quality control during antimicrobial susceptibility tests

4.10. The growth potential of *Salmonella* and *Shigella* isolated from diarrheal Out- patients on selected food

The growth potential of *Salmonella* and *Shigella* isolated from diarrheal Out-patients was assessed on two food items (gruel and firfir), because firfir is frequently utilized by the community as a breakfast and gruel is utilized by babies .About two hundred grams of each food item was steamed at 100°C for one minute to kill any vegetative cell, which might be present in the items. Then 100 g of each food item was challenged with overnight culture of the reference isolates to give an inoculum level of 10^2 - 10^3 cfu/g. The challenged foods were left at ambient temperature for 24 hours. To investigate the initial inoculum level, inoculated foods (10geach) were homogenised separately in90mI of buffered peptone water and 0.1 ml of appropriate dilutions were spread plated on XLD agar to count *Salmonella* and *Shigella*. Ten gram Portions of the food samples were further sampled aseptically at 6 hour intervals from 0 – 24 hours

3.11. Statistical Analysis

Data were organized and summarized using simple descriptive statistics. Moreover, all components of the data were entered and analyzed using SPSS computer software (version 16.0).The socio-demographic characteristics of the diarrheal out-patients were

compared with the prevalence rate using one way ANOVA. Statistical significances were considered at $P<0.05$.

4.12. Ethical consideration

The study was ethically approved by Research Review and Ethical committee of college of Natural Sciences, Jimma University. Official permission was requested from Jimma University Specialized Hospital. The purpose of the study was explained to all respondents and concerned officials and informed consent for participation in the study was obtained prior to sample collection. Professional support was obtained by Medical Laboratory Technicians of Jimma University Specialized Hospital.

5. Results

A total of 176 diarrheal patients who were attending Outpatients department of Jimma University Specialized Hospital were involved in the study with 100% response rate. 19(10.8%) and 2(1.1%) were found positive for *Salmonella* and *Shigella* respectively.

5.1. Socio-demographic characteristics of the study participants

Among the 176 study participants, 18.8%, n=33 were in the age category of <4 years. Concerning the educational background of the study participants, 18(10.2%) and 58(33.0%) educated to grade1-4 and have educational level above grade 12, respectively. The proportions of female outpatients (52.3%) were higher than males 47.7% of which 63.6% were urban residents. Majority (54.5%) of the study participants was unemployed (Table 2)

Table 2. Socio-demographic characteristics of the study population, Jimma University Specialized Hospital January to March 2014.

Characteristics	Category	No Respondent	Percent (%)
Age	<4	33	18.8
	5-9	21	11.9
	10- 14	19	10.8
	15-19	20	11.4
	20-24	22	12.5
	25-29	12	6.8
	30-34	14	8.0
	35-39	12	6.8
	40-44	13	7.4
	>45	10	5.7
Education	Illiterate	52	29.6
	1-4	18	10.2
	5-8	21	11.9
	9-12	27	15.3
	>12	58	33.0
Sex	Male	84	47.7
	Female	92	52.3
Residence	Urban	112	63.6
	Rural	64	36.4
Occupation	Unemployed	96	54.5
	Business men	28	15.9
	Farmer	26	14.8
	Civil servant	26	14.8

5.2. Prevalence of *Salmonella and Shigella*

Frequency of isolation of *Salmonella* was high among the age group between 20-24 and 5-9 with 5 positive samples (2.8%), and there was no *Salmonella* isolated form age group above 40. In this study, only two *Shigella* isolate (1.1%) were encountered in the age category of less <4, which were the only isolates of the study (Table 3).

Table 3. Prevalence of *Salmonella* and *Shigella,* against different age groups at Jimma University Specialized Hospital from January to March 2014.

Age group	Frequency (%)	*Salmonella* Positive No. (%)	*Shigella* positive No. (%)
<4	33(18.8)	1(0.6)	2(1.1)
5-9	21(11.9)	5(2.8)	0(0.0)
10-14	19(10.8)	2(1.1)	0(0.0)
15-19	20(11.4)	1(0.6)	0(0.0)
20-24	22(12.5)	5(2.8)	0(0.0)
25-29	12(6.8)	2(1.1)	0(0.0)
30-34	14(8.0)	2(1.1)	0(0.0)
35-39	12(6.8)	1(0.6)	0(0.0)
40-44	13(7.4)	0(0.0)	0(0.0)
>45	10(5.7)	0(0.0)	0(0.0)
Total	176(100.0)	19(10.8)	2(1.1)

Concerning the educational background of the study participants, 18(10.2%) and 58(33.0%) educated to grade1-4 and have educational level above grade 12 respectively. The numbers of *Salmonella* and *Shigella* positive samples were the highest among illiterates at the prevalence rate of 4% and 1.1% respectively (Table 4).

Table 4. Association of prevalence of *Salmonella* and *Shigella* with educational background of out patients in Jimma University Specialized Hospital from January to March 2014.

Educational	Frequency (%)	*Salmonella* positive No. (%)	*Shigella* positive No. (%)
Illiterate	52(29.6)	7(4.0)	2(1.1)
1-4	18(10.2)	2(1.1)	0(0.0)
5-8	21(11.9)	5(2.8)	0(0.0)
9-12	27(15.3)	1(0.6)	0(0.0)
>12	58(33.0)	4(2.3)	0(0.0)
Total	176(100.0)	19(10.8)	2(1.1)

In the current study, the proportions of female outpatients (53.2%) were higher than the males 47.7% of which 63.6% were urban residents. Regarding prevalence of *Salmonella,* 10 were isolated from among the male outpatients. There were relatively high frequency of isolation of *Salmonella* and *Shigella* isolates in rural residents with detection rates of 5.7% and 1.1% respectively (Table 5**).**

Table 5. Sex and residence distribution of patients and prevalence of *Salmonella* and *Shigella* isolates at Jimma University Specialized Hospital, January to March 2014**.**

Sex	**Frequency (%)**	***Salmonella* positive No. (%)**	***Shigella* positive No. (%)**
Male	84(47.7)	10(5.7)	1 (0.6)
Female	92(52.3)	9(5.1)	1 (0.6)
Residence			
Urban	112(63.6)	9(5.1)	0(0.0)
Rural	64(36.4)	10(5.7)	2(1.1)
Total	176(100)	19(10.8)	2(1.1)

Majority (54.5%) of the study participants were unemployed. There were 14 *Salmonella* isolates among unemployed and 2 isolates of *Shigella* were encountered among farmers (Table 6).The frequency of isolation of *Salmonella* among Business men and farmers appeared relatively low although it needs more data to make generalizations

Table 6. Occupational status of diarrheal outpatients and prevalence of *Salmonella* and *Shigella* isolate at Jimma University Specialized Hospital from January to March 2014.

Occupation	Frequency (%)	*Salmonella* positive No. (%)	*Shigella* positive No. (%)
Unemployed	96(54.5)	14(8.0)	0(0.0)
Business men	28(15.9)	3(1.7)	0(0.0)
Farmer	26(14.8)	2(1.1)	2(1.1)
Civil servant	26(14.8)	0(0.0)	0(0.0)
Total	176(100)	19(10.8)	2(1.1)

5.3. Antimicrobial susceptibility pattern of *Salmonella* and *Shigella* spp

All the 19 isolates of *Salmonella* spp were susceptible (100 %) to ciprofloxacin and norflaxacin followed by gentamycin (94.7%), chloramphenicol (94.7%), and amikacin (89.5%) (Table 7).However, the highest frequency of resistance to ampicillin (100%) was observed followed by tetracycline (47.4%) and nalidixic acid (26.3%). Regarding *Shigella* spp, the two isolates were susceptible (100%) to ciprofloxacin, norflaxacin and gentamycin whereas the highest resistance (100%) was observed in ampicillin and tetracycline.

Table 7.Antimicrobial susceptibility pattern of *Salmonella* and *Shigella* spp isolated from diarrheal Out-patients in Jimma University Specialized Hospital from January to March 2014.

		***Salmonella* spp.**			***Shigella* spp.**		
Antimicrobial agents	Disc potency (μgml^{-1})	Resistance (%)	Intermediate (%)	Sensitive (%)	Resistance (%)	Interme diate	Sensitive (%)
Amikacin	30	-	2(10.5)	17(89.5)	-	1	1
Ciprofloxacin	5	-	-	19(100)	-	-	2
Chloramphenicol	30	1(5.2)	-	18(94.7)	-	1	1
Gentamycin	10	1(5.2)	-	18(94.7)	-	-	2
Cotrimoxazole	25	1(5.2)	5(26.3)	13(68.4)	1	-	1
Norflaxacin	30	-	-	19(100)	-	-	2
Nalidixic acid	30	5(26.3)	4(21.05)	10(52.6)	1	-	1
Ampicillin	10	19(100)	-	-	2	-	-
Tetracycline	30	9(47.4)	3(15.7)	7(36.8)	2	-	-

The MDR profile of *Salmonella* spp indicated that, the highest resistance (42.1%) 0f the isolates towards two antibiotics followed by three (26.3%) and four antibiotics (21.0%) (Table 8.). In case of *Salmonella* spp, the maximum number of antibiotics resisted was four. However, the highest MDR (26.3%) was observed to TE/AMP. Over all, two antibiotic resistance patterns was dominated (42.1%) the multidrug resistance profile of *Salmonella* spp. The highest MDR profile of *Shigella* spp was also observed towards four antibiotics.

Table 8. MDR of *Salmonella* spp. and *Shigella* spp isolated from diarrheal Out- patients in Jimma University Specialized Hospital from January to March 2014

	Salmonella spp.			*Shigella* spp.	
No. of antimicrobial resistance	Antimicrobial resistance patterns	No. of isolates (%)	Total (%)	Antimicrobial resistance patterns	No. of isolates
Two	TET/AMP	5(26,3)	8(42.1)	-	
	SXT/AMP	1(5.2)		-	
	NAL/AMP	2(10.52)		-	
				-	
Three	TET/NAL/AMP	2(10.52)	5(26.3)	-	
	SXT/AMP/TET	2(10.52)		-	
	NAL/SXT/AMP	1(5.2)		-	
Four	NAL/AMP/TET/SXT	1(5.2)	4(21.0)	TET/AMP/NAL/SXT	1
	NAL/TET/AMP/C	1(5.2)		C/TET/AMP/AMK	1
	AMP/SXT/CN/NAL	1(5.2)			
	AMK/AMP/NAL/TET	1(5.2)			

TET-tetracycline, AMP-ampicillin, SXT-cotrimoxazole, NAL-nalidixic acid, C-chloramphenicol, CN-gentamycin, AMK-amikacin, CIP-ciprofloxacin, NOR-norflaxacin.

5.4. The growth potential of *Salmonella* and *Shigella* in selected food items.

The growth potential of *Salmonella species* was analyzed in gruel and firfir over a period of 24hr.During the first 6hr, nearly similar growth was observed in both food items. Then after, the growth rate was increased by 3 log cfu/g in gruel (3.6-6.6 log cfu/g) and relatively slow growth was observed in the case of firfir (3.8-4.2 log cfu/g) until 12hr. Finally, *Salmonella* count as high as 7.2 log cfu/g and 6.2 log cfu/g was observed in gruel and firfir, respectively at the end of 24hr storage (Fig. 2)

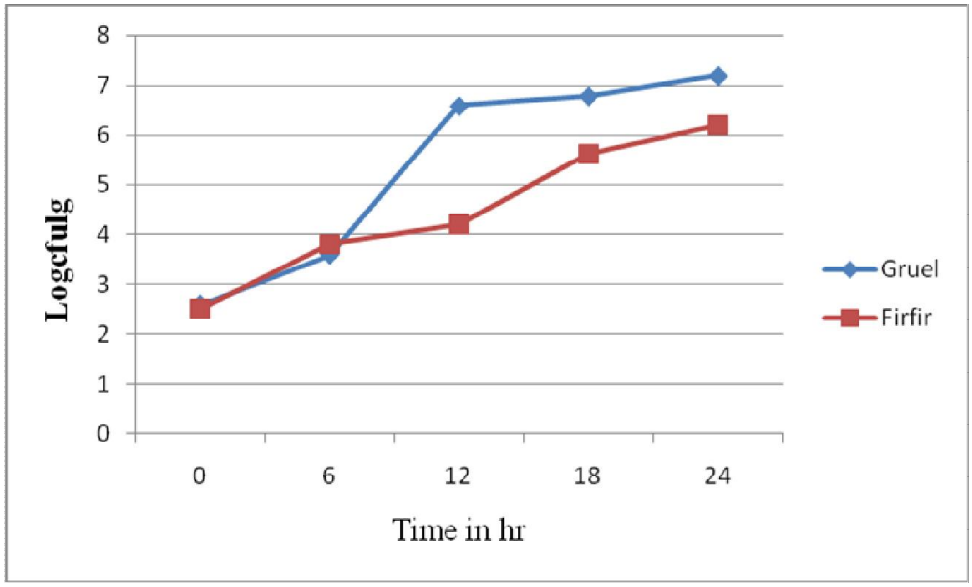

Figure 2-The growth potential of *Salmonella* species isolated from diarrheal out-patients, Jimma University Specialized hospital, in selected foods, 2014

The pH value of sampled food varied during the 24hr period storage. At the beginning (0hr), the pH value of gruel (6.32) was greater than the pH value of firfir (5.14.).Then after, the pH value of gruel was reduced from 5.66 to 5.00 between 6 to 12hr where as that of firfir increase from 5.14 to 5.22 .Finally it slightly rised up and reached 5.1 for gruel and 5.28 for firfir (Fig.3).

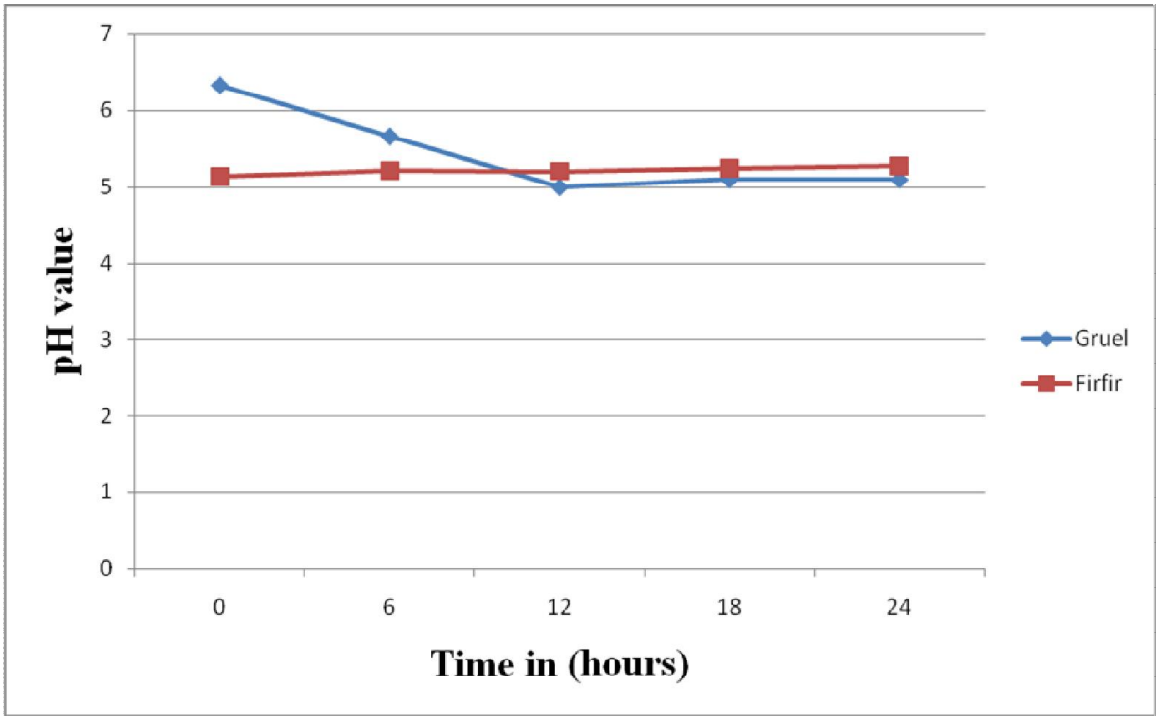

Figure 3. pH-values of gruel and firfir challenged with *Salmonella* species isolated from diarrheal out-patients in Jimma University Specialized Hospital, 2014.

Similarly, the growth potential of *Shigella* spp was assessed in two different food items (gruel and firfir).The growth rate was higher in the gruel (2.51-3.8 logcfu/g) than in the firfir (2.5-3.5logcfu/g) in the first 6hr (Fig. 4). The growth rate increased by 1.5logcfu/g(3.5-5.14logcfu/g) in firfir within 12hrs and 3log cfu/g (3.8-6.9 logcfu/g) in gruel within 18hrs.The maximum growth potential (7.5 log cfu/g) was observed in gruel and 7.3 log cfu/g was observed in firfir at 24hr.

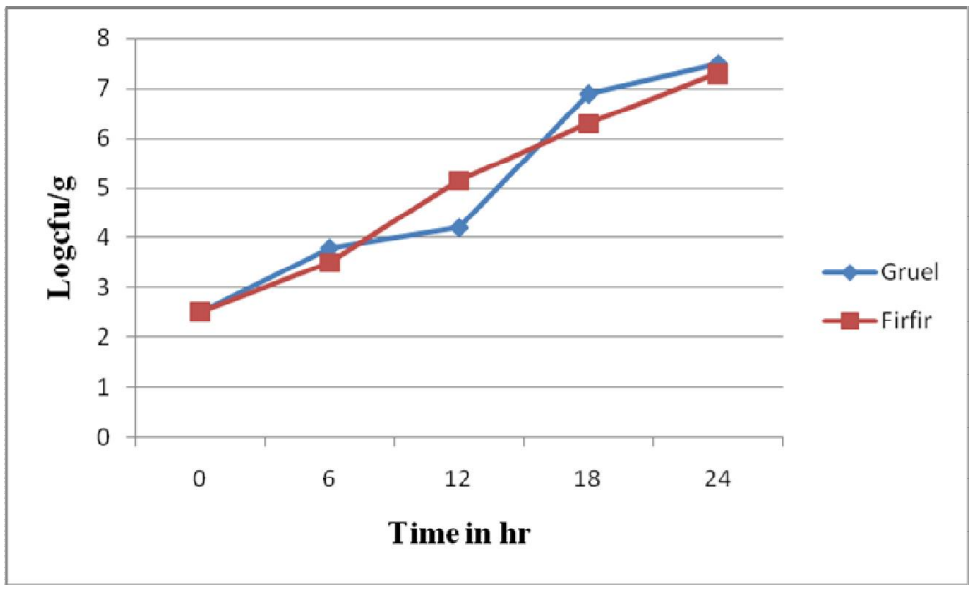

Figure 4.The growth potential of *Shigella* species isolated from diarrheal out-patients in Jimma University Specialized hospital.

At 0hr the pH of gruel (6.32) was greater than the pH of firfir (5.14). Then after a slight pH reduction were observed in gruel up to 24. The pH of firfir stays the same with only minor fluctuation(5.23 to 5.22) between 6 and 12hr, and slightly increase and reach to 5.28 at the end of 24hr (Figure 5).

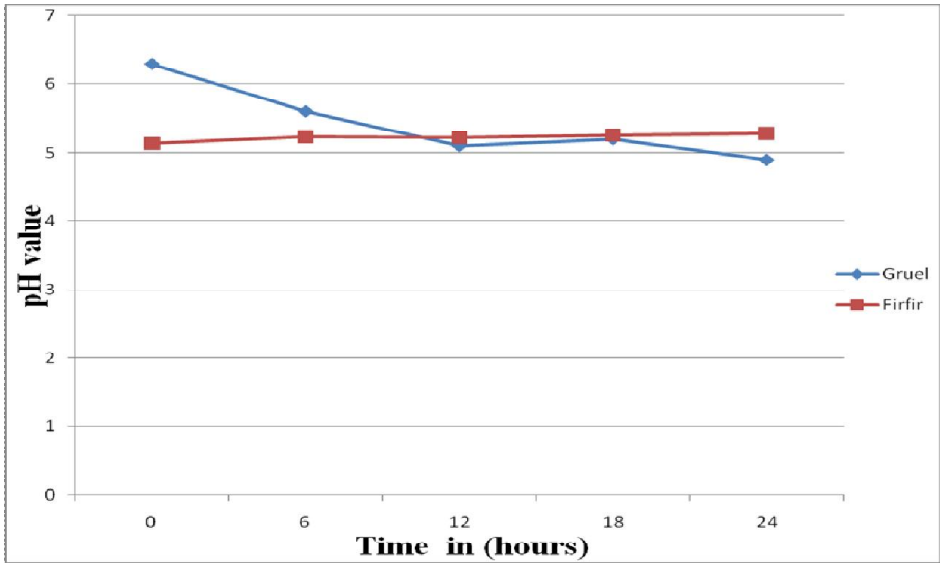

Figure 5-pH-values of gruel and firfir challenged with *Shigella* Spp isolated from diarrheal out-patients, Jimma University Specialized Hospital, 2014.

6. Discussion

Food and water borne illnesses comprise a broad spectrum of diseases and are responsible for substantial morbidity and mortality worldwide. It is a growing public health problem in developing as well as developed countries. Although these illnesses cause substantial morbidity in the developed countries, the main burden is borne by developing countries (Bhunia, 2008) .In the United States, food-borne illnesses affect up to 80 million people and cost an estimated $5 billion US on an annual basis and the consequence is devastating in developing countries (WHO, 2011).The major enteric infections contributing to this problem are *Salmonella* and *Shigella*.

The widespread occurrence and distribution of *Salmonella* and *Shigella* is indicated in Ethiopia (Awole *et al*., 2002; Mache, 2002; Andualem and Geyid, 2005; Reda *et al*., 2011; Addis *et al,* 2011; Mengistu *et al*., 2014). In recent years, the number of *Salmonella and Shigella* related out breaks in humans has increased considerably in the country (Mengistu *et al*., 2014). Much more is known now about the extent of food borne illness and how severe it can be, not just in terms of acute illness, but also in terms of long term (Mengistu *et al*., 2014). Accurate estimates of the burden of diarrheal diseases caused by *Salmonella* species and other foodborne pathogens are needed to effectively set public health goals and allocate resources to reduce disease burden (WHO, 2011).

Our finding indicated that, of the total 176 diarrheal Out-patients, 10.8% were positive for *Salmonella* and 1.1% were positive for *Shigella***.** The prevalence rate of *Salmonella* in this study is in line with the earlier studies reported as 10.7% (Andualem and Geyid, 2005), 11.5% (Reda *et al*., 2011), 13.6% (Addis *et al,* 2011) and 10.5% (Mengistu *et al*.,2014 but lower than a study reported as 15.4% (Mache, 2002) and higher than the 7.2% prevalence report by Awole *et al*. (2002).

In this study, the prevalence rate of *Shigella was* 1.1%. This rate was lower than that report by Ashenafi (1983) (9%) and Asrat *et al* (1999) (11.7%) from Tikur Anbessa, Ethio-Swedish children's hospital and a report by Reda *et al*.,(2011) (6.7%) from Harar, Ethiopia. The low isolation rate of *Shigella* in this study is comparable with the very

recent report (2.3%) made from among diarrheal children in Jimma health center, (Beyene and Tasew, 2014). The low prevalence of *Shigella* in this study could be due to increased awareness of the community about personal and environmental hygiene from the continuous interventions made by the Health Science students from Jimma University during their filed practice.

Several studies showed that, there is a difference in the distribution of *Salmonella* and *Shigella* infection among different age groups (Karim *et al*., 2001; Mengistu *et al*., 2014). In this study the highest isolation rate of *Salmonella* was observed in the age group between 20-24 (26.3%) and 5-9 (26.3%). This is inline with earlier reports made from Ethiopia (Mache, 2002; Mengistu *et al*., 2014).

Concerning *Shigella* isolates, it was observed only in the age group below five. This is in agreement with reports from different parts of the world including Ethiopia (David *et al*., 2001; Tiruneh, 2009).Therefore; shigellosis occurs worldwide but is most common among pediatric age group in under developed tropical countries including Ethiopia. Community based data on shigellosis are incomplete but most hospital suggested that the case-fatality rate is highest among children less than 5 year of age particularly if there is malnutrition. In epidemic Shigellosis, the rate is as high as 3.9% in children under age of 1 and 19.3% for infants less than 4 month of age.The case fatality declines with increase in age (David *et al*., 2001; WHO, 2007). Understanding the prevalence rate among different age groups is important to target intervention and preventive measures based on their age group.

Concerning educational background, this study showed that there was high isolation rate of *Salmonella* and *Shigella* among people with no education (illiterate), which is 36.8% and 100%, respectively. This result is comparable with earlier study made in (Aziz *et al*., 1990). Education is vital to create awareness in the community with regard to the mechanism of management of infectious diarrhea and control of other factors that leads to this disease. It also used to develop knowledge about hygienic condition and nourishments. Poor environmental sanitation, malnutrition, inadequate water supply,

poverty and limited education are the major factors implicated in the occurrence, spread and severity of diarrheal disease (Nath *et al.,* 2006).

Of the total isolates, 73.7% were observed among unemployed participants. This is in agreement with earlier report made by Jill and colleague. Income of the study participants is also one of the contributing factors for the prevalence of *Salmonella* and *Shigella,* because living standard of individuals can be influenced by their income. Developed countries have high standard of living which made them get safe water, hygienic environment, better food handling practice and sanitary disposal (Molbak, 2000).

Due to selective pressure created by the use of antimicrobials in food processing animals, the risk of antimicrobial resistance among food borne pathogens has increased (chui *et al*., 2002).Mobile elements such as plasmids and transposons facilitate the rapid spread of antibiotic resistant genes among bacteria (Sunde, 2005).In addition, high rates of antibiotic resistance bacteria may possibly result from inappropriate or uncontrolled use of antibiotics. Therefore, it is necessary to pay attention to hygienic food hundling practice as well as avoiding uncontrolled use of antibiotics (Van *et al*., 2007). An increase in the antimicrobial resistace in *Salmonella* and *Shigella* make the treatment of infection more challenging. Therefore, epidemiological information and monitory system are necessary to control *Salmonella* and *Shigella* infection in public health sectors.

In agreement with studies conducted by (Beyene and Tasew, 2014), *Shigella* isolates were susceptible to ciprofloxacin, gentamicin and norflaxacin. The highest resistance of *Shigella* spp (100%) in the current study towards ampicillin and tetracycline is in agreement with studies conducted by Roma *et al.* (2000) reported high rate of resistance of *Shigella* Spp to ampicillin (93%), erythromycin (90%), tetracycline (90%) and cotrimoxazole (56%). Asrat (1999) also reported high rate of resistance of *Shigellla* species to tetracycline (97.3%) and ampicillin (78.7%).

The high level of antibiotic susceptibility of *Salmonella* which was observed in the current study in ciprofloxacin and norflaxacin is in agreement with studies in Ethiopia (Mengistu *et al*., 2014; Beyene and Tasew, 2014).The resistance of *Salmonella* towards ampicillin (100%) and Tetracycline (47.4%) was in agreement with report made by

(Beyene and Tasew, 2014) in which case most of the *Salmonella* isolates were resistant to ampicillin. In the current study, multidrug resistance towards four drugs was observed in *Salmonella* and *Shigella*.

In the current challenge study, the growth rate of *Salmonella* was faster during the earlier fermentation (storage) time although it tended to decrease with drop in pH , this is in agreement with Kingamkono *et al*., (1996) who added several enteropathogens to cereal gruels prepared from sorghum and inoculated with a lactic acid starter culture .According to this report, in the gruels prepared without the lactic acid starter culture, all enteropathogens increased in number during incubation at 32^{o}C except for *Campylobacter* strains which decreased after 12 h.

The growth potential of *Shigella* isolated from diarrheal Out-patients was also examined in this study. The maximum count was obtained in gruel challenged with *Shigella* with in 24hr. *Shigella* species grow to the level of infective doses within 6 to 12hrs.The pathogen could initiate a successful infection at this cell number. The maximum growth observed in the current study was relatively lower as compared to studies reported by Muleta and Ashenafi (2001).The reason for this discrepancy is the relatively acidic nature of gruel at the end of 24hr. Even though the gruel is relatively acidic, *Shigella* manage to grow to the maximum of >7logcfu/g with in 24hr period. This is because the pathogen can manage to grow in low pH food items (Bagamboula *et al*., 2002). This result demonstrates the ability of *Shigella* to grow and survive in gruel. Since this food item is frequently utilized by babies, care should be taken when handling the food, extension of the food before use should also be avoided. The maximum growth of *Shigella species* in firfir was almost similar with a very minor increment in gruel. The growth of *Shigella species* in firfir was steady than in gruel. The pathogens reach to its infective dose within 6 to 12hrs. This is in agreement with studies reported by Muleta and Ashenafi (2001).

The challenge studies revealed that *Salmonella* species reached to the infective dose (5logcfu/g) within 12 and 18hr in gruel and firfir, respectively. The maximum count obtained was 7.2 log cfu/g in gruel and 6.2 log cfu/g in firfir. As compared to the previous study (Muleta and Ashenafi, 2001), the maximum count obtained in this study

was relatively smaller. The reason for this difference can be the acidic nature of the food and the nature of the ingredients from which the food was prepared. For the cause of typhoid an individual should have a minimum oral dose of 10^5 *S. typhimurium* where as at least 10^9 *S .typhimurium* cells are required to cause symptoms of toxic infection. It takes 12-24hr incubation after a person takes contaminated food containing sufficient number of *Salmonella* to manifest disease symptoms such as diarrhea, vomiting and fever (Toder, 2012).

The growth of challenged pathogens increases as pH rises to the neutral, where as their count decreases when the pH fall from neutral (Sahlin, 1999). This fluctuation in pH is due to change in the source of carbon and nitrogen. When small amount of the original carbon and energy substrate present some microbial cultures tend to generate enzyme to utilize new carbon and energy. In the current study, the fluctuation in the pH of gruel is relatively higher than firfir. The reduction in microbial growth could not be explained only on the bases of reduction in pH but intrinsic and extrinsic factors should also be considered.

7. Conclusion

Based on result of this study, the following conclusions were made:

- The overall prevalence of Salmonellosis and Shigellosis among out-patients in Jimma University Specialized Hospital was 10.8% and 1.1% respectively.
- The frequency of isolation of *Salmonella* and *Shigella* was relatively high in the age group between 20-24 and less than 10 years.
- In this study, more *Salmonella* and *Shigella* were isolated from the illiterate group
- The rate of isolation of *Salmonella* was greater than *Shigella*
- In the food samples, the pathogens were found to grow to infective dose within 24hr.Thus, food items assessed in this study support growth of pathogens and consumption of unhygienically prepared food could cause health risk.
- In the current study, isolates of *Salmonella* and *Shigella* showed resistance towards chloramphenicol, cotrimoxazole and nalidixic acid and multidrug resistance towards four drugs were also observed in both pathogens

8. Recommendations

From the findings of this study, the following recommendations were made:-

- Result of the present study will strengthen the knowledge in the field of epidemiology of *Salmonella* and *Shigella* to generate further trials which may help policy makers in planning interventions for the at risk population in the field of water sanitation, hygienic food handling practice, vaccine development and implementation.

- Health sectors, non-governmental organizations and other responsible bodies should work with the community in awareness development on food and water borne diseases and improving sanitary facilities in order to reduce burden posed due to the disease.

- Consumption of food items used in this study is at high risk of food poisoning due to *Salmonella* and *Shigella* if stayed for more than a day . Therefore, consumption before 6hr of preparation could significantly minimize health hazards associated with it.

- In this study, relatively high rate of prevalence of *Salmonella* and *Shigella* is observed in the age group between 20-24 and children less than ten years, however, it doesn't describe why? Therefore, further investigations have to be carried out on why these groups are more prevalent than others.

- In this study, identification of the isolate is a presumptive ,not show exact identity; therefore molecular approach should be used to overcome this problem

- The observed drug resistance in *Salmonella* and *Shigella* can be used as an input to the health institutes to give due attention to the problem and use drug susceptibility test for appropriate subscription.

Reference

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Appendices

Appendix I

Jimma University

College of Natural Sciences

Department of Biology (Applied Microbiology)

Questionnaire designed for determination of the prevalence of *Salmonella* and *Shigella* in out- patients in Jimma University specialized hospital:

Dear Respondents,

Your response to the questions has significant impact on the quality of data and result. Thus, you are kindly requested to respond genuinely.

Back grand Information of diarrheal out patients

1. Code: _____
2. Age:_____
3. Sex A. Male B. Female
4. Educational background of patient/patient parents
   A, Illiterate B, 1-4 C, 5-8 D, 9-12 E, Above 12
5. Occupation A, Unemployed B, Private Sector /business
   C, Farmer D, Civil Servant E, Others
6. Residence A, Urban B, rural
7. Religion A, Muslims B, Orthodox C, Protestant D, Catholic E, Others

Main Information

1. The type of water that you drink is:-
   A Tape Water B, River Water C, lake water D, Others
2. Do You have an experience of eating food stayed more than a day ?
   A Yes B, No
3. Hand washing practice
   2.1. Before preparing food A, Yes B, NO
   2.2. After toilet use A, yes B, No

4. If your answer for question no 3 is yes how often?

A, always B, sometimes C, rarely D, others (please specify)_____

5. How do you Wash your hand
   A. Using water Only
   B. Using water and soap
6. Use of antibiotic before diagnosis
   A. Yes B, No
7. History on previous antibiotic use
   A, Yes B, No
8. If your answer for question no 6 is yes
   What are the antibiotics used _____
9. Do you have any information about food and water born diseases? A, Yes B, NO

10. If your Answer for question No 8 is yes can you mention at least two_____

11. Have you ever get education about personal hygiene from concerned bodies?

A, Yes B, No

12. If your answer for question 10 is yes how often?

A, weakly B, monthly C, yearly D, others (specify)_____

Thank you for your Cooperation

Annex 2

Binary logistic regression: the risk of *Salmonella* by demographic characteristics

Variables in the Equation

		B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Age	-.057	.110	.266	1	.606	.945	.762	1.172
	Sex	-.152	.535	.081	1	.776	.859	.301	2.448
	Education	.518	.229	5.131	1	**.024**	1.679	1.072	2.629
	Occupation	-.644	.322	4.006	1	**.045**	.525	.280	.987
	Residence	1.895	.733	6.682	1	**.010**	6.653	1.581	27.991
	Constant	-4.967	2.122	5.479	1	.019	.007		

a. Variable(s) entered on step 1: age, sex, Education, occupation, residence.

Annex 3

Binary logistic regression: the risk of *Shigella* by demographic characteristics

Variables in the Equation

		B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	Age	-14.414	852.981	.000	1	.987	.000	.000	.
	Sex	-.693	1.871	.137	1	.711	.500	.013	19.562
	Education	-4.950	3052.846	.000	1	.999	.007	.000	.
	Occupation	8.672	1191.608	.000	1	.994	5837.725	.000	.
	Residence	5.845	11399.182	.000	1	1.000	345.349	.000	.
	Constant	-17.648	25364.073	.000	1	.999	.000		

a. Variable(s) entered on step 1: age, sex, Education, occupation, residence.

Annex 4

Biochemical test procedures

Procedure for Simon citrate agar

A small amount of growth is harvested with a sterile (1uL) loop.

Lightly inoculate the surface of the agar slant.

Do not use a heavy inoculum.

Tubes are incubated under aerobic conditions at 36°C (+/- 1°C) with caps loosened.

Tubes should be examined and results recorded at 24 hours, 48 hours, and 5-7 days.

Procedure for lysine iron agar

A small amount of growth is harvested with a sterile (1uL) loop.

Lightly inoculate the surface of the agar slant

Make a single stab in the butt of the tube

Tubes are inoculated under aerobic conditions at 36°C (+/- 1°C) with caps loosened.

Tubes should be examined and results recorded at 24 hours, 48 hours, and 5-7 days.

Procedure for motility medium

A small amount of growth is harvested with a sterile (1uL) loop.

Make a single stab into the tube of MIO agar. The stab should be made straight into the agar and stop approximately 1 cm from the bottom of the tube.

Do not make multiple stabs into the agar and do not twist the needle into the media.

Tubes are inoculated under aerobic conditions at 36°C (+/- 1°C) with caps loosened.

Tubes should be examined and results recorded following overnight (18-24) incubation

Procedure for triple sugar iron agar

A small amount of growth is harvested with a sterile (1uL) loop.

Lightly inoculate the surface of the agar slant.

Make a single stab in the butt of the tube

Tubes are inoculated under aerobic conditions at 36°C (+/- 1°C) with caps loosened.

Tubes should be examined and results recorded at 24 hours, 48 hours, and 5-7 days.

Procedure for urea agar

A small amount of growth is harvested with a sterile (1uL) loop.

Lightly inoculate the surface of the agar slant.

Tubes are inoculated under aerobic conditions at 36°C (+/- 1°C) with caps loosened.

Tubes should be examined and results recorded at 24 hours, 48 hours, and 5-7 days