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ADDIS ABABA UNIVERSITY COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

OCCURRENCE OF SALMONELLA IN SELECTED BEEF CHAIN LOCATIONS AND STOOL OF DIARRHEIC PATIENTS IN ADAMA, ETHIOPIA

A THESIS SUBMITTED TO COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE, ADDISABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MVSC. IN VETERINARY PUBLIC HEALTH

BY

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AUTHOR STATEMENT

First, I declare that this thesis is my genuine work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MVSc. degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate. Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however permission must be obtained from the author.

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LIST OF ABBREVIATIONS

AAAO	Adama Abattoir Adminstiration Office
ATHO	Adama Twon Health Office
BGA	Brilliant Green Agar
BGSA	Brilliant Green Sulfa A
BPW	Buffered Peptone Water
CDC	Center for Disease prevention and Control
CLIS	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic Acid
ECDC	European Center for Disease prevention and Control
EFSA	European Food Safety Authority
ELISA	Enzyme-linked Immunosorbent assay
HCI	Health Care Institutions
HEA	Hektoen Enteric Agar
ISO	International Organization for standardization
LIA	Lysine Iron Agar
MKTTn	Muller-Kauffmann tetrathionate/novobiocin broth
PPE	Personal Protective Equipment
RVS	Rappaport Vasiliadis Soya broth
SIM	Sulfide Indole Motility
SSA	*Salmonella*- Shigella Agar
TSI	Triple Sugar Iron
WHO	World Health Organization
XLDA	Xylose Lysine Deoxycholate Agar

ABSTRACT

Salmonella is one of important zoonotic pathogens and its occurrence in animals poses a continuous threat to human health. The occurrence, public health aspect and associated factors of *Salmonella* in most low and middle income countries like Ethiopia is largely unknown and this demands a study in local context. Therefore, a cross-sectional study was conducted aimed to determine the occurrence of *Salmonella* in slaughtered cattle, carcasses at an abattoir, meat at butcher's shop and diarrheic persons attending outpatient clinics. The study also determined the determinant factors for the occurrence of *Salmonella* and its antimicrobial susceptibility test. A total of 461 sample were collected which constitute 168 feces, 105 carcass swabs, 122 stool samples, 66 meat samples from ready to be slaughtered cattle, carcasses at an abattoir, diarrheic out patients and butcher's shop, respectively. The overall prevalence of *Salmonella* in the current study was 5.4% while the prevalence per sample type were 6.55%, 2.86%, 6% and 5.7% for fecal sample of ready to be slaughtered animals, carcass swabs at abattoir, meat at butcher's shop, and stool of diarrheic out patients, respectively. The degree of antimicrobial susceptibility for *Salmonella* isolates ranges from 0% to 96%. Out of antimicrobial tested isolates 84%, 92%, 96% of them were susceptible to chloramphenicol, ciprofloxacin and gentamycin, respectively. Among all isolates tested 76%, 88%, 92 and 96 % were resistant to cephalotin, ampicillin and streptomycin, and tetracycline respectively while 4%, 8%, 12%, 32%, 36% resistant to gentamycin, ciprofloxacin, chloramphenicol, nalidixic acid and kanamycin respectively. Most of the isolates were resistant to more than three antimicrobials. This study showed that sources of contamination to beef were more of associated with insufficient hygienic practices and improper handling of meat in the slaughterhouse and butcher shops. And also, consumption of raw meat was a significant determinant factor for *Salmonella* to occur in human population. Therefore, awareness creation is required regarding hygienic meat handling, regular monitoring, inspection and maintenance of machineries in slaughterhouse is needed and further study of *Salmonella* should be conducted to characterize the circulating serotypes.

Key Words: *Antimicrobial susceptibility, Beef, Determinant factors, Diarrheic patients, Occurrence, Salmonella*

1. INTRODUCTION

Safe and nutritious food with sufficient amount has a great importance in maintaining life and keeping oneself health. Unsafe food poses serious health risk to human beings ranging from diarrhea to cancers. Food-borne illnesses are a growing public health concern worldwide and caused by consumption of food contaminated by pathogenic microorganisms or chemical hazards (WHO, 2017). *Salmonella* takes a large share among costly foodborne pathogens worldwide (Reddy *et al*., 2016; Li *et al*., 2019). The genus *Salmonella* contains two species, called *Salmonella enterica* and *Salmonella bongori* (Lamas *et al.*, 2018) which together have more than 2500 serotypes (Quinn *et al*., 2016). Over half of them belong to *Salmonella enteric* subsp. *enterica*, the majority of which are recognized as cause of salmonellosis in human beings (Eng *et al*., 2015). Globally about 153 million cases of gastroenteritis and 57,000 deaths occur due to *Salmonella* infections each year (CDC, 2017).

In Africa, *Salmonella* burden is huge and it is a major cause of gastroenteritis and enteric fever. The disease causes substantial morbidity and mortality typically in immune-compromised individuals, malnourished and young children (Ao *et al*., 2015). Human salmonellosis is one of the major food borne diseases in Ethiopia (Tadesse, 2014) as a consumption of raw and undercooked meat is common in most parts of the country (Adimasu *et al*, 2014). Most often, meat becomes contaminated with *Salmonella* during processing, when meat and meat products come in contact with intestinal content or due to poor hygienic practices during processing (Tadesse and Gebremedhin, 2015).

Besides public health importance of food borne illness caused by *Salmonella* currently, additional important concern worldwide is the emergence and dissemination of antimicrobial-resistant pathogenic *Salmonella* species (Doménech *et al*., 2015). This could be due to the improper usage of antimicrobials in both human and veterinary medicine (Ventol, 2015). In Ethiopia, multidrug resistant *Salmonella* species have dramatically increased in humans and animals (Takele *et al*., 2018).

One of the major sources of food borne diseases was due to the widespread habit of raw beef consumption (Edeget *et al*., 2014) which is considered as potential risk of acquiring multidrug

resistant *Salmonella* (Wabeto *et al*., 2017). In the current study area, the information about occurrence of food-borne salmonellosis along meat supply chain is insufficient, and no studies identified sources of meat contamination and human illnesses with its antimicrobial susceptibility.

Therefore, the objectives of this research were: (1) to isolate *Salmonella* from apparently healthy cattle feces ready to be slaughtered, carcass at abattoir, meat at butcher shops, and human diarrheic patients and (2) to assess anti-microbial susceptibility profile of the isolates

2. LITERATURE REVIEW

2.1. Taxonomy and nomenclature of *Salmonella*

Theobald Smith in 1885 discovered *Salmonella* (Scott, 2012) but has got its name after a veterinarian called Daniel E. Salmon who first isolated *Bacillus choleraesuis* from porcine intestines and later in 1900 Lignieres changed it into *Salmonella choleraesuis.* Nowadays the *Salmonella* genus is divided into two species: *Salmonella enterica* and *Salmonella bongori*, with *S. enteric* further subdivided into 6 additional subspecies (Ryan *et al*., 2017). Its sero-typing is done based on their somatic -O and flagellar -H antigens identified. Additionally, capsular -Vi antigens rarely can be detected (Quinn *et al*., 2011; Quinn *et al*., 2016).

2.2. Physiological and cultural characteristics of *Salmonella*

Salmonella are categorized as facultative anaerobic gram-negative rods within the family *Enterobacteriaceae* (Markey *et al*., 2013). Almost all *Salmonella* species pocess flagella which is useful for motility except *S.* Pullorum and *S.* Gallinarum (Scott, 2012). Salmonellae characteristically ferment glucose and mannose without producing gas (Carroll *et al*., 2016) but do not ferment lactose. Rarely, lactose-fermenting strains are encountered (Quinn *et al*., 2011). They are oxidase negative, catalase positive, reduce nitrates to nitrites and can grow at 35^{o}C to 37^{o}C temperature range (Carr, 2017; Moxley, 2013).

Brilliant Green Agar (BGA), Brilliant Green Sulfa A (BGSA), *Salmonella- Shigella* Agar (SSA), Xylose Lysine Deoxycholate Agar (XLD), Hektoen Enteric Agar (HEA) are selective medias used to culture *Salmonella*. On BGA, colonies and the medium are red indicating alkalinity. On XLD agar, colonies are red (alkaline) with a black centre, indicating H_2S production (Markey *et al*., 2013; Moxley, 2013; Quinn *et al*., 2011). Up on inoculation of triple sugar iron agar /TSI/, they give an acid butt/alkaline slant with H_2S (Quinn *et al*., 2011), and alkaline or purple after inoculating to Lysine iron agar (LIA) (Markey *et al*., 2013). They are indole negative which forms yellow ring after addition of kovacs reagent on the top of *Salmonella* inoculated and incubated SIM medium. In addition to this they are urea negative and citrate positive (Mikoleit, 2015).

2.2. Epidemiology of *Salmonella*

2.2.1. Reservoir host

Gastrointestinal tract of warm and cold-blooded animals serve as reservoir for *Salmonella* (Moxley, 2013). Their occurrences are worldwide and have a potential to infect many mammals, birds and reptiles (Quinn *et al*., 2016). Humans and animals may act as carriers and reservoirs without actually showing any symptoms of the disease (Curtis *et al*., 2013). Additionally, reptiles have been reported to be the cause for human sporadic infection from snakes, lizards, and turtles and other species (Marin *et al*., 2013; Pees *et al*., 2013; Whiley *et al*., 2017).

2.2.2. Modes of transmissions

Most species of *Salmonella* cause gastrointestinal infection worldwide. The infection is restricted to mild gastroenteritis that rarely needs antimicrobial treatment (Chen *et al*., 2013). There are several ways for transmission of *Salmonella* to human beings including non-food vehicles (McEntire *et al*., 2014).

Salmonella is a natural inhabitant of the intestinal tract of food animals which serves as reservoir of the pathogen. Hence, contaminated foods with intestinal content of carrier animals or poorly handled meet during processing are main sources of infection for human being (Glenn Morris and Potter, 2013). These bacteria are usually transmitted to people by consuming raw meat, poultry products (McEntire *et al*., 2014) and fresh produces (Reddy *et al*., 2016) that are directly or indirectly contaminated with animal feces and other potential sources (Scott, 2012)

The level of contamination of chicken and beef at retail shops may vary based on sanitation measures that they follow and meat processing which includes temperature control, handling (mincing and cutting), storage and packing. Poor sanitation and management of meat at retail shop creates a fertile ground to the multiplication of the microorganism especially of *Salmonella* species (Shafini *et al*., 2017). The risk factor for meat to be contaminated by pathogenic organisms at retail shop is that cross contamination between meat, personnel and equipment. Contamination arises from improper and ineffective cleaning and disinfection of materials, tools and equipment like chopping

boards, knives and tables (Balakrishnan *et al*., 2018). During slaughter, some procedures such as evisceration and splitting may also contribute to carcass contamination (Hui, 2012).

There is a high probability of transmission for *Salmonella* at all stage of food chain from animal feed, primary production, and all the way to households or food-service establishments and institutions (WHO, 2018). At the farm level, cattle may become exposed to *Salmonella* through contact with contaminated feces, feed, or the environment (Nazareth, 2017), which poses a risk to food safety if these organisms are transferred on the carcass during slaughter (Edeget *et al*., 2014). Cattle may also be contaminated with *Salmonella* during transportation through contact with feces of other infected animals (Hui, 2012).

Poultry is widely acknowledged to be a reservoir for *Salmonella* and contamination of chicken eggs can occur in a number of ways. Basically, egg contents may be contaminated with *Salmonella* by two routes: transovarian and trans-shell (Holly, 2016). In addition to this, chickens acquire infection from contaminated feces, feed, or the environment (Steven and Richard, 2017).

Contact with infected animals, including pets, can induce infection to human. These infected animals often do not exhibit signs of disease. In addition, fecal-oral route is the other mechanism by which person to person transmission of infection will occur (WHO, 2018).

2.3. Clinical presentation

Infection of *Salmonella* commonly occurs as acute gastroenteritis mode and there should be ingestion of 10^6-10^8 *Salmonella* organism to cause symptomatic disease in healthy adults (Chen *et al*., 2013).

The incubation period of salmonellosis may vary from 6 hours to 72 hours after the ingestion of contaminated food or water. The symptoms are relatively moderate and manifested by acute onset of fever and chills, nausea and vomiting, abdominal cramping, and diarrhea. More often the illness takes 2–7 days, and most patients recover without medication (WHO, 2018). Infants, older adults, and people with immunosuppressive conditions (including HIV), hemoglobinopathies, and malignant neoplasms the infection is severe and life-threatening. Antibiotic-resistant organisms caused infection will lead to bloodstream infection and hospitalization. The ratio of people that

develop bacteremia or focal infection (such as meningitis or osteomyelitis) is estimated to be 5%. Outcome of infection varies among its sero types. Most of them are more likely to result in invasive infections (CDC, 2017; Santos, 2015).

2.4 Pathogenesis

The host immunity status and the organism serotype that involved in inducing infection determine the severity of the disease. The infection is more severe in patients with immune-suppression, children below the age of 5 year and aged individuals than the one with good immune status. Most of the *Salmonella* serotypes can induce infection which ranges from mild to life threatening (Edeget *et al*., 2014).

Pathogenic *Salmonella* strains are endowed with the capability to infect host cells and reside within an intracellular, vacuolar compartment called the *Salmonella*-containing vacuole (Singh *et al*., 2018). Microfold or M-cells, which are part of the epithelia covering intestinal villi associated with lymphoid follicles that are abundant in the Peyer's patches is the most prominent means of pathogenesis of *Salmonella* (Santos, 2015). It does have its own phagocytosis mechanism that can facilitate entree to the susceptible host cell. In order to gain access into the host cell, pathogenic *Salmonella* uses *Salmonella* pathogenicity islands (SPIs), gene clusters located at the large chromosomal DNA and encoding for the structures that are responsible for the invasion process (Grassl and Finlay, 2008).

Effectors of *Salmonella* can be injected across gut epithelial cell membrane into the cytoplasm with aid of multi-channel proteins through encoding of SPIs for type III secretion systems. Then effectors activate the signal transduction pathway and initiate re-arrangement of the actin cytoskeleton of the host cell. At the end epithelial cell membrane extend out ward or ruffle to engulf the bacteria (Arya *et al*., 2017). The engulfed bacterium is then encased in a membrane compartment called a vacuole, which is composed of the host cell membrane. Then *Salmonella* uses the SPIs type III secretion into the vacuole and causing the alteration of the compartment structure. The remodeled vacuole blocks the fusion of the lysosomes and this permits the intracellular survival and replication of the bacteria within the host cells; and carried in the reticulo endothelial system (Arya *et al*., 2017; Santos, 2015).

2.5. Diagnosis

2.5.1 Culture method

The transfer of microorganism from its natural habitat to artificial growth-permitting laboratory medium is referred to as culturing (Yousef, 2008). Infections that are clinically suspected are ultimately confirmed by isolation and identification of the causative agent. To provide effective and efficient anti microbial therapy, appropriate and accurate identification of the microorganism and antibiotic susceptibility tests is required (Han, 2013). Conventional culture methods are traditional method which is technically simple, sensitive and capable of reliable isolation and identification of food borne bacterial pathogens. Their main limitation is that they are labor-intensive and time consuming (Jasson *et al*., 2010). In culture based method the targeted pathogen is isolated from enrichment after inoculation and incubation of selective and differential media with specified pathogen, and then confirmed depending on biochemical properties (Yousef, 2008).

Culture based methods are commonly used techniques and remain the gold standard for the detection of *Salmonella* due to their selectivity and sensitivity (Garrido-Maestu *et al*., 2019). A series of steps are employed including nonselective enrichment, selective enrichment, and selective/differential plating biochemical and serological confirmations (Lee *et al*., 2015).

Non-selective pre-enrichment media, such as Buffered Peptone Water (BPW) are used to increase the number of target cells as these are generally not uniformly distributed in foods, typically occur in low numbers, and may be present in a mixed microbial population. Next, primary enrichment cultures are typically inoculated into secondary selective enrichment broths, such as Selenite Cystine broth (SC), Rappaport Vasiliadis Soya broth (RVS), Muller Kauffmann Tetrathionate- Novobiocin broth (MKTTn) and incubated at elevated temperatures (37°C or 42°C for 18-24 hours) before being struck onto selective agars such as XLD (ISO-6579, 2017).

2.5.2 Immunological based methods

Rapid agglutination assays: numerous rapid latex agglutination assay tests are broadly in use for the quick detection of *Salmonella*. However, the assays are mainly used as a confirmation screen for presumptive *Salmonella.* Colonies after culture isolation from selective agar plates, with further

confirmation and identification is done on those organisms giving a positive latex reaction. A single colony from selective media or enrichment suspension taken and is allowed to be mixed with latex reagent and after a little time rotation, the results are easily appreciated. Smoothness and retaining its original color of latex indicates test result is negative where as distinct color agglutination against an altered background indicates a positive result. The test result is at least 24 hours earlier than conventional culture methods. However, non-specific agglutination of some organisms will result in lack of specificity. Some commercial kits include Remel Wellcolex Colour tests for the presumptive identification of *Salmonella* serogroups A, B, C, D, E, and G, and the Vi- antigen using just two reagents. Similar tests include Oxoid *Salmonella* latex test, Microgen *Salmonella* Latex test, and Denka-Seiken, among others (Odumeru and León-Velarde, 2012).

Enzyme-linked immunosorbent assay (ELISA): is also used to detect the presence of an antibody or an antigen in a *Salmonella* suspect samples. Accordingly, a sample with an unknown amount of antigen is immobilized on a solid support either non-specifically or specifically. Then, Horse Radish Peroxidase (HRP) is added to form a complex with the antigen. Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. Following the final wash, enzymatic substrate (ABTS or 3, 3', 5, 5'-tetramethylbenzidine) is added to produce a visible signal (colorimetric or fluorescent product) due to the enzymatic cleavage of the substrate. The presence of target antigen in the sample can be measured by indicating colorimetric equipment (Odumeru and León-Velarde, 2012).

ELISAs are very sensitive, rapid, specific, and easy to carry out. Most laboratories are able to easily adopt the technology for regular microbiological testing. However, different constituents of the enrichment medium and incubation conditions used during test process influences its reactivity. Most often, majority of ELISA methods, negative results can be obtained within 24 hrs after an overnight incubation in selective broth. Positive results may still subjected to further cultural isolation and biochemical and serological confirmation according to regulatory interest (Lee *et al*., 2015).

Lateral immune diffusion assays: A sandwich type of ELISA that uses a monoclonal antibody as the detection antibody and polyclonal antibody as a capture antibody is used in lateral flow immunoassays. The antibodies are set on a hydrophobic poly-vinylidine difluoride-based membrane.

A drop of an enrichment sample is placed in a reaction window and travels by capillary action across the membrane to react with the antibodies and provide a color change. Within 24 hrs results can be obtained. Denaturation or degradation of the capture antibody may result in false positive results and it is likely that detection antibody or enzyme-conjugated antibody may also bind non-specifically to denatured capture antibody (Odumeru and León-Velarde, 2012).

2.5.3. Molecular method

Polymerase chain reaction (PCR): there is modernized microbiological analysis allowing detection of harmful microorganisms in food, without classical isolation and identification (Chapela *et al*., 2019) and rapid and reliable technique in detecting *Salmonella* (Jinu *et al*., 2014). It is the amplification of a nucleic acid target sequence. The targeted sequences can be a specific gene, redundant areas in the sequence or random sequences. However, for the synthesis of the oligonucleotides based on the amplification of a specific portion of the DNA, the targeted DNA sequence must be known (Gorski and Csordas, 2010). For food borne bacterial pathogens, commonly targeted DNA areas are virulence factors, toxins, cellular metabolites (Liu *et al*., 2019; Wang and Salazar, 2016).

Detection of food borne pathogens is effective if done by PCR because of its high sensitivity, specificity, and rapidity, than conventional microbiological culture methods. However, PCR can be limited by several factors like: inhibitory compounds found in food components (Ahmed *et al*., 2014). The amount of pathogen /low/ that found in food and quantity of food/ large/ is another factor that inhibits the reaction (Jasson *et al*., 2010). In order to solve the problems caused by PCR inhibitors, all the steps leading up to DNA amplification must be optimized for the sample type in question (Hedman and Rådström, 2019).

The use of real-time PCR is become a promising alternative approach in food diagnostics. It possesses a number of advantages over conventional methods, in terms of rapidity, excellent analytical sensitivity and selectivity, and potential for quantification (Rodríguez-Lázaro *et al*., 2013). A real-time PCR method has been developed with custom designed primers and a TaqMan probe to detect the presence of a 262-bp fragment of the *Salmonella*-specific invA gene (Cheng *et al*., 2008).

2.5.4. Biosensors

Food borne pathogens are a growing concern with respect to human illnesses and death. In order to reduce and combat the risk there should be a mechanism that rapidly identifies the source of the bacteria. The development of biosensor designs come up with the solution for rapidly detection of food borne pathogens and other microorganisms. Biosensors can automate this process and have the potential to enable fast analyses that are cost and time-effective (Yasmin *et al*., 2016). Biosensors are analytical devices that convert a biological response into an electrical signal (Mehrotra, 2016; Velusamy *et al*., 2010). Various biosensor techniques are available that can identify food borne pathogens and other health hazards: i.e. nucleic acid biosensors. Enzyme based biosensors, antibody based biosensors, micro and nano biosensors (Lee *et al*., 2015**)**

Biosensor-based methods rely on the recognition of antigen targets or receptors by antibodies, aptamers or high-affinity ligands. The captured antigens may be then directly or indirectly detected through an antibody or high-affinity and high-specificity recognition molecule (Poltronieri *et al*., 2014).

2.6. Antimicrobial resistance

Antimicrobial resistance is a growing public health issue that threatens the effective prevention and treatment of disease that induced with pathogenic microorganisms (Ravi Shankar and Balasubramanium, 2014). Therefore it became one of the most serious risks to global public health (Carlos, 2010). Nowadays modern human and veterinary medicine and safety of our food and environment are under jeopardize due to antimicrobial resistance (FAO, 2016) and each year in the U S, millions of people suffer from infections with bacteria that are resistant to one or more of the antibiotics intended to treat those infections. At least 23,000 people die each year as a direct result of these antibiotic-resistant infections (CDC, 2013).

Antimicrobials are substance that destroy or hinder the growth of micro-organisms and are used to treat infections caused by pathogenic organisms (Muktar *et al*., 2015). They can be produced/ designed naturally from microbes or synthetic (Muiru, 2016). Micro-organisms are considered resistant if their growth and multiplication is not hindered by an antimicrobial to which they were

sensitive. Such resistance is called ‘acquired resistance’ and is encoded by resistance genes in the DNA of the microbe. Resistance genes can arise through spontaneous mutations in the microbial DNA, but some have evolved over many years due to natural selection by natural antimicrobials in the environment. These genes can also transfer from drug-resistant microbes to drug-sensitive ones (ECDC, 2018; EFSA and ECDC, 2013).

The development of antibiotics resistance is the main undesirable side effect of antimicrobial use in both humans and animals and results from the continuous positive selection of resistant bacteria clones, whether these are pathogenic, commensally or even environmental bacteria. This will modify the population structure of microbial communities, leading to accelerated evolutionary trends with unpredictable consequences for human health. The use of antimicrobials can differ in humans and food-producing animals, in terms of both the methods of administration and the quantities administered; there are important variations between and within food-producing animal species, as well as between countries (EFSA, 2008).

Resistance against antimicrobials can be developed due to improper usage of antimicrobials for treatment of food animals and human (Hoelzer *et al*., 2011; Pui *et al*., 2011). the reasons for increasing resistance levels include: suboptimal use of antimicrobials for prophylaxis and treatment of infection, noncompliance with infection-control practices, prolonged hospitalization, increased number and duration of intensive care-unit stays, multiple co morbidities in hospitalized patients, increased use of invasive devices and catheters, ineffective infection-control practices, transfer of colonized patients from hospital to hospital, grouping of colonized patients in long-term-care facilities, antibiotic use in agriculture and household chores, and increasing national and international travel. The level of antibiotic resistance is dependent on the following: the populations of organisms that spontaneously acquire resistance mechanisms as a result of selective pressure either from antibiotic use or otherwise, the rate of introduction from the community of those resistant organisms into health care settings, and the proportion that is spread from person to person (Byarugaba, 2010).

The major mechanisms of active antimicrobial resistance are prevention of accumulation of antimicrobials either by decreasing uptake or increasing efflux of the antimicrobial from the cell *via* a collection of membrane-associated pumping proteins, qualitative drug target site alteration which

reduces the affinity for antimicrobials either by mutation or by target modification, or quantitative drug targ*et al*teration by overproduction of the target and inactivation of antibiotics either by hydrolysis or by modification(Muktar *et al*., 2015).

During the past few years studies conducted in different parts Ethiopia revealed that there is antimicrobial resistant *Salmonella* which is isolated from different food animals and food products (Amenu, 2012; Dagnachew, 2017; Ejo *et al*., 2016; Kemal *et al*., 2016; Teshome and Anbessa, 2012).

Moreover, the spread of resistant bacteria and resistance determinants within and between these animal, human and environment must be addressed. The most outstanding mechanism in which this problem is solved includes elimination of inappropriate use of drugs, mainly where there is high volume usage. Avoiding mass medication of animals with human antimicrobials especially with third generation like cephalosporins and fluoroquinolones, and in-feed use of antimicrobials, such colistin, tetracyclines and macrolides, for growth promotion can reduce the emergence of antimicrobial resistant bacteria. In people it is essential to better prevent infections, reduce over-prescribing and over-use of antimicrobials and stop resistant bacteria from spreading by improving hygiene and infection control, drinking water and sanitation. Pollution from inadequate treatment of industrial, residential and farm waste is expanding the resistant pathogens in the environment (Collignon and McEwen, 2019).

2.7. Status of *Salmonella* in human and beef in Ethiopia

Carcass contamination with *Salmonella* is of special public health significance for a country like Ethiopia, where consumption of raw and undercooked meat is common in most areas of Ethiopia. Contamination is likely to be further amplified as meat passes through various chains until it reaches the final consumers (Tadese and Gebremedhin, 2015).

Table 1: Status of *Salmonella* from human and beef in Different part of Ethiopia

Study area	Sample type	Prevalence (%)	References
Harar	Stool	11.50	(Reda *et al*., 2011)
Jima	Blood	4.30	(Tizazu et al., 2011)
Bishoftu	Carcass swab	2	(Sibhat et al., 2011)
Jimma	Feces	3.30	(Dabasa and Bacha, 2012)
	Beef	13.30	
Jimma	Stool	6.20	(Beyene and Tasew, 2014)
Gonder	Stool	1.08	(Demissie et al., 2014)
Butajira	Stool	10.50	(Mengistu et al., 2014)
Harar	Stool	25	(Adimasu et al., 2014)
Addis Ababa	Stool	3.95	(Mamuye et al., 2015)
Addis Ababa	Stool	6.20	(Eguale et al., 2015)
Bahirdar	Carcass	7.60	(Muluneh and Kibret, 2015)
Hawasa	Stool	2.50	(Mulatu et al., 2015)
Gondar	Raw meat	12.50	(Ejo et al., 2016)
	Minced meat	8	
Addis Ababa	Carcass swab	5.70	(Kebede et al., 2016)
Hawasa	Stool	41.70	(Amsalu et al., 2017)
Haramaya	Meat	6.70	(Edget et al., 2017)
Bahirdar	Meat	70	(Azage and Kibret, 2017)
Haramaya	Cutting board surface swab	9.10	(Bekele and Lulu, 2017)
	Carcass swab	7.80	

Wolaita Sodo	Carcass	12.50	(Wabeto et al., 2017)
Bishoftu and Addis Ababa	Minced beef	9.80	(Dagnachew, 2017)
Addis Ababa	Feces	4.10	(Ketema et al., 2018)
	Carcass swab	2.50	
Jimma	Feces	11.30	(Takele et al., 2018)
	Carcass swab	5.60	
	Stool	8	
Adama	Feces	12.50	(Abunna et al., 2018)
	Carcass swab	3.70	
Ambo	Feces	4.60	(Mustefa and Gebremedhin, 2018)
Gondar	Meat samples	17.30	(Garedew et al., 2018)
	Hand swabs		
	Knife swabs		
	chopping board		
Addis Ababa	Feces	6.70	(Banti, 2018)
	Carcass swab	10	
Mekele	Carcass	7.29	(Atsbha et al., 2018)
Nekemte	Stool	7.10	(Terfassa and Jida, 2018)
Hosaina	Stool	1	(Abebe et al., 2018)
Arbaminch	Stool	12.60	(Ameya et al., 2018)
Bonga	Beef	18.20	(Tegegne, 2019)
	Stool	11.30	
Jigjiga	Stool	11	(Admassu et al., 2019)

3. MATERIALS AND METHODS

3.1. Study area

Adama is a city found about 100 km away from Addis Ababa, in the south eastern direction located in Oromia regional state, East Shewa Zone at 8.55°N 39.27°E at an elevation of 1712 meters above sea level. The town has a total human population of 234,231 (CSA, 2014). Because of its strategic position, the city of Adama has been serving as the centre of trade and tourism for decades (All African, 2018). There are more than 250 butcheries in the city and one municipal abattoir which provide slaughtering service. On average about 120 cattle are slaughtered every slaughter day (AAAO, 2017).

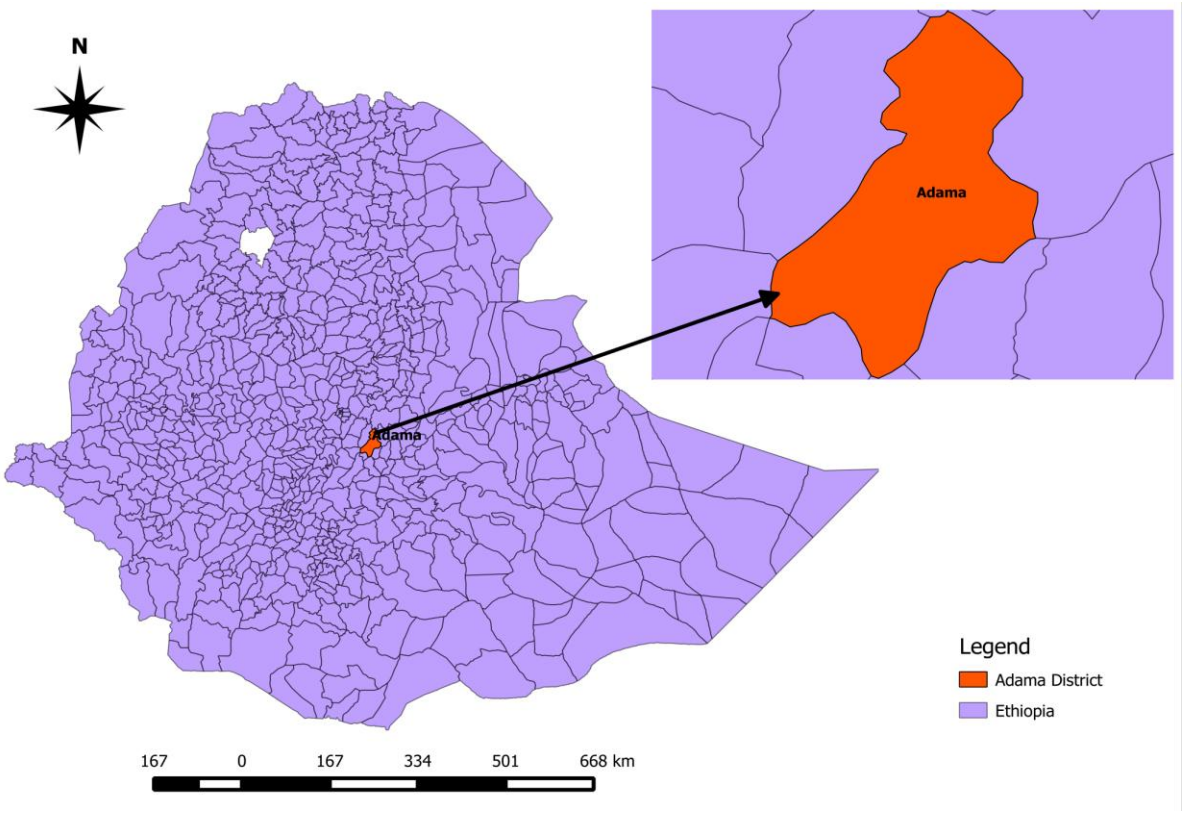

Figure 1: Map of study area.

3.2. Study design and study population

A cross-sectional study design was conducted from October 2018 to May 2019 at an abattoir supplying meat for local consumption, butcher shops and Health Care Institutions (HCI) in Adama. The study populations were apparently healthy cattle ready to be slaughtered at the abattoir. Human study was conducted on diarrheic out-patient visiting HCI. The animals presented to abattoir for slaughter were from extensive or semi-intensive farms around the study area.

3.3. Sample size determination and sampling strategy

The sample sizes for feces collection from ready to be slaughtered animals, beef carcass swab, meat at butcher shops and stool from diarrheic out patients were determined using the formula for simple random sampling given below (Thrusfeild, 2005).

$$n = \frac{Z^2 * p(1-p)}{d^2}$$

Where:

n = number of required sample size

z = normal distribution (95%)

p = expected prevalence

d = desired absolute precision

For all the sample size calculations, 95% confidence interval and 5% precision were used. Accordingly, the sample size of apparently healthy to be slaughtered cattle and beef carcass swab at abattoir was determined based on a previous study report on prevalence of *Salmonella* in cattle as 12.50 % and beef carcass 3.70% (Abunna *et al*, 2018). Thus, 168 ready to be slaughtered animals. A total of 55 beef carcass swabs were calculated but the sample size was nearly doubled. Besides, to determine the sample size of beef meat at butcher shops, 4.53 % expected prevalence was used (Tadese and Gebremedhin, 2015), hence, 66 meat samples were collected. On the other hand 122 samples from human diarrheic out patients were collected based on previous report of 8.72% prevalence of *Salmonella* in Ethiopian diarrheic patients (Tadese, 2014).

3.4. Sample collection and processing

3.4.1. Sample collection

Study area visit was done to facilitate research collaboration prior to the sample collection and cooperation letter was sent to Adama City Abattoir administration and health office. The study was in effect from October 2018 to May 2019. The sampling days were randomly assigned to abattoir, butcher shops and HCI. During the study period, sampling at abattoir was done once a week. At each sampling day in abattoir 15 fecal and carcass swab samples were collected using systematic random sampling method based on order of animal to be slaughtered. Fecal samples were collected directly from the rectum using sterile arm length glove. Carcass swabs were collected from breast area, lateral and medial part of both hind limbs and forelimbs using sterile cotton swabs that were pooled together in sterile 50ml screw cupped test tubes containing 10ml BPW for each individual animal. The carcass samples were collected following the guidelines of the International Organization for Standardization (ISO 17604, 2013). Meat samples from butcher shops were collected by using sterile polyethylene bag once per week. Stool samples were collected from diarrheic out patients admitted to different HCI at Adama. According to Adama town health office 16 HCI are allowed to get involved in any kind of survey or study (ATHO, 2017). Among the 16 HCI, eight were selected by cluster sampling method (Adama east one, west one, south one, north two, center three). Stool samples were purposively collected from diarrheic patients whose ages were one year and above with collaboration from physicians and medical laboratory technology professionals. The consistency of stool was determined immediately after collection based on Bristol stool chart as type 4, 5, 6, and 7 (Lewis and Heaton, 1997). The samples were collected using sterile screw cupped universal bottle containing 10ml BPW.

3.4.2. Sample transportation

All samples were collected aseptically and labeled legibly with permanent marker identifying type, source of sample, sample code and date of sampling. The samples were transported in an ice box containing ice packs to the Veterinary Public Health Laboratory of College of Veterinary Medicine, Addis Ababa University (Annex 1).

3.4.3. Isolation and identification

Salmonella isolation and identification was carried out in line with the guidelines of the International Organization for Standardization, (ISO-6579, 2017). Within the protocol are steps that include primary enrichment in non-selective liquid medium (pre-enrichment), secondary enrichment in selective liquid media, plating out on selective and non-selective media and finally, confirmation by biochemical methods.

Primary enrichment in non-selective liquid medium (pre-enrichment): The chilled samples were left for 3 to 5 h at 20 to 22°C before being processed. Twenty-five grams of minced beef, 25 g of fecal samples, pooled beef carcass swab and 4 to 5 g of stool samples were added to BPW (Himedia, Mumbai, India) in 1:9 ratios. The mixture was homogenized using a laboratory blender at high speed for 2 min. The enrichments were incubated aerobically at 37 °C for 18 hr. Stool sample which weighs 1gram was added to screw cupped test tube containing 10ml BPW (Himedia, Mumbai, India) and incubated aerobically at 37 °C for 18 hr.

Enrichment in selective liquid media: For this purpose, RVS (HIMEDIA,Mumbai,India)/and MKTTn broth (HIMEDIA, Mumbai, India) were used. RVS broth is incubated at 41.5 °C for 24 hr after inoculating by 0.1ml product of primary enrichment, and the MKTTn broth at 37 °C for 24 hr after inoculating by 1ml product of primary enrichment.

Plating out and identification: Plating was carried out on XLD agar (OXOID, Basingstoke, England) plates. A loopful from enrichment broth culture was streaked separately onto the plating out media. The plates were then incubated aerobically at 37°C °C for 24 h. Then the plates were examined for the presence of *Salmonella* colonies. Typical colonies of *Salmonella* grown on XLD medium are small red translucent and/or dome shaped colonies, which may have central black spot due to hydrogen sulfide production. For confirmation, two presumptive *Salmonella* colonies (or less depending on ability to grow) were selected from selective plating media. The selected colonies were streaked on the surface nutrient agar (Accumix, Malaga, Spain) plates in a manner that allow isolated colonies to develop and incubated at 37℃ for 24 hours for further confirmation with biochemical tests.

Biochemical characterization: Colonies suspected to be *Salmonella* were examined biochemically using triple sugar iron (TSI) agar slants (HIMEDIA, Mumbai, India), lysine decorboxylase test using lysine iron agar (LIA) (HIMEDIA, Mumbai, India), urease test using urea broth (HIMEDIA, Mumbai, India), indole test using SIM (HIMEDIA, Mumbai, India) and citrate utilization test using Simmon's citrate agar (HIMEDIA, Mumbai, India). TSI, lysine decarboxylase , urease and citrate utilization tests were conducted according to ISO-6579,2017.

3.5. Antimicrobial susceptibility test

All biochemically confirmed *Salmonella* isolates were tested for susceptibility against ten antimicrobial agents (TM Media, New Delhi, India) in accordance with the National Clinical and Laboratory Standards Institute (NCCLS, 2013). Disk diffusion technique was used to test for the susceptibility of *Salmonella* to antibiotics. Three colonies of *Salmonella* from nutrient agar plate were transferred to 5 ml of saline water to standardize bacterial suspension, the density of suspension was adjusted to 0.5 McFarland standard and 0.1ml of the broth was then spread onto Mueller Hinton agar (CM 0337, OXOID, Basingstoke, England) using a sterile spreader. Each disk was placed using sterile forceps and distributed evenly in the plate and pressed gently to ensure contact with the agar followed by incubation of the plates at 37 °C for 24 hours. Finally, the zone of inhibition was measured by Venire caliper after 24 hours of incubation. The antimicrobials used were selected from the currently available and commonly used chemotherapeutic agents for the treatment of *Salmonella* infection in humans and animals. *Escherichia coli* ATCC 25922 were used as a quality control and standard strain. The susceptible, intermediate and resistant categories were assigned on the basis of zone of inhibition (CLIS, 2018). *Salmonella* isolates were considered as resistant if they are no longer inhibited by an antimicrobial to which they were previously sensitive to at least one antimicrobial drug and multiple resistant if it was resistant to two or more antimicrobial drugs tested (Muktar *et al*., 2015).

Table 2: Antimicrobials used and their break points for Entrobacteriaceae

Antimicrobials	**Concentration (^g/disk)**	**Susceptible (≥mm)**	**Intermediate (mm)**	**Resistant (≤mm)**
Ampicillin (AMP)	10 µg	17	14-16	13
Cefoxitin (CX)	30 µg	18	15-17	14
Cephalotin (CEP)	30 µg	18	15-17	14
Chloramphenicol (C)	10 µg	18	13-17	12
Ciprofloxacin (CIP)	5 µg	21	16-20	15
Gentamycin (GEN)	10 µg	15	13-14	12
Kanamycin (K)	30 µg	18	14-17	13
Nalidixic Acid (NA)	30 µg	19	14-18	13
Streptomycin (S)	10 µg	15	12-14	11
Tetracycline (TET)	30 µg	15	12-14	11

Source: CLIS, 2018

3.6. Questionnaire survey

Semi-structured questionnaire was prepared and presented to abattoir workers, butchers and diarrheic patients whose samples were taken for this study. The questionnaire addressed questions related to habits of consumption of raw meat, age, cattle farming, contact with cattle feces and presence or absence of immune comprising disease. In addition to this clinical record of diarrheic patients were consulted for confirmation of status of immune comprising diseases. Meat handling and hygiene practiced in the abattoir and butcher shops were assessed by the use of semi-structured questionnaire and thorough close personal observation. The questionnaire was originally prepared and written in English. For better understanding of the interview, it was translated into two local languages; Amharic and Afaan Oromo. It was presented to interviewees' based on their preference (Annex 6, 7 and 8).

3.7. Data management and analysis

The data generated from the study was arranged, coded and entered to Microsoft Excel spreadsheet (Microsoft® office excel 2007). Descriptive analysis was used to describe the result of proportion analysis and questionnaire survey. Proportion was estimated as the number of samples detected

positive to *Salmonella* from the total sample size analyzed. Fisher's exact test was observed to assess association between *Salmonella* prevalence between different samples. Firth logistic regression was used to associate the presence of *Salmonella* in diarrheic patients and determinant factors. In addition to this to evaluate the association of determinant factors and presence of *Salmonella* in butcher shop firth logistic regression was used and reported using odds ratio, 95% confidence interval and P-value. Statistical software STATA® version 14 (STATA corp. College Station, TX, USA) was used for the analysis. The significance level was set at 95% confidence level.

3.8. Ethical considerations

Ethical clearance for the study was obtained from Oromia health bureau with Ref. No of BEFO/HBI8H/1-8/666 (Annex 8). Verbal consent (Annex 5) was obtained from the diarrheic out patients, abattoir and butcher shop workers during questionnaire survey.

4. RESULTS

4.1 Distribution of *Salmonella*

A total of 461 samples were examined for detection of *Salmonella*. The overall prevalence of *Salmonella* was 5.42% for all samples. Positive samples were found in all sample categories. The highest proportion was observed in feces from apparently health slaughtered cattle while the lowest was from carcass swab. The association between samples types were not statistically significant (P>0.05).

Table 3: The overall occurrence of *Salmonella* from different sample sources.

Sample type	No. Tested	Positive	Prevalence (%)	95% CI	P- Value*
Feces	168	11	6.55	3.65-11.47	
Carcass swab	105	3	2.86	0.92-8.55	
Meat	66	4	6	2.27-15.19	0.59
Stool	122	7	5.74	2.75-11.60	
Total	**461**	**25**	**5.42**	**3.69-7.91**	

CI- Confidence interval, *Fisher's exact test

4.6. Occurrence of *Salmonella* and the associated determinant factors in butcher shop

As indicated in Table 3, the occurrence of *Salmonella* in meat from butcher shop was 6. There was statistically significant difference (P<0.05) between individuals who did medical checkup and who did not (Table 4).

Table 4: Occurrence of *Salmonella* and the associated determinant factors in butcher shop in Adama

Determinant Factors	Total No.	No. positive (%)	COR	P-value
Floor type				
Ceramic	27	1 (3.70)	*	
Concrete	39	3 (7.69)	1.69	0.60
Sign of dirty wall				
No	53	2(3.77)	*	
Yes	13	2(15.38)	4.48	0.12
Fly control				
No	32	3(9.38)	*	
Yes	34	1(2.94)	0.38	0.33
Storage of offal and meat in one				
No	44	1(2.27)	*	
Yes	22	3(13.64)	5.21	0.10
Use of the same utensil for cutting of offal and meat				
No	54	2(3.70)	*	
Yes	12	2(16.67)	5	0.09
Head cover				
No	31	2(6.45)		
Yes	35	2(5.71)	0.88	0.89
Interval of PPE wash				
Twice a day	12	0(0)	*	
Every day	54	4(7.55)	2.273	0.59
Hand wash in between cutting meat				
No	26	3(7.50)	*	
Yes	40	1(3.85)	0.63	0.65
Meat chopping board				
Plastic	26	1(3.85)	*	
Wood	40	3(7.50)	1.59	0.65
Medical check up				
Not checked	11	3(27.27)	*	
Checked	55	1(1.82)	0.07	0.01**
Training				
Not trained	58	4(6.89)	*	
Trained	8	0(0)	0.71	0.83

CI- Confidence interval; COR- Crude odds ratio;**Statistical significant; * Reference variable

4.7. The occurrence of *Salmonella* in diarrheic patients and its determinant factors

Among diarrheic patients (122) seven were positive for *Salmonella*. There was statistical significant difference (P<0.05) between individuals who consumed raw meat and who did not (Table 5).

Table 5: The occurrence of *Salmonella* in diarrheic patients and the associated determinant factors

Determinant factors	**Total No.**	**No. of positive (%)**	**COR**	**P-value**
Age				
1-14	30	2(6.67)	*	
15-29	40	1(2.50)	0.43	0.43
30-40	19	1(3.45)	0.59	0.63
41-60	14	0	0.39	0.55
>60	9	3(33.33)	6.14	0.06
Cattle for farming				
No	98	4(4.08)	*	
Yes	24	3(12.50)	3.42	0.10
Contact with diarrheic patients				
No	102	5(4.90)	*	
Yes	20	2(10)	2.39	0.28
Contact with cattle feces				
No	104	2(4.81)	*	
Yes	18	2(11.11)	2.74	0.21
Consumption of raw meat				
No	98	3(3.06)	*	
Yes	24	4(16.67)	5.99	0.02**
Attending large gathering				
No	103	5(4.85)	*	
Yes	19	2(10.53)	2.56	0.24

CI -Confidence interval; COR- Crude odds ratio;**Statistical significant; * Reference variable

4.4. Antimicrobial susceptibility test

The current study on antimicrobial sensitivity test of *Salmonella* revealed a varying degree of susceptibility to antimicrobial agents tested. The degree of susceptibility for *Salmonella* ranges from 0%-96%. Majority of the isolates showed resistance to more than three anti- microbial agents.

Table 6: Anti microbial susceptibility level of *Salmonella* isolates to different antimicrobial disks

Antimicrobials	**Concentration (^g/disk)**	**Susceptible No. (%)**	**Intermediate No. (%)**	**Resistant No. (%)**
AMP	10 µg	0	3(12)	22(88)
CEP	30 µg	1(4)	5(20)	19(76)
CX	10 µg	2(8)	8(32)	15(60)
C	10 µg	21(84)	1(5)	3(12)
CIP	5 µg	23(92)	0	2(8)
GEN	10 µg	24(96)	0	1(4)
K	30 µg	6(24)	10(40)	9(36)
NA	30 µg	16(64)	1(5)	8(32)
S	10 µg	3(12)	0	22(88)
TET	30 µg	0	2(8)	23(92)

Most of isolates showed resistance to three and above antimicrobials tested.

Table 7: Multidrug resistance patterns of *Salmonella*

Resistance pattern	**Anti microbial tested**	**Total number of isolates**
3	AMP S TET	6
4	AMP CEP S TET	5
5	AMP CEP GEN S TET	1
6	AMP CEP CIP GEN S TET	2
7	AMP C CEP CIP GEN S TET	3
	AMP CEP CX K NA S TET	6
	AMP CEP CIP CX NA S TET	2

4.5 Questionnaire survey

4.5.1. The meat handling and hygienic practices at Adama City Abattoir.

A total of 37 abattoir workers (26 males and 11 females) were interviewed. About half of respondents were between age ranges 31-40. The majority of the respondents in abattoir used aprons, overall coats and boots while few used head cover and none of them used glove while on work. PPE used by abattoir workers are washed every day after job. At the abattoir, manual stunning method was in use and vertical dressing method was used for dressing and evisceration. There was a sink for hand wash in abattoir. All respondents replied washing their hands with soap before starting job and after toilet. There was a hot water sink for knife but not functional. Therefore, they did not sink knife in hot water between and before flaying and evisceration. The knife used for evisceration and flaying was different. Workers who work at flaying and evisceration had their own knife to perform the task. The majority of respondents washed their hands after evisceration. At abattoir, chlorinated water from a municipal pipe line was used for carcass washing. More than half of respondents did medical checkup at an interval of three month and got training on meat handling and hygiene practices (Table 8).

Table 8: The meat handling and hygienic practices at Adama city abattoir.

Task	Performance	No. of respondents	%
Sex	Male	26	70.27
	Female	11	29.73
Age	18-30	13	35.14
	31-40	19	51.35
	>40	5	13.51
Apron	Yes	36	97.30
	No.	1	2.70
Over all coat	Yes	36	97.30
	No.	1	2.70
Boots	Yes	36	97.30
	No.	1	2.70
Head cover	Yes	8	21.62
	No	29	78.38
Hand wash after evisceration	Yes	13	35.14
	No	24	64.86
Medical Check Up	Yes	36	97.30
	No	1	2.70
Frequency of medical check up	3 month	20	55.56
	6 month	10	27.78
	Yearly	6	16.67
Training	Trained	23	62.16
	Not trained	14	37.84

4.5.2. The meat handling and hygienic practices at Adama butcher-shop.

A total of 66 butchers working at Adama butcher shops were interviewed. The majority of were between 20-30 age groups. Source of beef cattle for the majority of respondents were from fattening farm. Source of meat in all cases were from abattoir and transported with close vehicle uncovered with plastic or other coverage material. In all butcher shops meat was displayed on open air without covering and house ventilation is categorized as fair. There was no meat cooling facility up on display. Majority of butcher shop's floor was made up of concrete and almost all were painted with white color. Majority had no dirty on their walls. There was a routine fly control in about half of butcher shop. All Butcher shops had refrigerator for meat storage and in some cases meat and offal's were stored in one. All respondents in butcher shops use apron, overall coat and boots however, none of them use glove while on work. About 46.67% of the respondents use head cover. The majority of respondents wash their PPE every day. All respondents wash their hand before touching

meat and after toilet but none of them wash their hand in between cutting meat. Most of butchers use wood cutting board and in rare case there is the use the same knife for cutting meat and offal. After the end of job, all respondents wash material, tools, equipment used in butcher shop by hot water and soap. More than half of respondents did medical checkup at an interval of three month. Most of the respondents did not got training on meat handling and hygiene practices (Table 9).

Table 9: The meat handling and hygienic practices at Adama City butcher-shop.

Task	Performance	No. of respondents	%
Age	20-30	38	57.58
	31-40	20	30.30
	>41	8	12.12
Source of beef cattle	Fattening farm	40	60.61
	Local market	26	39.39
Floor type	Ceramic	27	40.91
	Concrete	39	59.09
Wall painting with white color	Yes	65	98.48
	No	1	1.52
Sign of dirty on wall	Yes	13	19.70
	No	53	80.30
Routine fly control	Yes	34	51.52
	No	32	48.48
Storage of offal and meat in one same place	Yes	22	33.33
	No	44	66.67
Use of the same knife for offal and meat cut	Yes	12	18.18
	No	54	81.82
Hand wash in between cutting meat	Yes	40	60.61
	No	26	39.39
Head cover	Yes	35	53.03
	No	31	46.67
Frequency of washing PPE	Twice per day	12	18.46
	Every day	53	81.54
Meat chopping board	Plastic	26	39.39
	Wood	40	60.61
Medical check up	Yes	55	83.33
	No	11	16.67
Frequency of medical check up	Monthly	6	10.53
	3 month	43	75.44
	6 month	5	8.77
	Yearly	3	5.26
Training	Trained	8	12.12
	Not trained	58	87.88

5. DISCUSSION

The present study assessed the occurrence of *Salmonella* along beef supply chain in typical large urban areas. In addition *Salmonella* was isolated from diarrheic patients. The occurrence of *Salmonella* in slaughtered cattle in the current study was 6.55% which is comparable with the previous reports (6.70%) of Banti (2018) and 5.60% of Takele *et al*. (2018) in different parts of Ethiopia.

Similarly the prevalence of current reort is similar with the report of 4.10 % Ketema *et al* (2018) and 4.60 %, Mustefa and Gebremedhin (2018). Conversily, lower prevalence than the current study was reported by other scholars as 3.30%, by Dabasa and Bacha (2012). On the other hand Abunna *et al*. (2018) reported a higher prevalence (12.50 %) than the current study in the same study area. The reasons for the lower in the prevalence of *Salmonella* in cattle in current study compared with the previous report might be due to the majority of the animals were brought for slaughter from fattening farm in and around Adama where antibiotics are potentially intensively used . There is a use of antibiotics for growth promotion and disease prevention in feedlot farms (Muktar *et al*., 2015) this will reduce the *Salmonella* fecal shedding in animal (Economou and Gousia, 2015; FAO and WHO, 2015; Jone, 2011). In addition the animals stay in lairage for less than 12 hrs. This decreases the probability of being stressed and cross contamination which creates unfavorable condition for *Salmonella* fecal shedding (FAO and WHO, 2015; Njisane and Muchenje, 2017).

The occurrence of *Salmonella* from carcass swab 2.85% at the current abattoir was in agreement with previous works of 3.70% (Abunna *et al*., 2018), 2% (Sibhat *et al*., 2011) and 2.50 % (Ketema *et al*., 2018). However, the current prevalence is lower than previous reports of 11.3% (Takele *et al*., 2018) and 12.5% (Wabeto *et al.,* 2017). The proportion of *Salmonella* from carcass at abattoir in this study is higher than 1% which was isolated from carcass at Dire Dawa slaughterhouse (Edget *et al*., 2017). The higher prevalence than previous report might be due to absence of hot water washing knife in between evisceration and flaying. Normally, knives must be immersed in hot water for two minutes at 82°C to reduce the number of contaminating microorganisms (Motsoela *et al*., 2002). Contradictory to these facts, in current study knife was used without sinking into hot water. This could cause carcass contamination with different food borne pathogens (Bekele and Lulu, 2017; Muluneh and Kibret, 2015).

The current finding of *Salmonella* from raw meat at butcher shop was 6% which is comparable to 6.7% reports of Edget *et al*. (2017) at Haramaya University retail meat shop. However it is higher than the 1% obtained from Dire Dawa meat retail shop. The reason for being higher in proportion might be due to cross contamination of carcass up on transport, cross contamination by butcher shop utensils and hand, storage of offal and meat in one and open display method without cover. This might increase chance of meat contamination (Edget *et al*., 2017; Garedew *et al*., 2015). The present finding of *Salmonella* from raw meat at butcher shop is lower than 10.20% (Hiko *et al*., 2016), 12% (Ejo *et al*., 2016), 18.2% (Tegegn. 2019) and 35.6% (Garedew *et al*., 2015). The occurrence of *Salmonella* at butcher shop that had medical checkup has significant difference from who did not. Those respondents who go for medical checkup may get appropriate treatment if they become positive for *Salmonella.* This will decreases chance of cross contamination of *Salmonella* from meat handlers to meat.

Out of the 122 diarrheic patients presented to Adama town HCI, seven (5.73%) of them were positive for *Salmonella.* This finding is similar with 6.20 % reports of Eguale *et al.* (2015) and Beyene and Tassew (2014). Similarly the current report is similar with reports of4% Dagnachew, (2017), 3.95% Mamuye *et al*, (2015), 4.30% Tizazu *et al*, (2011). Conversely, the current report was higher than the previous works of 1.08% Demissie *et al*, (2014), 2.5% Mulatu *et al*, (2014), but relatively lower than reports of 7.07% Garedew *et al*, (2018), 11% Admassu *et al*, (2019) and 11.30% Tegegne, (2019), 12% Feleke *et al*, (2018), 19.05% Tesfaye *et al, (*2014) and 25% Adimasu *et al*, (2014) which were conducted in different parts of the country.

The questionnaire survey of diarrheic out patients that presented to Adama HCI showed that cattle farming, contact with cattle feces, contact with diarrheic patients, attending large gatherings were recognized as determinant factor to be infected with *Salmonella* since their odd ratio is greater than one (COR>1) even though the P-value was not significant. This could increase the possibility to be infected with *Salmonella* (Addis *et al*., 2011; Hoelzer *et al*., 2011; Thompson *et al*., 2013). In addition to this there is high statistical significance (P<0.05) difference between patients who had history of raw meat consumption with who did not. This indicates that consumption of raw meat might have source of infection to *Salmonella* in human which is agreement with the work of Adimasu *et al*. (2014).

Antimicrobial resistance emerges from improper use of antimicrobials in animals and human, and the subsequent transfer of resistance genes and bacteria among animals, humans, animal products and the environment (Ventol, 2015; Argudín *et al*., 2017) which may pose serious health risks to human (Poole, 2015). The consumption of multidrug resistant *Salmonella* along with a raw meat dish is directly relevant to the global public health crisis due to antimicrobial resistance (Wabeto *et al*., 2017). Determining the scope of the problem is essential for formulating and monitoring an effective response to antimicrobial resistance (WHO, 2014).

The current study on antimicrobial sensitivity test on *Salmonella* revealed a varying degree of susceptibility 0 to 96% antimicrobial agents tested. Most isolates were 84%, 92%, 96% susceptible to chloramphenicol, ciprofloxacin and gentamycin, respectively which is in agreement with previous reports of other scholars 88% (Takele *et al*., 2018), 100% (Azage and Kibret, 2017; Takele *et al*., 2018) and 93% (Banti, 2018) for chloramphenicol, ciprofloxacin and gentamycin, respectively. While, the current result disagrees with reports of 55.40% (Wabeto *et al*., 2017), 53.30 % (Banti, 2018) susceptibility for chloramphenicol, 58.91% (Wabeto *et al*., 2017), 23.50% (Mustefa and Gebremedhin, 2018) susceptibility for ciprofloxacin and 55.40% (Wabeto *et al*., 2017) 58.80% (Mustefa and Gebremedhin, 2018) susceptibility for gentamycin. Among all isolates tested 76%, 88%, 92 and 96 % were resistant to cephalotin, ampicillin and streptomycin, and tetracycline respectively. This result agrees with report of 100% (Mustefa and Gebremedhin, 2018) and 88.7% (Garedew *et al.*, 2015) for ampicilline, 89.3% (Ketema *et al.*, 2018) for streptomycin and 90% (Ejo *et al*., 2016) for tetracycline. This report disagrees report of 39.3% (Ketema *et al.*, 2018) and 32.1% (Garedew *et al.*, 2015) for tetracycline, 41.1% (Mustefa and Gebremedhin, 2018) for streptomycin and 23.8% (Azage and Kibret, 2017) for ampcilline.

6. CONCLUSION AND RECOMMENDATIONS

The current study revealed that sources of beef contamination were more of associated with insufficient hygienic practices and improper handling of carcass in the slaughterhouse and butcher shops which significant proportion of the carcasses were found contaminated. Though the sources contamination of the carcass and meat were not specifically determined the slaughtering process and meat handling showed apparent unhygienic conditions. For example, unhygienic floor surface, cutting boards, butcher shop utensils, workers hands, storage of meat and offal in one and transport of meat without cover might have been potential sources of beef contamination at slaughter houses and butcher shops. In addition, having cattle for farming, contact with cattle feces, and contact with diarrheic patients and attending large gathering were potential determinant factors to *Salmonella* in human beings. Moreover, consumption of raw meat was significant determinant factor for *Salmonella* to occur in humans. Furthermore, high proportions of *Salmonella* isolates were resistant to three or more of the antimicrobials that are commonly used in the veterinary and human medical practices. This is should be taken as serious issue with a significant public health risk.

Therefore, based on the present study the following recommendations are forwarded:

- ✓ Relevant intervention program and awareness creation on hygienic meat handling is required for abattoir and butcher shop workers and consumers to minimize the public health significance of *Salmonella.*
- ✓ Further investigation need to be conducted in order to characterize the pathogens and identify their serotypes and
- ✓ Slaughter house and butcher shop machinery should be regularly inspected, monitored and maintained to reduce level of contamination of carcass.

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8. ANNEXES

Annex 1: Sample record and biochemical record sheet

No.	Sample type	Collection site	Sample code	Collection date	Isolation				Biochemical test					Remark
					BPW	RVS	MKTTn	XLD	TSI	Urease test	Citrate utilization	Indole test	L-LDT	

Key: BPW: buffered peptone water; RVS: Rappaport-Vassiliadis soya broth; MKTTn: muller kauffman tetrathionate novobiocin; XLD: Xylose Lysine Deoxycholate; TSI: Triple sugar iron; L-LDT:L- lysine decarboxylase test

Annex 2: Type and preparation of microbiological media used for isolation and antimicrobial susceptibility test of *Salmonella*

1. **Buffered Peptone Water (MH 14941-500G, HIMEDIA, Mumbai, India)**

Preparation: suspend 20.07 grams (the equivalent weight of dehydrated medium per litre) in 1000ml of distilled water. Heat if necessary to dissolve the medium completely. Distribute in tubes or flasks as desired. Sterilize by autoclaving at 121 ℃ for (15lbs pressure) 15 minutes. Final PH is 7.0 ± 0.2 at 25℃.

Composition (g/l): tryptone10.0gm; Sodium chloride 5.0gm; disodium hydrogen phosphate 9gm ; potassium hydrogen phosphate 1.5gm.

2. **Rappaport Vassiliadis soya broth (M1491-500G, HIMEDIA, Mumbai, India)**

Preparation: suspend 27.11 gm in 1000ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired in to tubes and sterilize by autoclaving at 115 ℃ (10lbs pressure) for 15 minutes. Final PH is 5.2 ± 0.2 at 25℃.

Composition (g/l): papaic digest of soya bean 4.5gm; sodium chloride 7.2gm; potassium dihydrogen phosphate 1.44gm; dipotasium phosphate 0.4, magnesium chloride hexahydrate 29.00; malachite green 0.036gm.

3. **Mueller Kauffman TetrathionateNovobiocin broth base (MH 14961-500G, HIMEDIA, Mumbai, India)**

Preparation: suspend 89.42grams (the equivalent weight of dehydrated medium per litre) in 1000ml of distilled water. Heat the medium just to boiling. Do not autoclave. Cool to 45 - 50^{o}C and just before use aseptically add 20ml of iodine solution (20gm iodine and 25gm potassium iodide in 100ml sterile distilled water) along with rehydrated contents of one vial of MKTT novobiocin supplement (FD203). Mix well before dispensing to sterile tubes to disperse calcium carbonate uniformly. Final PH is 7.0 ± 0.2 at 25℃.

Composition (g/l): P 4.30; casein enzymic hydrolysate8.60; ox bile 4.75; sodium chloride2.60; calcium carbonate 38.70; sodium thiosulphate $5H_2O$ 47.80; brilliant green 0.0095

Final PH is 8.2 ± 0.2 at 25℃.

4. **Xylose Lysine Desoxycholate Agar (XLD) (CM 0469, OXOID, Basingstoke, England**)

Preparation: Suspend 53grams in one liter of distilled water. Heat with frequent agitation until the medium boils. Do not over heat. Transfer immediately to a water bath at 50℃. Pour in to plates as soon as the medium has cooled. It is important to preparing large volumes which will cause prolonged heating. PH: 7.4±0.2 at 25 ℃

Composition (g/l): yeast extracts 3.0; l-lysine hydrochloric acid 5.0; xylose 3.75; lactose 7.5; sucrose 7.5; sodium desoxycholate 1.0; sodium chloride 5.0; sodium thiosulphate 6.8; ferric ammonium citrate 0.8; phenol red 0.08; agar 15.0.

5. **Nutrient Agar (AM5074, Accumix, Malaga, Spain)**

Preparation: suspend 28 grams in 100ml distilled water. Mix thoroughly. Boil with frequent agitation to dissolve the powder completely. Sterilize by autoclaving at 15 lbs pressure (121℃) for 15 minutes. Mix well and pour in to sterile Petri dishes.

Final PH (at 25℃): 7.4 ± 0.2.

Composition (g/l): peptone 5gm; sodium chloride 5gm; beef extract 1.5gm; yeast extract 1.5gm; agar 15gm.

6. **Triple sugar agar (M021I-500g, HIMEDIA, Mumbai, India)**

Preparation: Suspend 64.62 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the medium to set in sloped form with a butt of depth about 2.5cm-5cm.

Final PH: 7.4±0.2 at 25℃.

Composition (g/l): peptone 20gm; beef extract 3gm; yeast extract 3gm; sodium chloride 5.0; glucose1gm; lactose 10gm; sucrose 10gm; iron (48) citrate 0.3gm; sodium thiosulfate 0.3gm; phenol red 0.024gm; agar 12.0gm

7. **Simmons Citrate Agar (M 099-500g, HIMEDIA, Mumbai, India)**

Preparation: suspend 24.28 grams in 1000ml distilled water. Heat on boiling to dissolve the medium completely. Dispense as desired in tubes or flasks sterilize by autoclaving at 15 Ibs pressure (121℃) for 15 minutes.

Composition (g/l): magnesium sulphate0.20; ammonium dihydrogen phosphate 1.0; dipotasium phosphate 1.00; sodium citrate 2.00; sodium chloride 5.00; bromothymol blue 0.08; agar 15.00.

Final PH: 6.5-7 ± 0.2 at 25℃

8. **MR-VP Medium (M 070-500g, HIMEDIA, Mumbai, India)**

Preparation: suspend 17.0 gram in 1000ml distilled water. Heat if necessary to dissolve the medium completely. Distribute in to test tubes 10ml amounts and sterilize by autoclaving at 15 Ibs pressure (121℃) for 15 minutes.

Composition (g/l): buffered peptone 7.00; dextrose 5.00; dipotassium phosphate 5.00

Reagent required for voges- proskauer reaction

- ✓ α-Naphtanol, ethanolic solution

Preparation: dissolve α-Naphtanol in ethanol

Composition (g/l): α-Naphtanol 6 grams; ethanol 96 % (volume fraction) 100ml.

- ✓ Potassium hydroxide solution

Preparation: dissolve potassium hydroxide in distilled water

Composition (g/l): potassium hydroxide 40 grams; distilled water 100ml

9. **Mueller-Hinton Agar (CM 0337, OXOID, Basingstoke, England)**

Preparation: suspend 38 grams in 1litre of distilled water. Bring to boil to dissolve the medium completely. Sterilize by autoclaving at 121℃for 15 minutes.

Final PH: 7.3 ± 0.1 at 25℃

Composition (g/l): beef, dehydrated infusion 300.00; casein hydrolysate 17.5; starch 1.5; agar 17.00

10. 0.5 McFarland standards

Composition: 1.17% $BaCl.2H_2O$ solution and 0.36N of 1% sulfuric acid (H_2SO4). **Preparation: Add approximately 85Ml of** 1% H_2SO4 to a 100ml of volumetric flask, using a 0.5ml pipette add 0.5ml of 1.17% $BaCl.2H_2O$ drop wise to the H_2SO4 while constantly swirling the flask. Bring to 100ml with 1% H_2SO4.place a magnetic stirring in the flask and place on the magnetic stirrer for approximately three to five minutes. Examine solution visually to make certain it appears homogeneous and free of visible clumps. Dispense three to seven ml, cub tube tightly and seal with paraffin and keep at dark and room temperature.

11. Urea agar base (M112S-500g, HIMEDIA, Mumbai, India)

Preparation: Suspend 24.51 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add 50 ml of sterile 40% Urea Solution (FD048) and mix well. Dispense into sterile tubes and allow setting in the slanting position. Do not overheat or reheat the medium as urea decomposes very easily.
Final PH: 6.5-7 ± 0.2 at 25°C

Composition (g/l): Dextrose 1.000, Peptic digest of animal tissue 1.500, Sodium chloride 5.000, Monopotassium, phosphate 2.000, Phenol red 0.012, Agar 15.000

12. Lysine Iron Agar (M377-500g, HIMEDIA, Mumbai, India)

Preparation: Suspend 34.56 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense into tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubes in slanted position to form slants with deep butts.

Composition (g/l): Peptone 5.000, Yeast extract 3.000, Dextrose (Glucose) 1.000, L-Lysine 10.000, Ferric ammonium citrate 0.500, Sodium thiosulphate 0.040, Bromocresol purple 0.020, Agar 15.000
Final pH (at 25°C) 6.7±0.2)

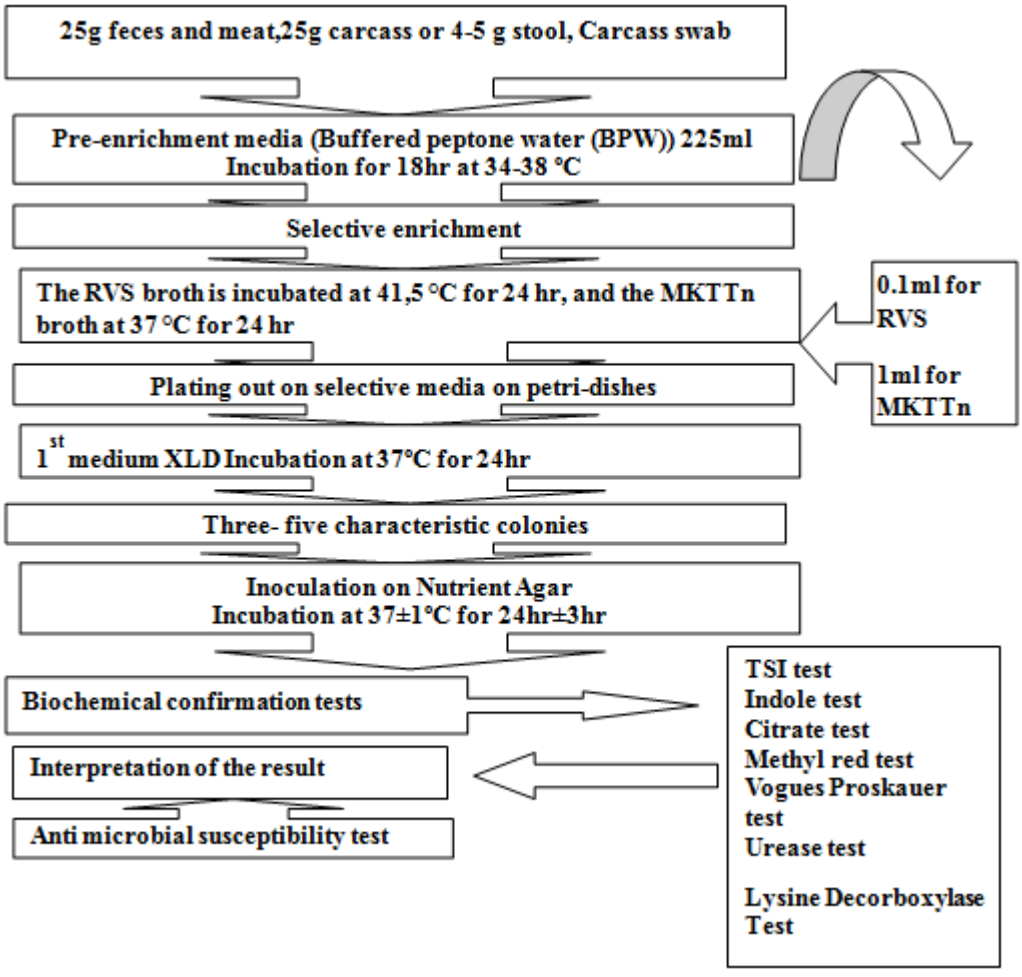
13.SIM Medium(M181-500g, HIMEDIA, Mumbai, India)

Preparation: Suspend 36.23 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubes to cool in an upright position.

Composition (g/l): HM Peptone B# 3.000, Peptone 30.000, Peptonized iron 0.200, Sodium thiosulphate 0.025, Agar 3.000

Final pH (at 25°C) 7.3±0.2

Annex 3: Flow chart for *Salmonella* Isolation



Annex 4: *Salmonella* Isolation

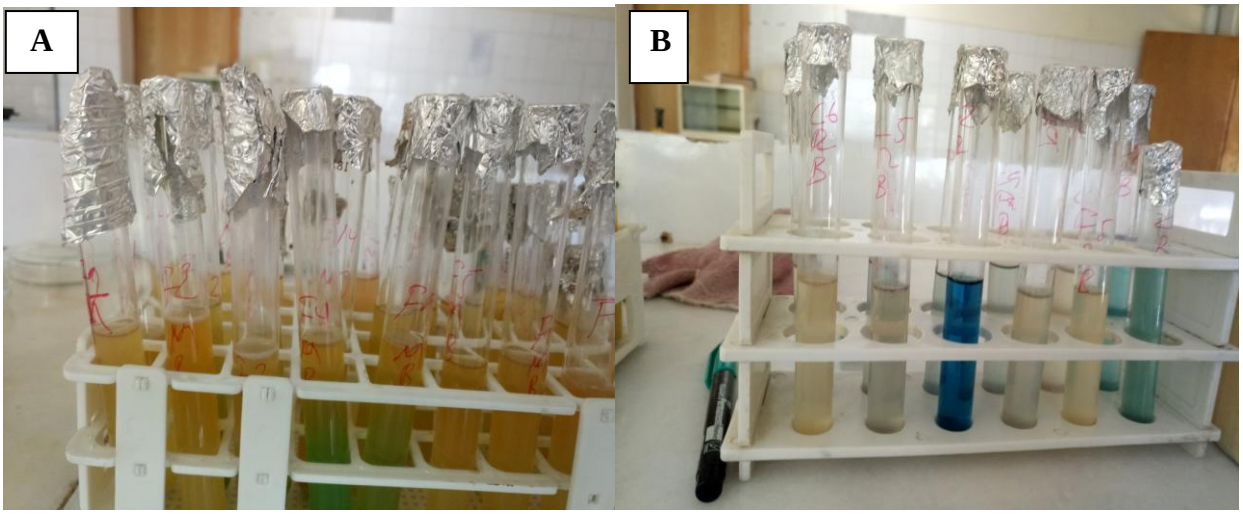


Figure 2: Erichment of sample.

A. Mueller Kauffman Tetrathionate Novobiocin Enrichment B. Rappaport Vassiliadis soya broth Enrichment

Figure 3: *Salmonella* appearance on XLD agar

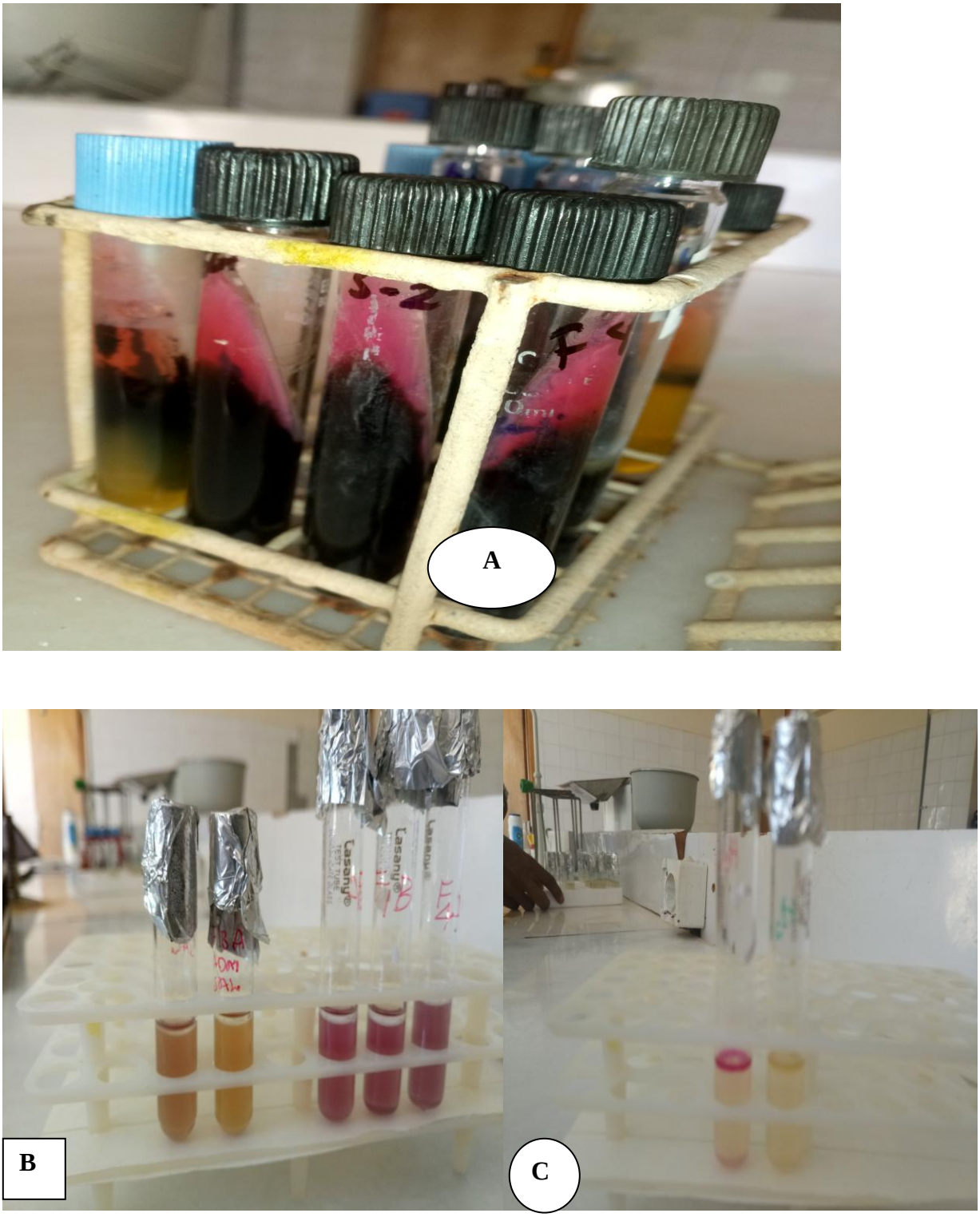


Figure 4: Biochemical Result of *Salmonella*

A. TSI Test B. Lysine Decorboxylase Test C. Indole Test

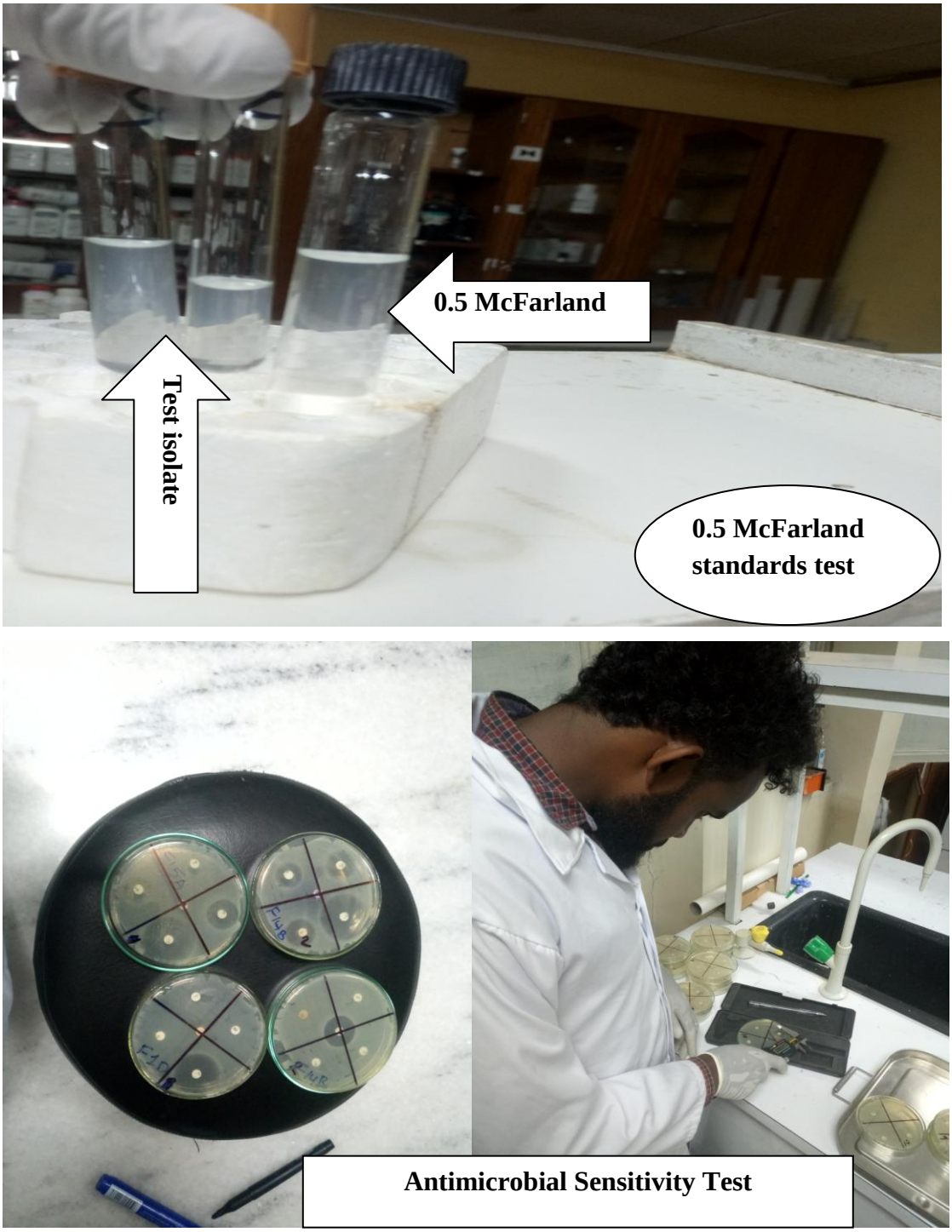


Figure 5: Antimicrobial Sensitivity Test

Annex 5: Verbal Consent

Greeting!

My name is Dr. Endalu Mulatu. I am from Addis Ababa University College of Veterinary Medicine and Agriculture. I am working a research entitled "**Occurrence of *Salmonella* in Selected Beef Chain Locations and Stool of Diarrheic Patients in Adama, Ethiopia".** The aim of the research is to assess the occurrence of *Salmonella* along beef supply chain. The interview will take about **20 minutes** to finish. Apart from the importance of your answers to all of my questions, you may decline to answer any of the questions if you don't feel comfortable. There is no immediate benefit to you now but as part of the research, the output will be useful prevention of the pathogen. On the other hand what I want to note you is that I respect your privacy and confidentiality. This means I will disclose your name and other personal identifiable information to anybody outside of the research.

Statement of the respondent

I confirm that I have understood the above description of the study and that I have had the opportunity to ask questions about this study. I confirm that I agree to provide answers.

Do you agree to participate? □ Yes □ No

If the person cannot read and write, let him/her to give verbal consent. In that case, put the letter 'V' in the box for agreement and get the signature of a person who can be a witness.

If the participant is literate:

Participant name: _____Telephone: _____

Signature (or other mark)_____

I would like to thank you in advance for your input in this study by answering the questions and sharing your experience.

Annex 6: Questionnaire for identification of source of infection on diarrheic patient's determinant factors

1. Address_____
2. Sex_____
3. Age_____
4. Educational Status_____
5. Marital status_____
6. Do you have cattle for farming? A. Yes B. No
7. Have you had direct contact with cattle feces before illness? A. Yes B. No
8. Had you contact with someone with diarrhea illness before becoming ill? A. Yes B. No
9. Can you tell me duration of diarrhea since its onset?_____
10. What is its consistency? A. bloody B.Mucoid C. Watery D. Mixed
11. What do you ate raw meat at last 6-72hrs A. yes B. no
12. If your answer on number five is yes, from where you got it.
    A. Butcheries of Adama town B. backyard slaughter C. other (specify)
13. What type of meat is it?
    A. beef B. mutton C. chevon D. other (specify)
14. Did you attend a large gathering like wedding ceremony the week before your illness? A. Yes B. No
15. If your answer of number 12 is yes, did others develop similar illness? A. Yes B. No
16. Do you have another illness? A. Yes B. No
17. If your answer for number seven is yes state the disease? _____

Annex 7: Questionnaire for abattoir workers on hygienic meat handling practices

1. Address_____
2. Sex_____
3. Age_____
4. Educational Status_____
5. Marital status_____
6. Your job position in abattoir _____
7. From where you got beef cattle? A. butcheries B own farm C. other (specify)_____
8. How long animals stay in lairage? A. >12 hrs B. <12 hrs
9. How often the lairage is cleaned? A. everyday B. per two days C. other (specify)
10. How often the slaughter house cleaned? A. everyday B. per two days C. other (specify)
11. Do you use the following personal protective equipments while at work?

Personal protective equipment	Response	
	Yes	No
Apron		
Boots		
Glove		
Overall coat		
Head cover		

12. How often you clean personal protective equipments? A. every day B. twice a day C. other (specify)_____
13. Method of carcass dressing and evisceration? A. vertical B. Horizontal
14. Do you have sink for hand wash? A. Yes B. No
15. Do you wash your hands before touching the carcass with soap? A. Yes B. No
16. Do you use the same knife for flaying and evisceration? A. yes B.no
17. Do you sink knife in hot water before and in between flaying and evisceration? A. Yes B. No

18. Do you wash your hands after evisceration? A. yes B. No
19. Does the carcass washed with chlorinated water after evisceration? A. Yes B. No.
20. Have gone for medical checkup before and after you employed? A. Yes B. No.
21. If your answer is yes for no. 12 at what frequency you for medical checkup?_____
22. Do you wash your hands after going toilet? A. yes B. no
23. Have you ever received any training on hygienic handling of carcass? A. yes B.No.

Annex 8: Questionnaire for meat handlers on hygienic practices at butcher shop.

1. Address_____
2. Sex_____
3. Age_____
4. Educational Status_____
5. Marital status_____
6. Your job position in butchery _____
7. From where you got beef cattle? A. fattening farm B. local market
8. Source of meat A. abattoir B. backyard slaughter
9. What is the means of transporting meat from abattoir to the retail shop?
   A. Cart B. Closed vehicle
10. What type of display method you use to display meat? A. open air B. Cabinet C. Other (specify)
11. Do you cover meat up on display and transport? A. yes B. no.
12. Do you cover meat up on display? A. yes B. no.
13. What is status of house ventilation? A. good B. fair C. satisfactory
14. From what type of butcher shop floor? A. ceramic B. concrete
15. Wall painted with white color? Observation: A. yes B. no
16. Is there sign of dirty on the wall? A. yes B. no
17. Do you perform routine fly control? A. Yes B. No
18. Do you have Refrigerator for storage of meat? A. yes B. no.
19. Do you have different storage and display for offal's and meat? A. yes B. no.

20. Do you use the same equipment while handling meat and the offal's? A. yes

B. no.

21. Do you use meat cooling facilities at the display cabinet? A. yes B. no.

22. What type of meat chopping material you use? A. plastic B. wood

23. Do you use the following personal protective equipments?

Personal protective equipment	Response	
	Yes	No
Apron		
Boots		
Glove		
Overall coat		
Head cover		

24. At what interval you wash it?

A. Everyday B. twice a day C Other (Specify)

25. Do you wash your hand before and in between contact with meat? A. yes B. no

26. Do you wash your hand after toilet? A yes B. No

27. Do you sanitize materials tools and equipments you use in shop? A. Yes B. No

28. What is your source of water A. municipal B. bore hole C. other (specify)

29. If yes what method you used for sanitizing? A. hot water with soap B. disinfectants C. Autoclaving . other (specify)_____

30. Have gone for medical checkup before and after you employed? A. Yes B. No.

31. If your answer is yes for no. 12 at what frequency you for medical checkup? _____

32. Have you ever received any training on hygienic handling of carcass? A. yes

B. No.

Annex 9: Ethical Clearance

BIIROO EEGUMSA FAYYA OROMIYAA
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OROMIA HEALTH BUREAU

Lakk/Ref. No. BEFO/HBTFH/1-8/666
Guyyaa /Date 14/3/2011

Waaj/Eeg/Fay/Bul/Mag /Adaamaa tiif
Adaamaa

Dhimmi: Xalayaa deggersaa ilaala

Akkuma beekamu Biiroon keenya ogeeyyii, dhaabbilee akkasumas namoota qorannoo gaggeessuuf piroppoozaala dhiyeeffatan piroppoozaala isaanii madaaluun akkanumas iddoo biraatti ilaalchisanii fudhatama argatee (approved) dhiyaateef, piroppoozaala isaanii ilaaludhaan waraqaa deggersaa ni-kenna. Haaluma kanaan mata duree**''Isolation, identification, antimicrobial susceptibilitytest of non-typhoidal salmonella and survey of determinant factors along with meat supply chain and diarrhoeic patients, Adama, Oromia, Ethiopia'' jedhamu irratti ''Endalu Mulatu''magaalaa keessan keessatti** qorannoo geggeessuuf piripoozaalii isaanii Koree "Health Research Ethical Review Committee" Biiroo keenyaatti dhiyeeffataniiru. Haaluma kanaan Koree "Health Research Ethical Review Committee" Biiroo keenyaa pirpoozaala kana ilaaluun mirkaneessee qorannoon kun akka hojiirra oolu murteessee jira. Waan kana ta'eef hojii qorannoo kanarratti deggersa barbaachisaa ta'e akka gootaniif, akkanumas akka hordoftan jechaa,**''Endalu Mulatu''** qorannoo kun qaacceffamee xumuramee fiiriisaa kooppii tokko Biiroo Eegumsa Fayyaa Oromiyaatiif akka galii godhu garagalcha xalayaa kanaatiin isaan beeksifna. Anis **''Endalu Mulatu''** wayitii qorannoon kun qaacceffamee xumurame fiiriisaa koppii tokko Biiroo Eegumsa Fayyaa Oromiyaatiif akka galii godhu mallattoo kiyyaan mirkaneessa.

Nagaa wajjin

Yaadataa Ayyaanaa

Mallattoo____________________
Maqaa _**''Endalu Mulatu''**
Guyyaa____________________
Lakk. Bilbila:

G/G

''Endalu Mulatu'' tiif
Bakka jiranutti

Biiroo Eegumsa Fayyaa Oromiyaa

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