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Prevalence, Antibiogram and Growth Potential of *Salmonella* and *Shigella* in Ethiopia: Implications for Public Health: A Review

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ABSTRACT

This article has reviewed the available researches that obtained from peer-reviewed literatures on Salmonellosis and Shigellosis in different parts of Ethiopia. The prevalence of *Salmonella* and *Shigella*, the causative agent for Salmonellosis and Shigellosis, respectively, their antibiogram and growth potential were the main objectives of this review. 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. In most studies of Antibiograms tests, *Salmonella* and *Shigella* spp. showed high resistance to the commonly used antibiotics, which indicate serious problems in antimicrobial therapy globally, especially in developing countries. In challenge studies, *Salmonella* and *Shigella* spp. reached the infective dose within 4-24 h of inoculation, respectively in various food samples. In this review, it is noted that these potentially pathogens are still public health problems. Therefore, there needs health education, frequent monitory and evaluation system of microbiological and antimicrobial surveillance so as to plan intervention strategies for at risk population in the area of water sanitation and hygienic food handling practice to minimize the burden posed by the diseases Salmonellosis and Shigellosis.

Key words: Antibiograms, diarrheal diseases, Ethiopia, food borne diseases, growth potential, salmonellosis, shigellosis

INTRODUCTION

As far back as the documentation of human history goes, consumption of food unsafe for health and its consequences have been one of man's major health problems. They still remain to be a major public health concern globally. Food-borne diseases are known to be responsible for a large proportion of adult illnesses and deaths, more importantly, as sources of acute diarrheal diseases, they are known to claim the lives of overwhelming numbers of children every day (Zeru and Kumie, 2007).

Globally, food borne diseases remain a major public health problem. The problem is severe in developing countries due to difficulties in securing optimal hygienic food handling practices. Evidently, in developing countries, up to an estimated 70% of cases of diarrheal disease 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 and water indicating the importance of faecal-oral human-to-human transmission (Andargie *et al*., 2008).

Furthermore, acute infective diarrhea and gastroenteritis are major causes of ill health and premature deaths in developing world due, in large part, to the lack of safe drinking water, sanitation and hygiene, as well as poorer overall health and nutritional status. According to the latest available figures, an estimated 2.5 billion people lack improved sanitation facilities and nearly one billion people do not have access to safe drinking water. These unsanitary environments allow diarrhea causing pathogens to spread more easily (UNICEF/WHO, 2009).

Ranging from mild annoyances during vacations to devastating dehydrating illnesses that can kill within hours, acute gastrointestinal illnesses rank second only to acute upper respiratory illnesses as the most common diseases worldwide. In children less than 5 years old, attack rates range from 2-3 illnesses per child per year in developed countries to as high as 10-18 illnesses per child per year in developing countries (Kasper *et al*., 2005).

Now-a-days, diarrhea is one of the most common childhood illnesses, in both developing and developed countries. Estimation by World Health Organization (WHO) indicates that the world population suffered from 4.6 billion incidences of diarrhea causing 2.2 million deaths in the year 2004. While the disease is rarely a cause of death in developed countries, it is estimated that approximately 1.6 million children die each year from diarrhea in the developing world. Africa and South Asia are home to more than 80% of child deaths due to diarrhea. In addition, by contributing to malnutrition and thereby reducing resistance to other infectious agents, gastrointestinal illnesses may be indirect factors in a far greater burden of disease (WHO., 2004a; UNICEF/WHO, 2009).

All over the world, severe acute bacterial gastroenteritis is caused mainly by *Shigella*, whereas, *Salmonella*, *E. coli* (mainly enteropathogenic *E. coli* or EPEC, but also enterohemorrhagic *E. coli* or EHEC, enteroinvasive *E. coli* or EIEC and other types), *Campylobacter* and *Vibrio* spp. have been shown to play a role in the epidemiology of diarrhea, particularly in certain areas of the globe (Diniz-Santos *et al*., 2005).

In past many decades, bacillary dysentery was first differentiated from amoebic dysentery in 1887 and an etiologic agent, *Bacillus dysenteriae*, was isolated and described by Shiga in 1898. On the other hand, *Shigella flexneri* was originally described by Flexner in 1900. *Shigella sonnei* was first isolated in 1904, but it was not until 1915 that its pathogenic potential was recognized by Sonne (Gillespie and Hawkey, 2006). The consequent pains taking process of epidemiological, physiological and serological characterization of related dysentery bacilli ended with the recommendations of the 1950. Moreover, Congress of the International Association of Microbiologists *Shigella* Commission that *Shigella* be adopted as the generic name and that species subgroups be designated A (*Shigella dysenteriae*), B (*S. flexneri*), C (*S. boydii*) and D (*S. sonnei*) (Hale, 1991).

Bacteria of the genus *Salmonella* are widespread and important causes of food borne infections in man and are the most frequent etiologic bacterial agents of food borne disease outbreaks. According to the latest nomenclature, which reflects recent advances in taxonomy (Grimont and Weill, 2007), the genus *Salmonella* consists of only two major species: *S. enterica* and *S. bongori*. *Serotypes typhi* (*S. typhi*), *S. paratyphi* are highly adapted to man. *S. typhi* and *S. paratyphi* have humans as their main reservoir and enteric fever (typhoid and paratyphoid fever) as their most important clinical manifestation. Enteric fever continues to be an important cause of morbidity and mortality in developing countries. Although, primarily intestinal bacteria, salmonellae are wide spread in the environment and commonly found in farm effluents, human sewage and in any material subject to faecal contamination. Typhoid fever is a life threatening illness caused by the bacterium *Salmonella typhi* and was observed by Eberth (1880) in the mesenteric nodes and spleen of fatal cases of typhoid fever.

Food hygiene and food safety practices in Ethiopia: In these modern days, in which food is usually not consumed immediately following and/or at the site of production, the risks of food-borne diseases are becoming increasingly important; the concern is obviously much more in areas where food storage and preparation safety measures are far below the optimum. Food borne diseases are common in developing countries including Ethiopia due to the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment and lack of education for food handlers (WHO., 2004b). Likewise, National Hygiene and Sanitation Strategy program. MoH. (2005) reported that in Ethiopia more than 250,000 children die every year from sanitation and hygiene related diseases and about 60% of the disease burden was related to poor hygiene and sanitation in Ethiopia. Unsafe sources, contaminated raw food items, improper food storage, poor personal hygiene during food preparation, inadequate cooling and reheating of food items and a prolonged time lapse between preparing and consuming food items were mentioned as contributing factors for outbreak of food borne diseases (Du Toit and Venter, 2005).

Evidently, several studies conducted in different parts of the country showed that the poor sanitary conditions of catering establishments and presence of pathogenic organisms among others, such as *Salmonella, Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter* and *E. coli* (Molla *et al*., 2003; Abera *et al*., 2006; Zeru and Kumie, 2007; Woldemariam *et al*., 2009; Haileselassie *et al*., 2013).

Among the foods intended for humans, those of animal origin tend to be most hazardous unless the principles of food hygiene are employed. Animal products such as meats, fish and their products are generally regarded as high-risk commodity in respect of pathogen contents, natural toxins and other possible contaminants and adulterants (Yousuf *et al*., 2008). Bacterial contamination of meat products is an unavoidable consequence of meat processing (Jones *et al*., 2008). Several studies noted despite the possibility that data regarding meat borne diseases in Ethiopia are very scarce; a few studies conducted in different parts of the country have shown the public health importance of several bacterial pathogens associated with foods of animal origins (Molla *et al*., 2003; Ejeta *et al*., 2004; Hiko *et al*., 2009; Woldemariam *et al*., 2009; Kumar *et al*., 2010).

Salmonellosis: As report indicated, an American veterinarian Daniel Elmer Salmon, who first isolated *Salmonella enterica* serotype Choleraesuis from pigs in 1885 (Rabsch *et al*., 2003). According to research investigations, human infections of *Salmonella* are divided into typhoid fever, caused by *Salmonella typhi* and *Salmonella paratyphi* and also other clinical syndromes, including diarrheal disease, caused by a large number of Non-Typhoidal *Salmonella* (NTS) Serovars. Microbiologically, *Salmonella* species are Gram-negative, flagellated, non-sporeforming, facultative anaerobic rods characterized by O, H and VI antigens belongs to the family Enterobacteriaceae. As reported by Foley and Lynne (2008) there are over 2,500 identified serotypes of *Salmonella*. *Salmonella typhi* is one of the member and its diagnostic identification can be attained by growth on MacConkey and EMB agars and the bacteria is strictly non-lactose fermenting. Furthermore, Flowers (1988) and D'Aoust (1989) stated that Salmonellae form gas, while growing in media containing glucose. They are mesophilic, with optimum growth temperature between 35 and 37°C,

Table 1: Extra-intestinal infectious complications of typhoid fever caused by *Salmonella enterica* serotype *Typhi* (Su *et al*., 2004)

Organ system involved	Prevalence (%)	Risk factors	Complications
Central nervous system	3-35	Residence in endemic region, malignancy, endocarditis, congenital heart disease, paranasal sinus infections, pulmonary infections, meningitis, trauma, surgery and osteomyelitis of the skull	Encephalopathy, cerebral oedema, subdural empyema, cerebral abscess, meningitis, ventriculitis, transient Parkinsonism, motor neuron disorders, ataxia, seizures, Guillain-Barre syndrome and psychosis
Cardiovascular system	1-5	Cardiac abnormalities, e.g., existing valvular abnormalities, rheumatic heart disease, or congenital heart defects	Endocarditis, myocarditis, pericarditis, arteritis and congestive heart failure
Pulmonary system	1-6	Residence in endemic region, past pulmonary infection, sickle cell anaemia, alcohol abuse, diabetes and HIV infection	Pneumonia, empyema and bronchopleural fistula
Bone and joint	<1%	Sickle cell anaemia, diabetes, systemic lupus erythematosus, lymphoma, liver disease, previous surgery or trauma, those at extremes of age, and steroid use	Osteomyelitis, septic arthritis
Hepatobiliary system	1-26	Residence in endemic region, pyogenic infections, intravenous drug use, splenic trauma, HIV and hemoglobin apathy	Cholecystitis, hepatitis, hepatic abscesses, splenic abscess, peritonitis and paralytic ileus
Genitourinary system	<1	Urinary tract, pelvic pathology and systemic abnormalities	Urinary tract infection, renal abscess, pelvic infections, testicular abscess, prostatitis and epididymitis
Soft tissue infections	Least cases reported	Diabetes	Psoas abscess, gluteal abscess and cutaneous vasculitis
Hematological	Least cases reported		Haemophagocytosis syndrome

but generally have a growth range of 5-46°C. They are killed by pasteurization temperature and time, sensitive to low pH (4.5 or below) and do not multiply at water activity (aw) of 0.94, especially in combination with a pH of 5.5 and below. The cells survive in frozen and dried states for a long time.

Regarding toxins production in *Salmonella*, following ingestion of *Salmonella* cells, the pathogens invade mucosa of the small intestine, proliferate in the epithelial cells and produce a toxin, resulting in an inflammatory reaction and fluid accumulation in the intestine. The ability of the pathogens to invade and damage the cells is attributed to the production of a thermostable cytotoxic factor. Once inside the epithelial cells, the pathogens multiply and produce a thermolabile enterotoxin that is directly related to the secretion of fluid and electrolytes. Production of the enterotoxin is directly related to the growth rate of the pathogens (Hornick *et al*., 1970; Pearson and Guerrant, 2000).

Extra-intestinal infectious complications of typhoid fever caused by *Salmonella enterica* serotype *Typhi* are presented in Table 1.

Salmonellosis in Ethiopia: *Salmonella* is the most frequently reported cause of food borne illness (Birhaneselassie and Williams, 2013). Food borne salmonellosis often follows consumption of contaminated animal products, which usually results from infected animals used in food production or from contamination of the carcasses or edible organs (Alemayehu *et al*., 2003). *Salmonella* infection in meat animals arises from intensive rearing practices and the use of contaminated feeds (Ejeta *et al*., 2004). Cross-contamination of carcasses with *Salmonella* can also occur during slaughtering operations (Baird-Parker, 1990). Stress associated with transport of animals to abattoir augments shedding of *Salmonella* by carrier animals and this may contribute to the spread of the organism to other animals in the slaughter plant (Isaacson *et al*., 1999).

According to several reports, *Salmonella* infection most commonly occurs in countries with poor standards of hygiene in food preparation and handling and where sanitary disposal of sewage is lacking. It mainly occurs in the tropics and sub tropics in Africa, India, Pakistan, South East Asia and South America (WHO., 1989; Lanata *et al*., 1989; Al-Lahham *et al*., 1990; Muleta and Ashenafi, 2001; WHO., 2003; Senthilkumar and Prabakaran, 2005).

Furthermore, studies showed that 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. Studies indicated various percentages of *Salmonella* isolates in towns of Ethiopia. Moreover, high percentages of *S. typhi* isolates have been found to be resistant for antimicrobial agents (Yismaw *et al*., 2007; Andargie *et al*., 2008; Abera *et al*., 2010). 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 (Woteki *et al*., 2001).

Nevertheless *Salmonella* populations in different geographical areas or different hosts and environmental niche may undergo different evolutionary change, due to centralization of food production and distribution and population movement, *Salmonella* strains found in different countries of the world are believed to be clonally related (Winokur *et al*., 2001). *Salmonella* isolates in Ethiopia may have similar phenotypic and genotypic characteristics with isolates elsewhere in the world and non-typhoidal *Salmonella enterica* infection in children in Ethiopia is a major health problem and is caused by similar serovars to these reported from elsewhere in Africa: *S. typhimurium* and *S. enteritidis* (Getenet, 2008).

According to Muleta and Ashenafi (2001) in Ethiopia, minced beef is usually used for the preparation of a popular traditional Ethiopian dish known as locally “Kitfo” and most of the time it is consumed raw or medium cooked. The habit of raw meat consumption and the presence of *Salmonella* in minced beef indicate, in addition to the poor hygienic standards in food handling in the country, the presence of great public health hazards of *Salmonella.*

Epidemiology: Typhoid cases are stable with low numbers in developed countries, but nontyphoidal Salmonellosis has increased globally. Evidently, typhoid fever usually causes mortality in 5-30% of typhoid-infected individual in the developing world. Recently, WHO estimates 16-17 million cases occur annually, resulting in about 600,000 deaths. The mortality rates differ from region to region, but can be as high as 5-7% 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 2-4 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 1-10% of cases are reported (Hu and Kopecko, 2003a, b).

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

Table 2: Species and serogroups of *Shigella* (Thomas and Gerald, 2000)

Species	Serogroups	Serotypes	Locality
Shigella dysenteriae	A	12	Most common, with outbreaks
Shigella flexneri	B	6	Developing countries
Shigella boydii	C	18	
Shigella sonnei	D	1	Developed countries*

*Al-Lahham *et al*. (1990), WHO. (2005), Todd *et al*. (2007), Emch *et al*. (2008) and Desta (2010)

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 in1938 (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.

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. They are generally catalase positive and oxidase and lactose negative. They ferment sugars, usually without forming gas. The strains grow between 7 and 46°C, with an optimum at 37°C and pH 4.5. The genus is divided into four serogroups with multiple serotypes: *S. dysenteriae*, 12 serotypes; *S. flexneri*, 6 serotypes; *S. boydii*, 18 serotypes and *S. sonnei*, 1 serotype (Thomas and Gerald, 2000) (Table 2). 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).

Epidemiology: Annually, there are 165 million cases of shigellosis resulting in 1.1 million deaths in the developing world (Emch *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 elsewhere (1.3%) and Ethiopia (3.1%) (Andargie *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%) (Winokur *et al*., 2001; Khurana *et al*., 2008). Other study in Ethiopia showed that, *Shigella dysenteriae* and *Shigella flexneri* have been identified as the species that account for about 80% of *Shigella* isolates.

Shigellosis in Ethiopia: As a global, Shigellosis is human health problem. It is still an important public health problem, especially in developing countries like Ethiopia, where there is substandard hygiene and unsafe water supplies (Gebre-Yohannes and Drasar, 1987; Niyogi, 2005). Moreover, *Shigella* still accounts for a significant proportion of bacillary dysentery in many tropical and subtropical countries (MoezArdalan *et al*., 2003; Zafar *et al*., 2005). It is the most prevalent etiologic agent in childhood diarrhea in most countries (Geo *et al*., 1998; Khan-Mohammad *et al*., 2005). In Ethiopia, *Shigella dysenteriae* and *Shigella flexineri* have been identified as the species that account for about 80% of *Shigella* isolates (Gebre-Yohannes and Drasar, 1987).

Evidently, Shigellosis is a highly infectious disease of world significance. Its prevalence is highest in tropical and subtropical parts of the world where living standards are very low and access to safe and adequate drinking water and proper excreta disposal systems are often limited (Geyid, 2004). Diarrheal diseases are the major causes of morbidity and mortality in developing world. Shigellosis is endemic throughout the world where it is held responsible for some 165 million cases of severe dysentery. The devastating majority of these cases occur in the developing countries (Gebre-Yohannes and Drasar, 1987). Unlike other secretory diarrheas, shigellosis is the result of invasion of the distal small bowel and/or colon by *Shigella* species (Bennish *et al*., 1990). Infections caused by *Shigella* species are associated with symptoms which range from abdominal pain, cramps, fever, vomiting to bloody mucoid diarrhea (Asghar *et al*., 2002).

Furthermore, Shigellosis and the emergence of antimicrobial resistant *Shigella* species is a major health problem in Ethiopia (Geyid, 2004). A few studies conducted previously in the country indicated high rate of resistance to commonly used antimicrobial agents, such as ampicillin, tetracycline, cotrimoxazole, chloramphenicol among the isolates (Gebre-Yohannes and Limenih, 1980; Senait *et al*., 1999; Aseffa *et al*., 1995; Belay *et al*., 2000). Since the prevalence and pattern of resistance of *Shigella* species in the country varies from one area to another, updated information on their resistance patterns is very important for the proper selection and use of antimicrobial agents in a setting.

Shigellosis is currently an important health problem around the world, occurring predominantly in children younger than five years old, mainly in developing countries (De Paula *et al*., 2010).

In Ethiopia, as in other developing countries, Shigellosis is a common cause of morbidity and mortality, particularly in children (Huruy *et al*., 2008). Its prevalence is high in tropical and sub-tropical regions of the world, where living standards are very low and access to safe and adequate drinking water and proper waste disposal systems are often very limited, even absent (Tiruneh, 2009).

Table 3: Prevalence of *Salmonella* and *Shigella* and Appearance of diarrhea in patients in Harar, eastern Ethiopia, between January to February, 2007 (Reda *et al*., 2011)

	Organisms isolated					
	Salmonella		*Shigella*		Total	
Appearance of diarrhea	n	%	n	%	n	%
Bloody	7	25.0	1	59.0	8	17.8
Mucoid	12	42.8	9	52.9	21	46.8
Watery	0	0.0	1	5.9	1	2.2
Mucoid and bloody	9	32.1	3	17.6	12	42.8
Other	0	0.0	3	17.6	3	6.7
Total	28	100.0	17	100.0	45	100.0

Table 4: Frequency of *Shigella* and *Salmonella* species isolated from diarrheic patients attending Gondar town health institutions, Northwest Ethiopia, February to May, 2014 (Tesfaye *et al*., 2014)

Species	Species groups	No. of isolate	Frequency (%)
Shigella spp.	*Shigella flexneri*	11	64.70
	Shigella dysenteriae	3	17.65
	Shigella boydii	2	11.77
	Shigella sonnei	1	5.88
Total		17	80.95
Salmonella spp.		4	19.05
Total isolates		21	100.00

Generally, Shigellosis continues to be an important public health problem since communication in the world has become more frequent. In addition, this disease is difficult to be prevented because only a small number of bacteria are required to cause infection and it has exhibited steady trends towards multiple drug resistance.

Prevalence of *Salmonella* and *Shigella* in Ethiopia: Recently, many studies have demonstrated the prevalence of *Salmonella* and *Shigella* in different parts of Ethiopia. For instance, study conducted in Gonder town showed that *Shigella* species were isolated from stool samples of four food-handlers (3.1%) out of 127 food handlers (Andargie *et al*., 2008). In the study conducted by Reda *et al*. (2011) 28 (11.5%) *Salmonella* and 17 (6.7%) *Shigella* were isolated (Table 3). Besides, the prevalence rate of *Shigella* were also showed in different studies conducted in Ethiopia, a report (9%) by Ashenafi (1998) 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% were observed (Beyene and Tasew, 2014).

Moreover, in a cross sectional study conducted in raw milk samples from dairy farms and individual farmers in Kersa District, Jimma Zone, Ethiopia, indicated that the prevalence of *Salmonella* spp. in raw milk was 20% from a total of 100 samples of raw cow milk (Tadesse and Dabassa, 2012). In the recent study conducted on prevalence and antimicrobial susceptibility patterns of *Shigella* and *Salmonella* species among patients with diarrhea attending Gondar town health institutions, Northwest Ethiopia by Gunasekaran *et al*. (2014) indicated that of the total of 372 stool cultures, 17(4.57%) *Shigella* spp. and 4(1.08%) *Salmonella* spp. were isolated. According to their report, most commonly isolated strains of *Shigella* were *S. flexneri* 11 (64.7%) followed by *S. dysenteriae* 3 (17.65%), *S. boydii* 2 (11.77%) and *S. sonnei* 1 (5.88%) (Table 4).

On the other hand, in recent investigation conducted on prevalence of *Shigella* and *Salmonella* species among apparently health food handlers of Addis Ababa University student's cafeteria, Addis Ababa, Ethiopia by showed that out of 172 food-handlers screened, stool cultures revealed only six

(3.5%) *Salmonella* isolates (Aklilu *et al*., 2015). In their report they noted that these bacterial isolates were identified from food handlers who did not have regular medical checkup. No *Shigella* species was isolated from any of the stool samples obtained from Food handlers.

Furthermore, in the survey by Mengistu *et al*. (2014) on prevalence and antimicrobial susceptibility patterns of *Salmonella* serovars and *Shigella* species in Butajira, central Ethiopia, showed that 40 (10.5%) *Salmonella* and 17 (4.5%) *Shigella* strains were isolated from 382 patients. The *Salmonella* strains isolated were 6 (15%) group A (Somatic antigen O, O:2), 5 (12.5%) each of group B (O:4), D1 (O:9) andD2 (O:9,46) and 3 (7.5%) group C (O:7/O8) isolates while 16 (40%) could not be typed with the available antisera. Among 17 *Shigella* species; *Shigella sonnei* founded as 6 (35.3%) followed by *Shigella flexneri* 5 (29.5%), *Shigella dysenteriae* 3 (17.6%) and *Shigella boydii* 3 (17.6%).

Another study conducted in Jimma health center, Jimma, Ethiopia by Beyene and Tasew (2014), indicated that from a total stool specimens of 260 diarrheal children collected and examined for the presence of *Salmonella* and *Shigella* species, a total of 22 (8.5%) samples were positive for *Shigella* species, 6 (2.3%) and *Salmonella* species, 16 (6.2%), respectively.

In the study conducted in Gedo Hospital, West Shoa, Oromia state, Ethiopia by Gunasekaran *et al*. (2014), out of 200 samples, 40 diarrhea genic bacteria were isolated and identified as *Shigella* (n = 22) and *Salmonella* strains (n = 18).

A cross-sectional study at selected public health facilities in Addis Ababa, Ethiopia on a total of 253 children under 5 with acute diarrhea by Mamuye *et al*. (2015) indicated that among a total of 190 entropathogens isolated 21 (9.1%) was *Shigella* spp. followed by 10(3.95%) *Salmonella* spp.

In addition, study conducted elsewhere reviled that from a total of 158 fecal samples collected in under-five children with diarrhea, 11 (7.0%) *Shigella* species and 4 (2.5%) *Salmonella* species were isolated, respectively (Mulatu *et al*., 2014). Moreover, they also noted that the Sero-grouping data indicating that all *Shigella* isolates were found to be *S. flexneri* whereas, *Salmonella* species were identified as serogroup B (3, 1.9%) and serogroup A (1, 0.6%).

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 *et al*., 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). Moreover, 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 Multi Drug Resistance (MDR) in *Salmonella enterica* (Crump *et al*., 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 food borne 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. 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 *et al*., 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% or more of isolates are resistant to nalidixic acid (Bennish *et al*., 1991). Resistance to nalidixic acid also been reported in England and the United States. Antimicrobial resistance is an increasing problem in the United States. Report between 1999 and 2002 indicated that allisolated *Shigella* were susceptible to ceftriaxone and ciprofloxacin whereas 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; Aklilu *et al*., 2015). 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.

In addition in the study elsewhere by Tadesse and Dabassa (2012), *Salmonella* isolates were showed highly resistance to Nalidixic acid (80%) followed by tetracycline and kanamycin (35% each) and amikacin (30%), gentamycin, Chloramphenicol and streptomycin (25% each) and ciprofloxacin (5%); however, most susceptible to ciprofloxacin (95%).

Moreover, in the recent study conducted on prevalence and antimicrobial susceptibility patterns of *Shigella* and *Salmonella* Species among patients with diarrhea attending Gondar town health institutions, Northwest Ethiopia by Tesfaye *et al*. (2014) reviled that strains of *Shigella* species were resistant to ampicillin (94.1%), tetracycline (88.2%), amoxicillin (88.2%), but susceptible to norfloxacin (100%) and ciprofloxacin (100%). *Salmonella* isolate were also resistant to ampicillin (75%), amoxicillin (100%) and tetracycline (75%), but highly susceptible to norfloxacin (100%), chloramphenicol (100%) and ciprofloxacin (100%). The emergence of antibiotic resistance among *Shigella* and *Salmonella* spp. isolates are serious problems in antimicrobial therapy globally, especially in developing countries.

In the study by Aklilu *et al*. (2015) on anti-microbial susceptibility patterns of *Salmonellae* species from food handlers in Addis Ababa University student's cafeteria, all isolates of *Salmonella* were sensitive to ciprofloxacin (100%), amikacin (100%) and gentamycin (100%) but resistant to ampicillin (100), clindamycin (100%) and erythromycin (100%).

Besides in study by Mengistu *et al*. (2014) high frequency of resistance for both *Shigella* and *Salmonella* isolates was observed to tetracycline (82.4, 52.5%), co-trimoxazole (76.5, 37.5%) and ampicillin (47.1, 60%), respectively. All isolates were sensitive to ceftriaxone except 6 intermediate level *Salmonella* isolates. Fifty three percent of *Shigella* isolates were Multi-Drug Resistant (MDR) (≥3 drugs) as compared to 27.5% of *Salmonella* isolates.

In the study by Beyene and Tasew (2014) in Ethiopia, indicated that *Shigella* species showed hundred percent resistances to ampicillin, amoxicillin and cotrimoxazole. All *Salmonella* isolates (16) were resistant against amoxicillin. All *Shigella* (6) and *Salmonella* species were susceptible to ceftriaxone, ciprofloxacin and gentamycin.

In the study by Mulatu *et al*. (2014), all the four *Salmonella* species and 11 *Shigella* species were subjected to antimicrobial susceptibility tests. Accordingly, *Shigella flexneri* showed high resistance against Amoxicillin (100%), Erythromycin (90.9%) and Ampicillin (63.6%). However, low resistance rate was observed against gentamycin (27.3%) and Chloramphenicol (9.1%) and there was no resistance rate observed against Ciprofloxacin, Nalidixic acid and Cotrimoxazole.

In research investigation by Gunasekaran *et al*. (2014) on antibiotic Susceptibility pattern of *Salmonella* and *Shigella* isolates among diarrheal patients in Gedo Hospital, West Shoa Zone, Oromia State, Ethiopia, among all antibiotics tested, of a 22 *Shigella*, 21 (95.5%) *Shigella* strain showed the resistance, 1 (4.5%) intermediate to amoxicillin (SMX) and 17 (77.3%) *Shigella* strain showed resistance, 5 (22.7%) intermediate against tetracycline (TEC); Gentamycin (GEM) showed activity against *Shigella* strain compared with other antibiotics tested, 16 (72.7%) were susceptible and 6 (27.3%) were intermediate. The ciprofloxacin (CIF) 11 (50%) and co-trimoxazole (COT) 10 (45.5%) showed more or less equal susceptibility pattern, 2(9.1%) were resistance, 9 (40.9%) were intermediate for ciprofloxacin (CIF) and 3 (13.6%) were resistance, 9 (40.9%) were intermediate for co-trimoxazole (COT). Chloramphenicol (CHP) showed 8 (36.4%) resistance, 9 (40.9%) intermediate and 5 (22.7%) were susceptible against *Shigella*.

In the recent report, Mamuye *et al*. (2015), the overall resistance rates of isolated *Shigella* and *Salmonella* spp were high for ampicillin (95.7, 80.0%) and augmentin (91.4, 80%), respectively. However, high sensitivity was observed among both isolates for ciprofloxacin (91.3, 100%) and ceftraxion (91.4, 100%). More than 87% of *Shigella* species were multiple resistances (resistance for two or more antibiotics), whereas, 70.0% for *Salmonella* species.

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.

Growth potential of *Salmonella* and *Shigella*: 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. Although, myriad foods can serve as, *Salmonella* sources, meat and meat products, poultry products and dairy products are significant sources of food borne pathogen infections in humans. Presence of *Salmonella* spp. in fresh raw products can vary widely. Frequency usually ranges from 1-10%, 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 (aw), pH, oxidation potential (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 *et al*., 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 [100]. 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). Furthermore, 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 8 h and reached counts as high as log 8 CFU mL^{-1} within 12 h. Another study in Ethiopian street vended foods, *Salmonella typhimurium* reached counts $>10^8$ CFU g^{-1} within 24 h in the entire food items tested Egg sandwich, macaroni and lentil sandwich). Counts increased by about 1 log unit in the first 4 h and showed a steady growth thereafter (Muleta and Ashenafi, 2001).

Study noted that 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). *Shigella 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, 2002).

On the other hand, Fruits and vegetables can support the growth or survival of *Shigella* artificially contaminated fresh cut papaya, jicama and watermelon with *S. sonnei*, *S. flexneri* or *S. dysenteriae* and within 6 h at room temperature, growth was observed (Escartin *et al*., 1989). Moreover, 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. Zaika (2002) demonstrated *S. sonnei* and *S. flexneri* survival in apple juice (pH 3.3-3.4) and tomato juice (pH 3.9-4.1) held at 7°C for 14 days.

Besides, prepared foods can also support the survival of *Shigella* 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 (Islam *et al*., 1993). 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 h. All of the foods tested supported growth up to 10^8-10^{10} cells per g or mL within 6-18 h after inoculation at 25 and 37°C (Islam *et al*., 1993).

Study conducted elsewhere noted that growth of *Shigella flexneri* was markedly fast in the first 4 h 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. Moreover, 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).

In summary, the indicated incubation hours, following ingestion of contaminated food containing a sufficient number of *Salmonella* and *Shigella* induces disease symptoms such as diarrhea, vomiting and fever (Feasey *et al*., 2012). This indicates that food handlers with hygienic problem could play role in food contamination that results health risk.

In several studies, growth of challenged pathogens increased as pH increased whereas their counts decreased as pH lowered in general (Don, 2008; Kun, 2011). On the other hand, the change in pH could be changing of source of carbon and nitrogen. Some microbial cultures generate enzymes to utilize a new carbon and energy substrate when a small amount of the original carbon and energy substrate is present (Tadesse *et al*., 2005; Kun, 2011). However, pH is not the only required for the growth microorganisms but also other conducive natures of intrinsic and extrinsic parameters (Cayre *et al*., 2003; Jay *et al*., 2005).

CONCLUSION

Salmonellosis and Shigellosis are still global health problems, especially, in developing countries like Ethiopia where poor sanitation, lack of clean water supply and proper sewage disposal system exist. *Salmonella* and *Shigella* spp. are the causative agents of diarrheal diseases and the major causes of morbidity and mortality. Moreover, in most studies conducted in the country, they revealed that isolates of *Salmonella* and *Shigella* showed high rate of drug resistance to the commonly used antibiotics. In challenge studies of *Salmonella* and *Shigella* spp., in various food items, they grew to their infective dose within 4-24 incubation hours. Following ingestion of contaminated food containing a sufficient number of *Salmonella* induces disease symptoms. Therefore, for combating the problem posed by these pathogens, measures including health education, using safe waste disposal method, improvement of safe water supply, sanitation facilities and continuous monitoring of microbiological and appropriate usage of antibiotics were suggested.

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