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Original Article

Isolation, Identification and Antibiotic Susceptibility Testing of Salmonella from Sheep and Goats Slaughtered at Haramaya Municipal Abattoir, Eastern Ethiopia

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ABSTRACT

A cross-sectional study was conducted from April to August 2021 at Haramaya municipal abattoir, eastern of Ethiopia with the aims to isolate, identify and to determine antimicrobial susceptibility of *Salmonella*. Isolation and identification was performed by conventional methods for detection and identification of *Salmonella* according to (ISO-6579, 2002). The antimicrobial susceptibility testing was performed by disc diffusion method. A total of 228 samples of meat, meat swab, cecum feces and skin swab was collected from sheep and goats, and examined for the presence *Salmonella*. Out of total samples, 34 (14.91%) was found positive for *Salmonella* and there was statistically significant variation between positive *Salmonella* and sample sources (*p=0.000*). All of 34 isolated *Salmonella* were exhibited 100% multi-drug resistance and highest percentages of resistance were observed for amoxicillin (100%), chloramphenicol (100%), ampicillin (94.1%) and tetracycline (70.6%). However, all isolates were susceptible to gentamicin and 97.1% were sensitive to kanamycin. The highest level of resistance of *Salmonella* against most commonly used antimicrobials detected by this study may pose challenges to veterinary and human health sectors. Therefore, it is advisable to work on improving a good hygiene in abattoir to minimize incidence of infection and it will be better if principle of antimicrobial stewardship applied and unregulated use of antibiotics avoided both in humans and animals.

Key words: Antimicrobials, Disc diffusion, Haramaya, Salmonella, Susceptibility

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INTRODUCTION

Ethiopia is home for a large and diverse livestock resources and favorable production environments. The vast majority of the rural population's livelihood is partly based on livestock production (Solomon *et al.*, 2010). The country had 59.5 million heads of cattle, 30.70 million heads of sheep, 30.20 million heads of goats, 56.53 millions of poultry and 1.21 million heads of a camel (CSA, 2017). It is central to the Ethiopian economy contributing about 45% to the agricultural GDP, supporting the livelihoods of 70 % of the population, 18.7% to the national GDP and 16–19% to the total foreign exchange earning of the country (MoA, 2012).The country was endowed with largest livestock population that ranks 1stin Africa and 10th in the world, which could enable the country to gain from the growing global markets for livestock products if

production and productivity will improve (CSA, 2011).

Meat is among the most valuable livestock products and for many people serve as their first-choice source of animal protein which provides all the essential amino acids and various micronutrients in proper proportion to the human beings(Ameha, 2008). Meat composition makes it an ideal medium for the growth of a good number of microorganisms due to richness in nutrients(Dawit*et al.*, 2020). It is prone to contamination at various stages from primary production to when it is ready for consumption. Microorganisms from exterior part and intestinal tract of animals can contaminate the meat in abattoir during slaughtering operations and fecal cross-contamination of edible organs (Wondimu*et al.*, 2017). Amongst the microorganisms, *Salmonella* most frequently found on animal body coat and feces and transferred to meat during slaughtering(Yan*et al.*, 2003). Contaminated meat is one of the main sources of food borne illnesses and death caused by agents that enter the body through ingestion(WHO, 2007). It is generally recognized that the most significant foodborne hazards from fresh meat are bacteria that can causedisease in humans (pathogenic bacteria), such as *Salmonellae*species(Bersisa*et al.*, 2019).

The genus *Salmonella* was named after Daniel E. Salmon who first reported the isolation of *Salmonella* from a pig in 1885 and named the organism *Bacterium choleraesu is* currently known as *Salmonella* entericaserovar Choleraesuis (Rao, 2004). Salmonellosis is an infectious disease of humans and animals caused by organisms of the genus *Salmonella*(A folamiand Onifade, 2018). Salmonellosis is one of the main food borne zoonotic and animal husbandry problem throughout the world. The bacteria cause food borne poisoning in humans, mainly through animal products that include poultry, cattle, and pig products. *Salmonella* infections of food animals play an important role in public health and particularly in food safety, as food products of animal origin are considered to be the major source of human *Salmonella* infections(Destaw*et al.*, 2020).Food-borne diseases occur commonly in developing countries because of 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, 2004).

The burden of food borne illnesses is tremendous, affecting 10% of global population with 33 million deaths annually. Numerous factors contribute to diarrheal diseases, and *Salmonella enterica* causes food borne illnesses with significant public health impact(Wang*et al.*, 2019).Most *Salmonella enterica*serovars cause gastroenteritis that is a self-limiting infection that does not require antibiotic treatment. More serious *Salmonella* infections such as enteric fever and infections in the elderly, infants, and immuno compromised individuals may require antibiotic therapy. Some *Salmonella* have acquired resistance to antimicrobials used in the treatment of salmonellosis, which can lead to difficulty in managing these infections. Antimicrobial-resistant *Salmonella* in food animals could potentially lead to human infections with food borne resistant bacteria (Glenn*et al.*, 2011).

Antimicrobial resistance is a global public health, animal health and welfare concern. Its development and spread is influenced by both human and animal antibiotic use.Veterinary antibiotics are medicines used to cure animals of bacterial infection(APVMA, 2017).Antibiotics used against bacteria are the most commonly recognized form of antimicrobials.

Resistance is the ability of bacteria against the antagonizing effect of an antibacterial agent upon reproduction prevention or bactericidal(Cesurand Demiroz, 2013).Antibiotics are used in food animal production to promote growth and to prevent (prophylactic), treat (therapeutic) and control (metaphylactic) infectious diseases. The extensive use of antibiotics in the animal production systems for the purposes mentioned above has contributed to the development of drug-resistant bacteria. The close association of these bacteria has also been identified in the human food chain(Divek*et al.*, 2018).

Antibiotic resistance in foodborne pathogens such as *Salmonella* is a major concern for public health safety(Divek*et al.*, 2018).Antibiotic resistant *Salmonella*is a major global health concern owing to the increase in resistance to conventional antibiotics and the rise in multidrug resistance in recent years(Van Honert*et al.*, 2018).Infections that are difficult to treat or untreatable increase with AR, particularly as resistance to multiple antimicrobials increases(Frye *et al.*, 2011).Bacteria, not humans or animals, become antibiotic-resistant. These bacteria may infect humans and animals, and the infections they cause are harder to treat than those caused by non-resistant bacteria(WHO, 2020).

Resistant bacteria are transferred from food animals to man via the food chain (Maka and Popowska, 2016). Spread of resistance can involve the movement of resistant pathogenic bacteria themselves from one ecological niche to another (e.g., between animal and humans) or by indirect means (e.g., via the food chain and water supply)(JETACAR, 1999).Pathogens and antibiotic resistance can arise from multiple sources, for example, the endemic pathogens circulating in the farm or pathogens introduced through the feed, water, workers, and equipment. Slaughterhouse is one of the most important risk factors that can act as a mixing vessel for any kinds and numbers of pathogens including *Salmonella* present in animals collected from different unrelated farms. Under favorable circumstances, such pathogens can be disseminated via the meat to the consumers (Jiang *et al.*, 2019).

Farm and slaughterhouse workers, including veterinarians, have a high risk of being colonized or infected with resistant bacteria via direct contact with infected or colonized food animals and derived products. *Salmonella enterica* have assumed epidemiological importance due the large number of outbreaks and infections caused by contaminated water and food consumption(Silva*et al.*, 2013).

Antimicrobial resistance (AMR) both in human and veterinary medicine has reached alarming levels in most parts of the world and has now been recognized as a significant emerging threat to global public health and food security(FAO, 2016). Currently, the prevalence of antimicrobial resistant pathogens has increased at a speed inversely proportional to the approval of new drugs (Silva*et al.*, 2013).Antimicrobial use has been identified as one of the driving factors behind the increase in AR, and use of antimicrobials in animals has been the subject of much debate (Frye *et al.*, 2011).

In Ethiopia, large populations of livestock are kept and similarly large populations of humans are in contact with animals and/or with animal products. Abattoir is among the places where animals are collected together from different farms or areas and there might be possibilities of contamination of meat and abattoir environment from sub-clinically ill animals when appropriate examinations are not employed on animals to be slaughtered. As a result, zoonotic pathogens can be transmitted to humans via ingestion or contact with contaminated meat and cancause public health

impacts. In Haramayaworeda, like other parts of Ethiopia, different antibiotics are used to treat *Salmonella* in animals and it may be resistant to these antibiotics. And there was scarce information on prevalence and antibiotic resistant *Salmonella* from animals slaughtered at Haramaya municipal abattoir, eastern of Ethiopia. Therefore, the objectives of this study were

To assess the prevalence of *Salmonella* from sheep and goats slaughtered in Haramaya municipal abattoir

To determine the antibiotic susceptibility of *Salmonella* isolates from slaughtered sheep and goats in Haramaya municipal abattoir

LITERATURE REVIEW

GENERRAL OVERVIEW OF SALMONELLA

The genus *Salmonella* contains more than 2400 serotypes based on their somatic (O), flagellar (H) and occasionally capsular antigens. *Salmonella* are rod-shaped, Gram-negative, non-sporulating organisms.All salmonellae except *S. enterica* serotype Pullorumand *S. enterica* serotype Gallinarum are motile. Motility is mediated by peritrichous flagella(McVey*et al.*, 2013).As other Enterobacteriaceae they are oxidase negative but catalase positive. They are non-lactose fermenter that is why the colonies of *Salmonella* species have pale-straw colour on MacConkey agar. Most of *Salmonella* species are H_2S, indole and citrate positives. Salmonellosis is considered as one of the most important life threating bacterial zoonotic disease of human as well as animals(El-Ghany, 2020).The majority of salmonellae of veterinary importance belong to *S. enterica* subspecies *enterica.* The subspecies are further qualified by the serotype to give a final designation such as *S.enterica* subspecies *enterica* serotype *Typhimurium*(Quinn*et al.*, 2011).In humans, *Salmonella enterica*Typhi (*S. Typhi*) and *Salmonella enteric* Paratyphi (*S. Paratyphi*) cause typhoid fever and paratyphoid fever, respectively, while salmonellosis is an overarching term which includes invasive infection with all serovars of *Salmonella*, as well as the normally gut restricted infections of food poisoning(El-Ghany, 2020).

Epidemiology

Salmonella species are ubiquitous geographically and zoologically(McVey*et al.*, 2013).*Salmonella* is found worldwide in cold- and warm-blooded animals (including humans), and in the environment. Some of the *Salmonella* are host-specific, whereas others may infect a wide variety of animal species. Some *Salmonella* serotypes such as *Salmonella Pullorum* and *Salmonella Gallinarum* in poultry, *Salmonella Choleraesuis* in pigs and *Salmonella Dublin* in cattle are relatively host-specific. In contrast, *Salmonella Typhimurium* has comparatively wide host range produces gastroenteritis, sometimes leading to septicaemia, in cattle, horses, pigs, humans, and other species(Vegad and Katiyar, 2008).

Clinical outbreaks are correlated with depressed immune states. Clinical disease may develop from subclinical and latent infections if affected animals are stressed. The stress factors which have been most often associated with the development of clinical salmonellosis are: intercurrent infections, transportation, overcrowding, pregnancy, extreme ambient temperatures, water deprivation, sudden changes in rations altering the intestinal flora, the ingested number of salmonellae, the virulence of the infecting serotype or strain and the susceptibility of the host. All animals are at increased risk of developing disease if their normal flora is disrupted (e.g., stress and antibiotics). These circumstances render animals susceptible to exogenous exposure or activation of

silent infections(Quinn *et al.*, 2011; McVey*et al.*, 2013).

Humans appear to be susceptible to all *Salmonella* serotypes, with typhoid fever caused by *S. Typhi*, a disease restricted to humans, and infections with other serotypes being food-borne zoonoses. Whether a person develops disease following ingestion of salmonellae from the environment depends upon the dose of organisms, the serotype of *Salmonella*, and the colonization resistance of the infected individual(McVey*et al.*, 2013).

Table 1:*Salmonella* species of clinical importance and the consequences of infection

Salmonella species	Hosts	Consequences of infection
Salmonella Typhimurium	Cattle, horses, sheep and goats, pigs, dogs and cats, poultry	Enterocolitis and septicaemia
	Humans	Food poisoning
Salmonella Dublin	Cattle, sheep and goats, horses, dogs, pigs	Abortion, Subclinical faecalexcretors, Enterocolitis and septicaemia
Salmonella Choleraesuis	Pigs	Enterocolitis and septicaemia
Salmonella Pullorum, *S. gallinarum*	Chicks, Adult birds	Pullorum disease, Fowl typhoid
Salmonella Anatum	Cattle, poultry, dogs and cats, horses	Septicemia, diarrhea, abortion
Salmonella Arizonae	Turkeys, sheep and goats	Arizona or paracolon infection
Salmonella Enteritidis	Poultry	Often subclinical in poultry
	Humans	Food poisoning
Salmonella Newport	Cattle, horses	Fever and diarrhea, weakness

Source:(Vegad and Katiyar, 2008; Quinn *et al*., 2011).

PATHOGENESIS

The bacteria adhere to enterocytes through fimbriae and colonize the small intestine. They then penetrate enterocytes, where further multiplication occurs before they cross the lamina propria. The virulence of *Salmonella* serotypes relates to their ability to invade and replicate in epithelial cells. Because salmonellae are facultative intracellular organisms which survive in the phagolysosome of macrophages, they can dodge the bactericidal effects of antibody and complement. They continue toproliferate, both free and withinmacrophages. Survival within macrophages is necessary for development of systemic disease. Many *Salmonella* infections do not progress further. Further multiplication ultimately leads to septicaemia, with localization of bacteria in many organs and tissues. This includes the spleen, liver, meninges, brain, and joints (Vegad and Katiyar, 2008).

Many of the virulence features of salmonellae are encoded on *Salmonella* pathogenicity islands (SPI) and on virulence plasmids. Once ingested, salmonellae must survive the barrier of gastric acid, and the organism possesses a number of strategies to avoid or repair damage caused by acid stress. There are two

principal types of acid tolerance response, one induced during the exponential growth phase and the other which operates during the stationary phase of growth (Quinn *et al.,* 2011). Stationary phase salmonellae appear best suited to initiate disease, because under these conditions, RNA polymerase initiates transcription of genes responsible for acid tolerance and subsequent survival through the stomach(McVey *et al.,* 2013).

Following attachment to the surface of intestinal mucosal cells, the bacteria induce ruffling of cell membranes. This ruffling is part of the mechanism whereby the organisms are taken up into non-phagocytic cells and is now known to be one of the functions encoded by genes on SPI-1. This pathogenicity island is found in all serotypes of *S.enterica*analysed to date and one of its major effectors is a Type III secretion system (TTSS). The TTSS is a complex of proteins which forms a needle like structure for the transfer of virulence factors from the bacterium into the host cell. Other products transferred by the TTSS activate secretory pathways and alter ion balance within the cell. In addition, effector proteins result in neutrophil recruitment, and the resulting inflammation, together with the disturbance of fluid and ion balance causes diarrhea(Quinn *et al.,* 2011).

TRANSMISSION

Salmonellae are spread by direct or indirect means. Infected animals are the source of organisms which they excrete and infect other animals directly, or indirectly by contamination of the environment, mainly feed and water supplies(Vegad and Katiyar, 2008). It is primarily transmitted by the fecal–oral route, often through ingestion of contaminated food and water (McVey *et al.,* 2013). Infection is acquired by ingestion of material contaminated with infected faeces from either clinically ill animals, or carrier animals. The carrier state is particularly important in the maintenance and transmission of the disease. Human salmonellosis is generally foodborne and is contracted through consumption of contaminated food of animal origin such as meat, milk, poultry and eggs(Jemaland Ebsa, 2016).

CLINICAL FEATURES

The disease can be described as three syndromes: Septicaemia, acute enteritis, and chronic enteritis. Septicaemia is the characteristic formin newborn foals and calves and guinea-pigs. Affected animals show profound depression, dullness, prostration, high fever, and death within 24-48 hours. Acute enteritis is the common form in adult animals of all species. There is a high fever with severe fluid diarrhoea, sometimes dysentery. Other signs include anorexia, and faeces having a putrid smell, containing mucus, and sometimes blood and fibrinous casts. Pregnant animals usually abort. In all species, severe dehydration and toxaemia occur; the animal becomes recumbent and dies in 2-5 days. Chronic enteritis is a common syndrome in pigs and occurs sometimes in cattle and adult horses. In calves, there is persistent diarrhoea, with the occasional passage of spots of blood, mucus, and firm fibrinous casts, moderate fever, loss of weight and emaciation (Vegad and Katiyar, 2008).

In human disease, the clinical pattern of salmonellosis can be divided into four disease patterns namely enteric fever, gastroenteritis, bacteremia and other complications of non-typhoidal salmonellosis as well as chronic carrier state(Afolami and Onifade, 2018). Enteric fever: *Salmonella* Typhi causes typhoid fever whereas Paratyphi A, B and C cause paratyphoid fever with symptoms which are milder and a mortality rate that is lower for the latter. Both serotypes are solely humanpathogens. Infection typically occurs due to

ingestion of food or water contaminated with human waste.Roughly 10% of patients may relapse, die or encounter serious complications such as typhoid encephalopathy, gastrointestinal bleeding and intestinal perforation. Relapse is the most common occurrence probably due to persisting organisms within reticulo endothelial system (RES).Gastroenteritis: Non-typhoidal salmonellosis or enterocolitis is caused by at least 150 *Salmonella* serotypes with *Salmonella Typhimurium* and *Salmonella Enteritidis* being the most common serotypes in the United States. Infection always occurs via ingestion of water or food contaminated with animal waste rather than human waste. The emergence of multidrug-resistant *S. Typhimurium* DT(definitive type)104 has been associated with outbreaks related to beef contamination and resulted in hospitalization rates twice than that of other food borne salmonellosis. Bacteremia and other complications of non-typhoidal salmonellosis: About 8% of the untreated cases of salmonellosis result in bacteremia. Bacteremia is a serious condition in which bacteria enter the bloodstream after passing through the intestinal barrier. It has been associated with highly invasive serotypes like Cholearaesuis or Dublin.Chronic carrier state: Salmonellosis can be spread by chronic carriers who potentially infect many individuals, especially those who work in food-related industries. Factors contributing to the chronic carrier state have not been fully explained. On average, nontyphoidal serotypes persist in the gastrointestinal tract from 6 weeks to 3 months, depending on the serotypes(Pui*et al.*, 2011).

Diagnosis, isolation and identification of *Salmonella*

Diagnosis is based on the identification of the *Salmonella* either from faeces or from tissues collected aseptically at necropsy, environmental samples or rectal swabs, feedstuffs and food products(Demirbilek, 2018). In cases of intestinal infection, fecal samples are collected. Fresh fecal samples are placed onto nutrient, for example, blood agar, and one or more selective media, including Xylose Lysine Desoxycholate Agar (XLD), MacConkey agar and brilliant green agar. In systemic or septicaemic disease, a blood sample is collected for standard blood culture. Spleen and bone marrow are cultured for the *Salmonellae* when postmortem diagnosis of systemic salmonellosis is required(McVey*et al.*, 2013).Organism may be identified using a diversity of techniques that may include pre-enrichment to resuscitate sub lethally damaged *Salmonellae*, enrichment media that comprise inhibitory substances to inhibit competing organisms, and selective agars to differentiate *Salmonellae* from other enterobacteria. Various biochemical, serological and molecular tests can be used to the pure culture to allow for a reliable verification of an isolated strain (Demirbilek, 2018).Molecular techniques are now frequently used for the detection of salmonellae in clinical and environmental samples, a major advantage being the speed with which a result can be obtained. PCR and real-time PCR-based tests can be applied directly to samples. Serological tests such as ELISA and agglutination techniques are of greatest value when used on a herd or flock basis(Quinn*et al*., 2011).

TREATMENT

Antibiotic therapy should be based on results of susceptibility testing because R-plasmids coding for multiple resistance are comparatively common in salmonellae (Quinn*et al.*, 2011).Nursing care and appropriate antimicrobial therapy is the principal treatment for the enteric andsystemicform of salmonellosis. Since salmonellae survive in the phagocytic cell, the antimicrobial drug shouldbe one that penetrates the cell (McVey*et al.*, 2013). Examples

of those that distribute in thismanner include ampicillin, amoxicillin, gentamicin, trimethoprim–sulfonamides, and chloramphenicol/florfenicol. Fluid and electrolyte replacement therapy is required to counteract dehydration and shock(Quinn*et al*., 2011).

Prevention and control

Control is based on reducing the risk of exposure to infection. Salmonellosis is controlled through strict attention to protocols designed to curtail the spread to susceptible animals of any contagious agent found in feces(McVey *et al.*, 2013). Protective clothing and footwear should be worn by personnel entering into farm units. Effective routine cleaning and disinfection of buildings and equipment are essential. Overstocking and overcrowding should be avoided. Steps should be taken to prevent contamination of foodstuffs and water. Vaccination procedures are used in cattle, sheep, poultry and pigs (Quinn*et al*., 2011).

ANTIBOITIC RESISTANCE

Antibiotics are chemical agents that prevent bacterial growth by stopping the bacterial cells from dividing or by killing them. The term antibiotic resistance (AR) is used to refer to the ability of bacteria to withstand the effect of one or more antibiotic agents at clinically attainable concentrations, usually resulting in therapeutic failure(FAO, 2016).Antibiotic resistance occurs when a bacterium that is normally susceptible to an antibiotic becomes able to grow in the presence of antibiotic levels that would normally suppress growth or kill susceptible organisms. Clinical resistance occurs when the bacterium can continue to divide in the presence of the antibiotic concentrations that normally occur during treatment (therapeutic doses) and the antibiotic is no longer effective for treatment(JETACAR, 1999).

Development of antimicrobial resistance by microbial pathogens and commensals represents a major threat to animal health and public health. Antimicrobial resistance is a concern for animal health, but little is known about the magnitude of this problem(McEwen and Paula, 2002). The potential threat to human health resulting from inappropriate antibiotic use in food animals is significant, as pathogenic-resistant organisms propagated in these livestock are poised to enter the food supply and could be widely disseminated in food products.Commensal bacteria found in livestock are frequently present in fresh meat products and may serve as reservoirs for resistant genes that could potentially be transferred to pathogenic organisms in humans(Landers*et al*., 2012).

TYPES OF ANTIBIOTICS RESISTANCE

Bacteria can be naturally resistant to certain antimicrobial groups or they can obtain resistance to antimicrobials through a variety of mechanisms (FAO, 2016).The bacterial abilities to adopt various strategies for antibiotic resistance are all genetically encoded. These genetic mechanisms are classified into intrinsic and acquired resistance (Kumar and Singh, 2013).

INTRINSIC RESISTANCE

Intrinsic resistance also known as primary or innate resistance describes a status of general insensitivity of bacteria to a specific antimicrobial agent (Van Duijkeren *et al.*, 2018).It is the innate ability of bacteria to resist antimicrobial effect of particular antibiotic class through its inherent structural or functional characteristics(Kumar and Singh, 2013).This kind of resistance is caused by the structural characteristics of bacteria and it is not associated with the use of antibiotics.It develops as result of the natural resistance, or the microorganisms not including the structure of the target antibiotic, or antibiotics not reaching to its target due to its characteristics(Cesurand Demiroz, 2013).Intrinsic

resistance is a genus- or species-specific property of bacteria (FAO, 2016).

ACQUIRED RESISTANCE

Acquired resistance is the ability of bacteria to resist the activity of a particular antimicrobial agent to which it was earlier susceptible. This is mediated by vertical (e.g., mutation) or horizontal gene transfer (e.g., transformation, transduction or conjugation) which brings changes in bacterial genome. This brings alteration in the bacterial structural and functional characteristics leading to resistance against a particular antibiotic (Kumar and Singh, 2013).Present only in certain strains of a species or genus; acquired resistance is a strain-specific (FAO, 2016). This kind of resistance occurs due to mainly structures of chromosome or extra chromosomal genetic elements (Cesur and Demiroz, 2013).

CHROMOSOMAL RESISTANCE

Chromosomal resistance arises from mutations in bacterial chromosome (Cesurand Demiroz, 2013). Mutation is defined as "spontaneous change in DNA sequence within the gene". It is a random event. A change within a single nucleotide base pair brings corresponding change in one or more amino acids. This consequently changes the affinity of antimicrobials towards the targeted site(Kumar and Singh, 2013).

When an antimicrobial attacks a specific target, whether it be cell wall peptides, ribosomes or nuclear DNA, it locks on to specific receptors on the target. Bacterial mutation results in the alteration of these receptors so that the antimicrobial can no longer fit and the organism is thus resistant to the effects of the antimicrobial(Dennis, 2017).In bacteria, mutations naturally occur due to errors in DNA polymerase activity, insertions, deletions, and duplications(Kumarand Singh, 2013).This can be a result of structural changes in bacterial cells(Cesur and Demiroz, 2013).Antimicrobials do not induce mutations but may exert a selecting out of resistant strains by suppression of susceptible bacteria(Hsu, 2008).

EXTRA CHROMOSOMAL RESISTANCE

Extra chromosomal resistance depends on extra chromosomal genetic elements that can be transferred in various ways like plasmids, transposons and integrons (Cesurand Demiroz, 2013).Genetic elements like plasmid, transposon and integrons carry the antibiotic resistance genes. These elements act as vectors and transfer resistance genes to other bacteria belonging to the members of the same species, or to another species or even a different genus (Kumar and Singh, 2013).

A plasmid is a small DNA fragments within a cell that is physically separated from a chromosomal DNA and can replicate independently. While the chromosomes are big and contain all the essential genetic information for living under normal conditions, plasmids usually are very small and contain only additional genes that may be useful to the organism under certain situations or particular conditions. In nature, plasmids often carry genes that may benefit the survival of the organism, for example antibiotic resistance(Dennis, 2017).Plasmid genes are usually responsible for the generation of enzymes which inactive antibiotics (Cesurand Demiroz, 2013).

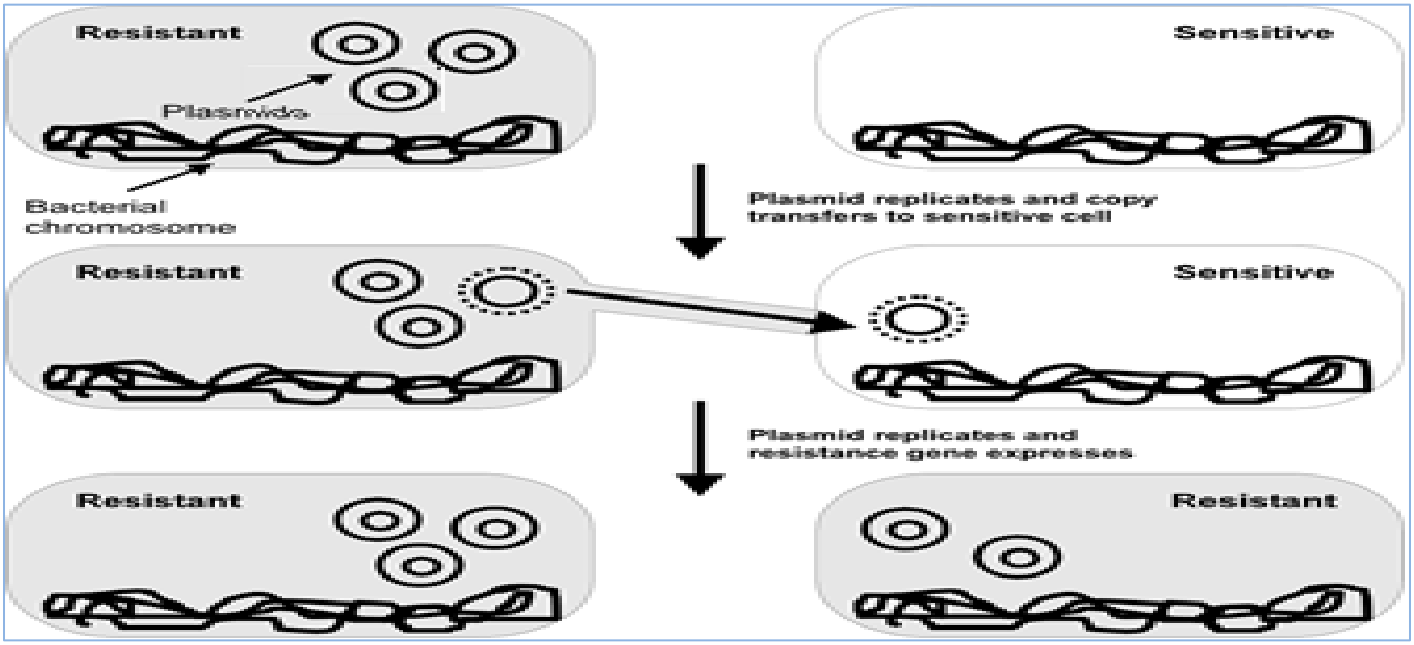
Transposon is a DNA sequence that can change its position within a genome, sometimes creating or reversing mutations and altering the cell's genome size. Also known as jumping genes because of their mobility they are the shuttles that also can mobilize genetic material from bacterial chromosome to plasmid and vice versa (Dennis, 2017).

Integron is a genetic element that can catch and carry genes, particularly those responsible for AMR. On their own they are immobile and rely on transposons to carry them around. Integrons are interspecies transferrable meaning that resistance genes can be transferred from one bacterial species to an entirely different one. Integrons often carry the resistance genes for several antimicrobials at the same time. Thus overuse of a less crucial antimicrobial, such as tetracycline may result not only in selection for resistance to tetracyclines but also to other, possibly more critically important, antimicrobials(Dennis, 2017).

Horizontal gene transfer is genetic modification by microorganisms themselves and is a very efficient and rapid way of transferring resistance between populations. It is the most relevant mode of resistance emergence and spread in microbial populations (Dennis, 2017). Transformation, conjugation and transduction are the three different mechanisms in bacteria for horizontal gene transfer (HGT) (Kumar and Singh, 2013).

Transformation involves the uptake of short naked DNA fragments and their homologous recombination in naturally competent bacteria (Kumar and Singh, 2013). It is the ability of microorganisms to utilise snippets of free DNA from their surroundings. DNA from dead cells gets cut into fragments and exits the cell. The free-floating DNA can then be picked up by competent cells (Dennis, 2017).Bacteria capable of taking up DNA from the environment are termed competent (Van Hoek *et al*., 2011). Exogenous DNA is taken up into the recipient cell from its surroundings through the cell membrane. The exogenous DNA is incorporated into the host cell's chromosome via recombination. Transformation results in the genetic alteration of the recipient cell (Dennis, 2017).

Conjugation involves the cell to cell contact via sexual pilli to transfer the piece of DNA (Kumar and Singh, 2013). Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells (Dennis, 2017). Sex pilli is formed by the responsible genes which is present only in the donor bacteria. Ultimately, the piece of DNA fragments having the resistance genes is transferred from resistant donors to previously susceptible bacteria(Kumar and Singh, 2013). During conjugation the donor cell provides a conjugative or mobilizable genetic element that is most often a plasmid or transposon.Transformation and transduction do not involve cell-to-cell contact(Dennis, 2017).



Donor Recipient

Figure 1: Movement of antibiotic-resistance genes from one bacterium to another by conjugation(JETACAR, 1999).

Transduction involves the transfer of DNA from one bacterium into another via bacterial viruses called bacteriophage (Kumar and Singh, 2013). It is the process by which viruses that prey upon bacteria, known as bacteriophages, can transmit genetic material from one organism to another (Dennis, 2017). It is less commonly linked with the transfer of antibiotic resistance genes compared to transformation and conjugation (Kumar and Singh, 2013).

Antibiotic resistant *Salmonella* in raw meat

Antimicrobial resistant *Salmonella* have been isolated globally from human infections, clinically ill animals, healthy food animals, food animal products, and fresh produce (Frye and Jackson, 2013). The antibiotic resistance of *Salmonella* species to a single antibiotic was first reported in the early 1960s. Since then, the isolation frequency of *Salmonella* strains resistant to one or more antibiotics have increased in the Saudi Arabia, United States, United Kingdom and other countries of the World. And multi drug resistance among many *Salmonella*serovars has become a big challenge to infectious disease management (Afolami and Onifade, 2018).

Strains of *Salmonella* spp. with resistance to antimicrobial drugs are now widespread in both developed and developing countries. In developed countries it is now increasingly accepted that for the most part such strains are zoonotic in origin and acquire their resistance in the food-animal host before onward transmission to humans through the food chain (Threlfall, 2002).The consumption of multidrug-resistant *Salmonella* isolates along with a raw meat dish is directly relevant to the global public health crisis of antimicrobial resistance (Gould and Russell, 2003).Microorganisms contaminate meat in abattoir during slaughtering and spread from the exterior part of animals and from the intestinal tract. Moreover, they are added from knives, cloths, air, workers, carts, boxes, and equipments. These microorganisms begin to multiply and spoil the meat if the environment is suitable for their growth. Food handlers in a carrier state or having an acute infection play a significant

role in transmitting infection (Ikeme, 1990; Bhandare *et al.*, 2007; Wales*et al.*, 2011).

In Ethiopia, eating raw meat is an indication of wealth. There is a popular traditional dish known locally as "KITFO" which is prepared from minced beef and, most of the time, it is consumed raw or moderately cooked. The habit of raw meat consumption and the presence of *Salmonella* in minced beef create a conducive environment to develop infection in the community (Wondimu*et al.*, 2017).Because of the impact on human health, zoonotic transmission, and ability to acquire antimicrobial resistance, *Salmonella* has been chosen as the sentinel organism for food borne disease and for antimicrobial resistance monitoring. Due to observed increases in morbidity and mortality in antimicrobial resistant infections, it has been suggested that resistant *Salmonella* are more virulent than sensitive strains (Frye and Jackson, 2013).

Mechanisms of Antibiotic Resistance in *Salmonella*

There are many mechanisms that bacteria, including *Salmonella,* exhibit to protect themselves from antibiotics and understanding the mechanisms by which bacteria resist antibiotics will become critical to solving the crisis. Antibiotic resistances in *Salmonella* species can result from enzymatic inactivation, decreased permeability, and development of efflux pump systems, alteration of target sites and in most cases in many serovars the overproduction of target sites to overwhelm the used antibiotics (Afolami and Onifade, 2018).

INACTIVATION OF THE ANTIMICROBIAL AGENT

This is a common cause of resistance that destroys or inactivates antimicrobial agents (Harriet and Nandita, 2014). Bacteria use enzymatic hydrolysis for inactivation of antibiotics. The production of β-lactamases that hydrolyze the β-lactam ring of penicillins is the classical example of antibiotic inactivation. The enzymes can often be excreted by the bacteria, inactivating antibiotics before they reach their target within the bacteria (Shimels, 2020).

EFFLUX OR TRANSPORT OF THE ANTIMICROBIAL

Efflux pumps are transporter proteins involved in the removal of toxic substances from the interior of the cell to the external environment. Efflux pumps in bacteria are major contributors to drug resistance; they extrude antibiotics to the exterior of the organism. The genes of efflux pumps can be intrinsic or acquired. The intrinsic efflux mechanism of resistance is chromosomally encoded and is activated by environmental signals or by mutation in regulatory genes (Shimels, 2020).

MODIFICATION OF THE ANTIMICROBIAL TARGET SITE

Modification of the antibiotic target site makes the antibiotic unable to bind properly. Microorganisms cannot evade antimicrobial action by dispensing with them entirely because of the vital cellular functions of the target sites. In this mechanism, bacteria found ways to alter the targets of antimicrobial agents (Shimels, 2020).

REDUCED PERMIABILIT OF THE ANTIMICROBIAL AGENT

Pathogens often become resistant simply by preventing entrance of the drug. The alteration in membrane permeability occurs when new genetic information changes the nature of proteins in the membrane. Such alterations change a membrane transport system pores in the membrane, so an antimicrobial agent can no longer cross the membrane(Harriet and Nandita, 2014).

ANTIBIOTIC RESISTANCE OF SALMONELLA SPECIES

Antibiotic resistant *Salmonella* species counteract or inactivates the action of different antibiotics through its resistance mechanisms (see Table 2).

Table 2: Mode of action and resistance mechanisms of antibiotics used to treat *Salmonella*

Antibiotics	Mode of action	Resistance mechanisms	Resistance genes
β-lactams- e.g., penicillins, cephalosporins	Inhibits cell wall synthesis	β-Lactamases, Modification of porin (*omp*F), Efflux of β-lactam(*omp*C)	*omp*C, *omp*F, *bla*OXA-1,
Aminoglycosides-e.g., Neomycin, Kanamycin Gentamycin, Streptomycin	Inhibits protein synthesis	Enzymatic modification and inactivation of aminoglycoside	*aac*C(3), *aac*C(3'),*aad*A, *str*A,*str*B
Phenicols e.g., Chloramphenicol,	Inhibits protein synthesis	Efflux pumps(*flo*R, *cml*A),chloramphenicol acetyltransferase	*flo*R, *cml*A, *cat*1
Tetracyclines	Inhibits protein synthesis	Efflux pumps, Modification of rRNA target, Inactivation of compound	*tet*(A),*tet*(B), *tet*(C), *tet*(D), *tet*(G), *tet*(H)
Sulfonamides	Inhibits metabolism	Dihydropteroate synthase	*Sul*1, *sul*2 *sul*3

Source:(JETACAR, 1999; Akinyemi and Ajoseh, 2017).

RESISTANCE TO AMINOGLYCOSIDES

Aminoglycosides exert their antibacterial action by irreversibly binding to one or more receptor proteins on the 30S subunit of the bacterial ribosome and thereby interfering with several mechanisms in the mRNA translation process (Papich and Riviere, 2018). Aminoglycosides are bactericidal. There are various mechanisms of aminoglycoside resistance, including alteration of the ribosomal binding sites, decreased uptake, decreased accumulation in bacteria, and the expression of enzymes which modify and inactivate these antibiotics. Of these mechanisms, enzymatic inactivation seems to be the most important and most common type of aminoglycoside resistance among *Salmonella* species. There are three types of aminoglycoside modifying enzyme: acetyltransferases, adenylytransferases and phosphotransferases (Maka and Popowska, 2016). Aminoglycoside inactivating enzymes may be encoded by plasmids or associated with transposons. Major, resistance genes include *str*A, *str*B, *aac*, *aad* (Akinyemi and Ajoseh, 2017).

RESISTANCE TO TETRACUCLINE

Tetracyclines possess antimicrobial activity by binding to the 30S ribosomal subunit of susceptible organisms. After binding to the ribosome, the tetracyclines interfere with the binding of aminoacyl-tRNA to the messenger RNA molecule/ribosome complex, thereby interfering with bacterial protein synthesis in growing or multiplying organisms. Tetracyclines are generally considered bacteriostatic. Resistance mechanisms include efflux, modification of the rRNA target, and inactivation of the compound. However, in *Salmonella*, active efflux systems are most commonly observed and include *tet*A, B, C, D, G and H (Frye and Jackson, 2013).

RESISTANCE TO B-LACTAMAS

β-lactam antibiotics exert their effects by preventing bacterial cell wall synthesis and disrupting bacterial cell wall integrity (Papich, 2018). They kill bacteria by inhibiting or weakening the cell wall. Most resistance to β-lactams is conferred by β-lactamases that enzymatically cleave the β-lactam ring and prevent it from bonding to and inactivating cell wall enzymes. Efflux of the β-lactam or modification of porins (e.g., *omp*F and *omp*C) is also a resistance mechanism to β-lactams. Often these different mechanisms are found in the same bacterium, resulting in high level β-lactam resistance. Most of the β-lactam resistance in *Salmonella* is encoded by horizontally acquired β-lactamases (Frye and Jackson, 2013).

RESISTANCE TO PHENICOLS

Chloramphenicol and related compounds such as florphenicol inhibit protein synthesis by binding to the 50S ribosomal subunit.The action of chloramphenicol (and florfenicol) is regarded as bacteriostatic, rather than bactericidal (Papich, 2018).Chloramphenicol has a broad spectrum of activity. It is primarily used for treatment of systemic salmonellosis caused by bacteria that are resistant to other drugs of choice (Frye and Jackson, 2013). One of the most common mechanisms of resistance against chloramphenicol in *Salmonella* is its inactivation by chloramphenicol acetyltransferases (CATs) enzyme (Maka and Popowska, 2016). Chloramphenicol resistance in *Salmonella* species can also be mediated by chloramphenicol efflux pumps encoded by the genes *cml*A and *flo*R. Resistance to chloramphenicol is highly prevalent in developing countries based on its cheapness and easy accessibility, despite its ban in developed countries, based on its toxicity (Akinyemi and Ajoseh, 2017).

RESISTANCE TO SULPHONAMIDES

They are also called folate pathway inhibitors. These are compounds that compete for substrates of the essential folic acid pathway in bacteria at two different steps, the sulfonamides, which inhibit DHPS (dihydropteroate synthase) and trimethoprim, which inhibit DHFR (dihydrofolate reductase). Both sulfonamides and trimethoprim act on the folic acid pathway in bacteria by interfering with the production of dihydrofolic acid. They have been used extensively in food animals as growth promoters in swine and for treatment of diseases such as coccidiosis in poultry. Sulfonamides are bacteriostatic when used alone or bacteriocidal when used in combination (trimethoprim-sulfamethoxazole). Resistance to both of these antimicrobials occurs by acquisition of genes encoding enzymes that do not bind these compounds. These include the *sul* genes, *sul*1, *sul*2, and *sul*3, which encode an insensitive DHPS enzyme and are found in *Salmonella* globally (Frye and Jackson, 2013).

Factors Influences the Emergence of Antibiotic Resistance

The emergence of resistance is the natural response of microbes to the presence of antimicrobial agents. Several factors contribute to the increase in antibiotic resistance by *Salmonella* species. These factors are: misuse of antibiotics,an incomplete course of antibiotics, unregulated sales of antibiotics, inappropriate prescription and dispensing practices and poor hygiene practices, the spectrum of activity of the antibiotics, the number of animals exposed to antibiotics, the total amount of antibiotic used which in turn is influenced by the dose and duration (JETACAR, 1999; Akinyemi and Ajoseh, 2017).

Transmission of Antibiotic Resistant *Salmonella* to Humans

Bacteria, particularly enteric bacteria, are commonly spread from animals to humans. Animal bacteria, including resistant strains, can spread to humans by

direct contact, through the food chain and by environmental contamination. Both pathogenic and non-pathogenic resistant bacteria can be transmitted from livestock to humans via food consumption, or via direct contact with animals or their waste in the environment.Fomites can also play an important role in the local and wider spread of resistant bacteria. Any mechanism that helps spread bacteria has the potential to transfer resistant bacteria (FAO, 2016).Spread of resistant bacteria from animals to humans can occur either by the spread of the resistant bacteria themselves (bacterial spread) or spread of the resistance genes (genetic spread) to potential human pathogenic bacteria (JETACAR, 1999).

Resistance may also be conferred by the exchange of genetic elements between bacteria of the same or different strains or species, and such transfer can occur in any environment where resistant bacteria have the opportunity to mix with a susceptible bacterial population, such as in the human or animal gut, in slurry spread on agricultural soil, or in aquatic environments (FAO, 2016).In particular, a bacterium can spread from an animal to human and then transfer its resistance gene to a human pathogen. Resistance genes that confer antibiotic resistance on one type of bacterium can be transferred to other bacteria, including from animal to human bacteria.

For many important human pathogens, resistance develops mainly as a result of the acquisition of antibiotic resistance genes by movement from a resistant bacterium into a formerly sensitive pathogen. This movement of genes occurs by a process known as horizontal gene transfer. It is possible for gene transfer to occur in animals, in the environment and in humans who have acquired animal bacteria, even if that species fails to establish itself in humans. The resistant bacteria donate resistance genes to other bacteria in the same ecological niche (e.g., animal gut, human gut, pond, river) leading to the potentially rapid and extensive transfer of genes in the bacterial population. Thus, a harmless bacterium that is resistant can donate its resistance gene(s) to a pathogen. Each animal type has its own range of bacterial flora and pathogens, some of which do not appear to spread to or colonise other animal species or humans easily. Thus, a bacterial species selected for resistance in a single animal type may not necessarily be transferred to humans. Nevertheless, it is possible that the selected resistant strain may transfer resistance genes into another bacterial species that is more easily transferred to humans. The spread of resistant organisms globally has also been documented, and presumably occurs because of the movement of the hosts (animals or human) or contaminated products (food, water) from one location to another, even across country borders and between continents (JETACAR, 1999).

Public Health Impacts

Antimicrobial resistance of bacterial origin, known as antibiotic resistance (ABR), of zoonotic food-borne pathogens is now regarded by the World Health Organization (WHO) as one of this century's leading global health challenges. The potential threat to human health from the misuse of antibiotics in food animals is significant(Van Honert*et al*., 2018).The use of antimicrobial agents in animals can result in antimicrobial-resistant bacteria and their resistance genes reaching the human population through a variety of routes. Antibiotic resistant zoonotic food-borne pathogens in food-producing animals can spread to humans via consumption of contaminated food or water, and direct contact with animals.

Antibiotic resistance can affect anyone, of any age, in any country(WHO, 2020).A concern to human health is the transfer of antibiotic resistant bacteria in food to

humans and subsequent colonization of the human intestine. This highlights the importance of correct food handling and preparation by consumers to avoid transmission because the presence of antibiotic resistant bacteria could affect the future of human health adversely as certain infections become more difficult to treat or infections occur if pathogenic antibiotic resistant bacteria are ingested(Van Honert*et al*., 2018). Most antimicrobial resistance in human pathogens comes from antimicrobial use in human medicine (McVey *et al*., 2013). However, antimicrobial-resistant bacteria of animal origin, such as *Salmonella*, can colonize the intestines of people. There are several clinical and public health consequences associated with antimicrobial drugs resistance in *Salmonella* species. These includes failure in therapy, increased burden of illness and outbreaks, increased virulence of *Salmonella* species, increased mortality and morbidity, increased cost of treatments, longer stay in hospital, increased transmission of resistant *Salmonella* strains.

Detecting Methods of Antibiotic Resistance

Antibiotic susceptibility testing (AST) methods are *in vitro* procedures used to detect antibiotic resistance in individual bacterial isolates. AST determine or predict which antibiotic will be most successful in treating a bacterial infection *in vivo*. Those laboratory-based detection methods can determine resistance or susceptibility of an isolate against any therapeutic candidates (Shimels, 2020). Those methods can also be used for monitoring the emergence and spread of resistant microorganisms in the population.

Susceptibility testing can involve *phenotypic* testing (i.e., determining the growth response of the organism of concern when exposed to the antibiotic) and *genotypic* testing (using polymerase chain reaction [PCR] to detect antimicrobial resistance genes of interest or whole genome and plasmid sequencing to detect the presence of antimicrobial resistance genes) (APVMA, 2017). Some examples of antibiotic sensitivity testing methods are dilution method (broth and agar dilution method) and disk-diffusion method. Among the available tests, agar disk-diffusion and the broth micro dilution methods are the two most commonly used methods in veterinary laboratories (Shimels, 2020).

It is essential to follow standardized procedures to ensure results are repeatable and that results from one laboratory are comparable to those from another for the compilation of common data (APVMA, 2017). Guidelines and recommendations for these are continuously updated by certain organizations worldwide, those which specify antimicrobial testing methods and interpretative criteria for veterinary pathogens are the Clinical Laboratory Standards Institute (CLSI) in the USA, OIE in EU and Calibrated Dichotomous Sensitivity (CDS-AST) in Australia.

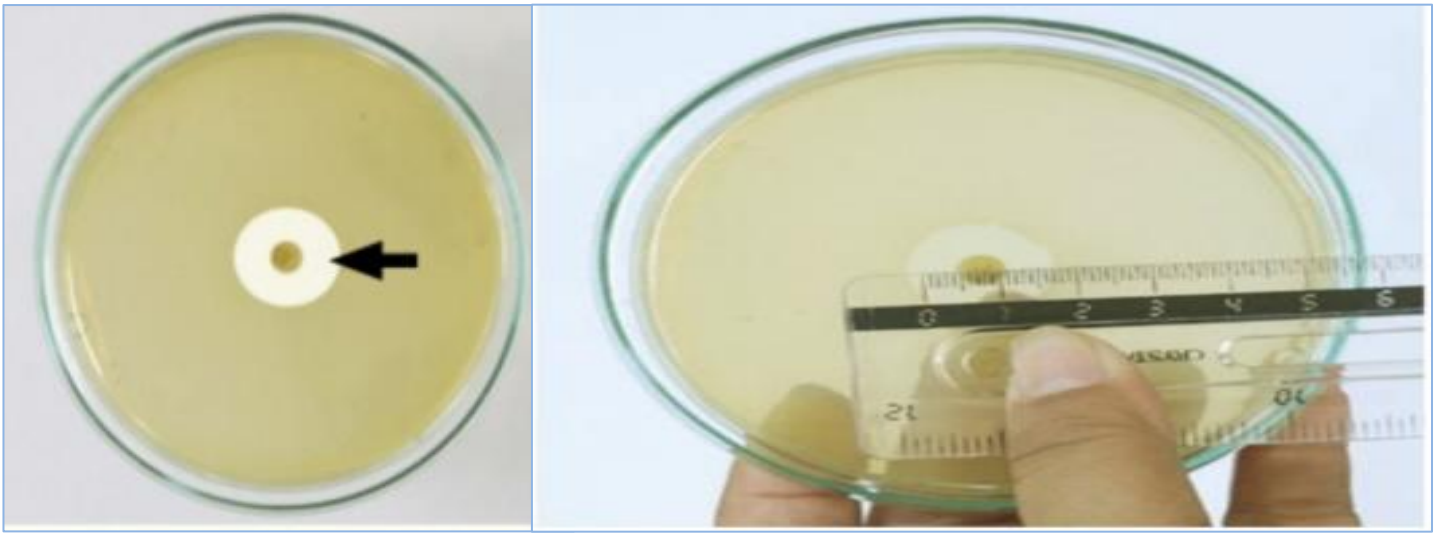
Disk-diffusion method

The application of commercially available drug-impregnated filter paper disks to the surface of an agar plate that has been inoculated to confluence with the organism of interest is called disk diffusion. Disc diffusion methods use filter paper discs impregnated with specific concentrations of antibiotics. The drug diffuses radially through the agar, the concentration of the drug decreasing logarithmically as the distance from the disk increases and results in a circular zone of growth inhibition around the disk, the diameter of which is inversely proportional to the MIC(Shimels, 2020).

The diameter of the zone shows the susceptibility of the isolate and the diffusion rate of the drug through the agar medium. The diameter of the zone of growth inhibition is measured with calipers or a ruler and

recorded in millimeters (Figure 2). Zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter. The zone diameters are interpreted on the basis of guidelines published by CLSI, and the organisms are reported as susceptible, intermediate, or resistant.

Disk diffusion can only be used to test rapidly growing organisms, for which criteria interpretation of zone sizes are available (Jones*et al.*, 2001). Generally, having larger the zone of inhibition, the lower the concentration of antimicrobial required to inhibit the growth of the organisms. The test is straightforward to perform, reproducible, and does not require expensive equipment.



A. B.

Figure 2:Disk-diffusion method of AST; (A) the zone of inhibition (arrow) is the point at which no growth is visible to the unaided eye, (B) record of the diameter of zones of inhibition(Ruangpan and Tendencia, 2004).

Dilution Method

The aim of the broth and agar dilution methods is to determine the lowest concentration of the antimicrobial that inhibits the visible growth of the bacterium being tested in either broth or on agar(OIE, 2019).Agar dilution and broth dilution are the most commonly used techniques to determine the minimal concentration of antibiotics that kill (bactericidal activity, MBC) or inhibit the growth (bacteriostatic activity, MIC) of bacteria(Wiegand*et al*., 2008).For both broth dilution methods, the lowest concentration at which the isolate is completely inhibited is recorded as the minimal inhibitory concentration or MIC. In clinical practice, this *in vitro* parameter is used to classify the tested microorganism as clinically susceptible, intermediate or resistant to the tested drug. Dilution methods are considered as reference methods for *in vitro* susceptibility testing and are also used to evaluate the performance of other methods of susceptibility testing.

Broth Dilution: The broth dilution technique of antibiotic susceptibility testing is also known as the minimal inhibitory concentration (MIC) technique (Shimels, 2020). Broth dilution uses liquid growth medium containing geometrically increasing concentrations (typically a twofold dilution series) of the antimicrobial agent, which is inoculated with a defined number of bacterial cells. It involves subjecting the isolate to a series of concentrations of antimicrobial agents in a broth environment. The

concentration of the antibiotic in each tube is double that in the previous tube (Figure 3). The final volume of the test defines whether the method is termed macrodilution, when using a total volume of 2 ml, or microdilution, if performed in microtiter plates using ≤ 500 µl per well. Tubes are incubated under optimum conditions for the test microorganism from 16 to 24 hours. After incubation, the presence of turbidity or sediment indicates growth of the organism. Antimicrobial effect could be determined by spectrophotometry or by plating counting(Wiegand *et al*., 2008).

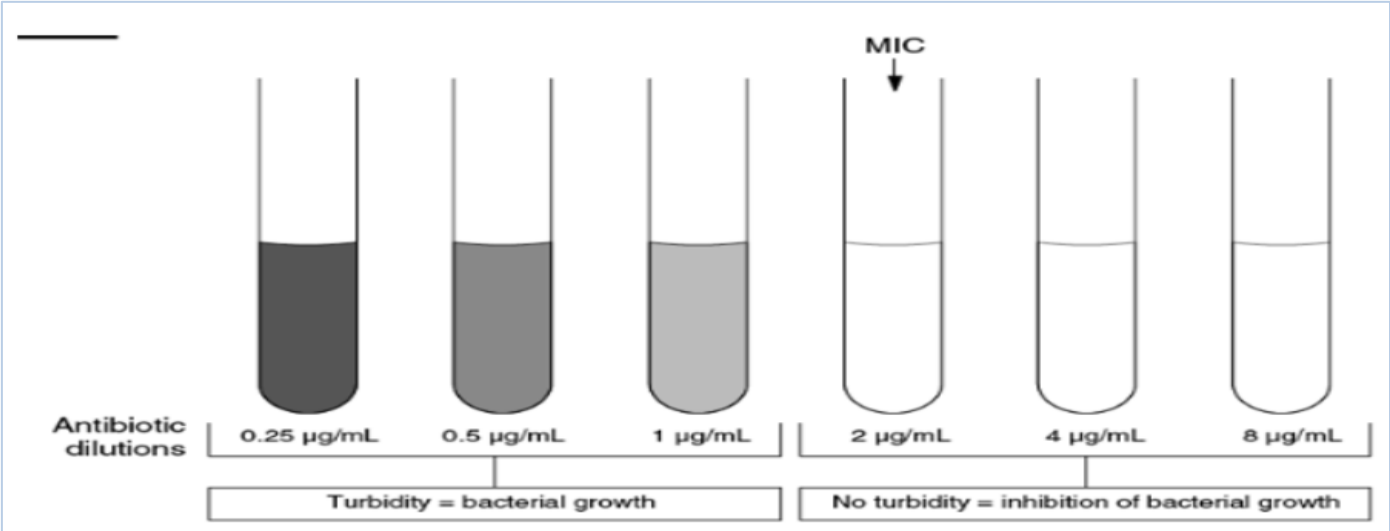


Figure 3:Diagram of broth dilution method(Hendry and Dennis, 2010).

Agar Dilution: Agar dilution involves the incorporation of varying concentrations of antimicrobial agent into an agar medium, usually using serial twofold dilutions, followed by the application of a defined bacterial in oculum to the agar surface of the plate (OIE, 2019). For agar dilution, solutions with defined numbers of bacterial cells are spotted directly onto the nutrient agar plates that have incorporated different antibiotic concentrations. After incubation, the presence of bacterial colonies on the plates indicates growth of the organism (Wiegand *et al*., 2008). The advantages of agar dilution testing include the reproducible results and satisfactory growth of most non-fastidious organisms. Agar dilution testing generally is not performed in routine clinical laboratories but can be ideal for regional reference laboratories or research laboratories that must test large numbers of isolates (Shimels, 2020).

Prevention and Control of Antibiotic Resistance

Antimicrobial resistance is a complex issue and requires human, animal and environmental health experts to work together to mitigate the continued development and spread of resistance. It is important to recognize that we cannot eliminate the emergence of resistance due to the rapid replication of bacteria, their ability to share resistance genes with other bacteria or to acquire them from their environment and any use of antimicrobials will continue to select for resistance. Therefore, our efforts must be focused on assuring that we are using antimicrobials as judiciously as possible and only in situations where the health or welfare of the patient would be compromised by a failure to treat (AVMA, 2020).

To prevent and control the spread of antibiotic resistance: only give antibiotics to animals under veterinary supervision, not use antibiotics for growth

promotion or to prevent diseases in healthy animals, vaccinate animals to reduce the need for antibiotics and use alternatives to antibiotics when available, promote and apply good practices at all steps of production and processing of foods from animal, improve biosecurity on farms and prevent infections through improved hygiene and animal welfare (WHO, 2020).

MATERIALS AND METHODS

Study area

The study area, Haramaya town, is situated in Haramaya woreda, East Hararghe zone, Oromia regional state, Ethiopia (Figure 4). It is located 21 km Northwest of Harar town and 505 km East of Addis-Ababa, capital city of Ethiopia. The altitude of this woreda ranges from 1400 to 2340 meters above sea level. It is characterized by "Woina-Dega" agro-climatic zone that receives mean annual rainfall of 775.9 mm. The monthly rainfall in the site is more than 100mm from April to September, except June 48.4 mm. The wettest month is August, 151.9 mm. The daily temperature in the site ranges from 10 ºC to 25 ºC. The livelihood in the area is based on agriculture(Shishaye and Nagari, 2016).The livestock population of Haramaya district is estimated at 71,205 heads of cattle, 15,294 sheep, 28,990 goats, 11755 donkeys, and 250 camels(Belay, 2013).

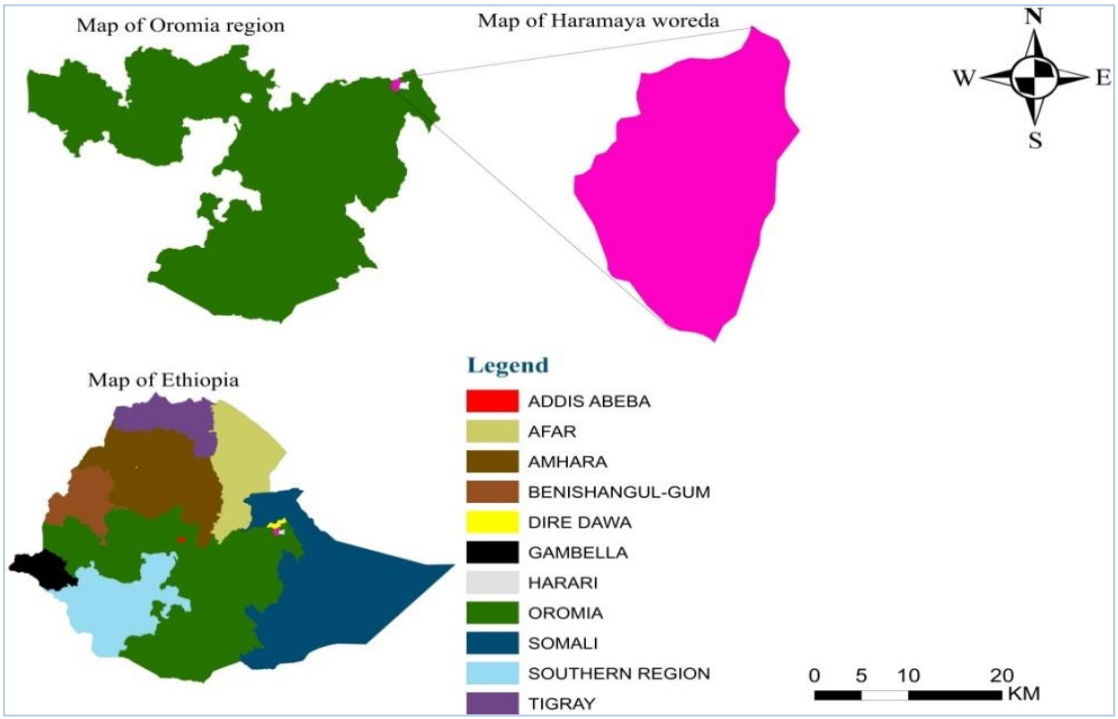


Figure 4: Map of Haramayaworeda and its location in a region.

STUDY SAMPLES

The study samples were meat sample, meat swab, ceacalfeces and skin swab of sheep and goats slaughtered at Haramaya municipal abattoir.

STUDY DESIGN

A cross-sectional study was conducted from April 2021 to August 2021 to determine the prevalence of *Salmonella* and its sensitivity to antibiotics on samples collected from sheep and goats slaughtered at Haramaya municipal abattoir.

Sample size determination

For isolation and identification of *Salmonella*, the sample size was determined based on Feyisa *et al.* (2017) expected prevalence in sheep and goats samples with 5% desired absolute precision and 95% confidence interval using the formula recommended by Thrusfield (2007):

$n = Z^2 \times P_{exp} (1\text{-}P_{exp})/d^2$, where

n is required sample size, Z is 1.96, P_{exp} is expected prevalence, and d is desired absolute precision of 0.05.Accordingly, the sample size was 196 but to increase the precision it was inflated by 16% and 228 samples was subjected to bacteriological examinations.

Sampling method, sample collection and transportation

The samples were collected randomly from slaughtered sheep and goats with proper labeling by sample type, sources and animal type (i.e., sheep or goat). All samples except skin swab (which is collected before slaughtering) were collected immediately after slaughter. The samples were collected from both sheep and goats slaughtered at Haramaya municipal abattoir during this study. Buffered peptone water, a transporting media, was used to transport the samples within required time to a laboratory. Each sample was placed within sample collection container depending on its type (e.g., feces, skin swab, meat sample, meat swab).

For skin and meat swab sampling, approximately a 10×10 cm area was sampled. Skin swab samples were sampled from thigh, abdomen and thoracic area. For meat swab, abdomen (flank), thorax (lateral), and breast were sampling sites. Sterile cotton tipped swab that fitted with wooden shaft was first soaked in 10 ml of buffered peptone water and rubbed over the sampling area of meat and skin horizontally and then vertically many times. At the end of rubbing process, the wooden shaft of soaked cotton swab was broken off by pressing against inside wall and cotton swab was left in test tube containing buffered peptone water media. The meat samples from cervical (neck), abdomen (flank) and breast were collected in screw-cap jar containing buffered peptone water (BPW). Cecum feces was placed in test tube containing 10 ml of buffered peptone water and finally packed into ice box containing ice packs to transport samples to Veterinary microbiology laboratory of Haramaya University.

Isolation and Identification of *Salmonella*

Isolation and identification of *Salmonella* was performed by conventional methods for detection and identification of *Salmonella* according to (ISO-6579, 2002).The samples was pre-enriched in Buffered peptone water media, a non-selective pre-enrichment liquid media, and then incubated at 37^0Cfor 24 hours. Then, 0.1 ml of pre-enriched samples were transferred into 10 ml of Rappaport Vassiliadis soya (RVS) broth and incubated at 42^0C for 24 hours. After incubation, a loop-full of selectively enriched culture from RVS broth was streaked onto the surface of Xylose lysine deoxycholate (XLD) agar media and incubated at 37^0C for 24 hours.

Following incubation, presence of typical and suspected *Salmonella* colonies was examined on XLD plate. The presence of pink colonies with black centers, the typical *Salmonella* colonies on XLD plate, was subjected to biochemical tests for identification after subculture on nutrient agar (Annex 8.3). All colonies of presumptive *Salmonella* were sub cultured onto nutrient agar and incubated at37^0C for 24 hours and further identified by conventional biochemical tests such as Triple sugar iron(TSI) agar, Simmon's citrate agar, Urease, methyl red (MR), Voges-Proscauer (VP) and indole test.

Antimicrobial susceptibility tests

The antimicrobial susceptibility testing of *Salmonella* isolates was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Oxoid, England) according to the recommendations of (CLSI, 2012). Biochemically confirmed *Salmonella* colonies that grown on nutrient agar were transferred into test tubes containing 0.8% saline solution until it have achieved 0.5 McFarland turbidity standards. Sterile cotton swab was dipped into the suspension, rotated several times and the bacteria swabbed uniformly over the surface of Mueller-Hinton agar plate (Oxoid, England). The plates were held at room temperature for 15-30 minutes to allow drying. Antibiotic discs with known concentration were dispensed by disc dispenser and the plates were incubated at 37^0Cfor 24 hours. The isolates were tested for the susceptibility of the following antibiotic discs: Ampicillin (AMP)10 µg, Amoxicillin-Clavulanic acid (AML)25 µg, Gentamicin (GM)10 µg, Kanamycin (K) 30µg, Tetracycline (TE) 30µg, Chloramphenicol (C) 30 µg, Nitrofurantoin (F)300µg and Erythromycin (E)15µg, discs were placed 23 mm apart and from plate edge. The plates were incubated at 37^0Cfor 24 hours and interpretation of break points was recorded according to (CLSI, 2021) (Annex 8.2).

Statistical data analysis

The collected data was entered into Microsoft excel 2010 and then, Statistical Package for Social Science (SPSS) version 2020 software were used to analyze the collected data. During data analyzing, 95% confidence of interval and 5% precession was considered to analyze the obtained data.

RESULTS

Prevalence of *Salmonella*

A total of 228 samples were collected from sheep and goats slaughtered in Haramaya municipal abattoir for detection and identification of *Salmonella*. A bacteriological examination by conventional culture and biochemical test methods was employed on 116 samples from sheep (meat sample, meat swab, skin swab and feces from cecum; each n= 29) and 112 samples from goat (meat sample, meat swab, skin swab and feces from cecum; each n= 28).

Out of total samples collected and processed, 34 (14.91%) was found positive for *Salmonella*and statistically significant variation between positive *Salmonella* and sample sources was observed (p=0.000) (see Table 4). From a total of 116 samples collected from sheep, 16 were positive for *Salmonella* isolates. Of these, 3 (10.3%), 5 (17.2%), 2 (6.9%), 6 (20.7%)were found to be *Salmonella* positive from meat sample, meat swab, skin swab and feces, respectively. Out of 112 samples collected from goats, 18 were found to be positive for *Salmonella*,7 (25%)from meat sample and 11 (39.3%)on feces from cecum of goats (See Table 3).

Table 3: Prevalence of *Salmonella* and type of sample from sheep and goats slaughtered in Haramaya municipal abattoir

Species	Type of sample	No Examined	Number of *Salmonella* isolate	
			Number positive	Total prevalence
Ovine	Meat sample	29	3(10.3%)	1.32%
	Meat swab	29	5(17.2%)	2.19%
	Skin swab	29	2(6.9%)	0.88%
	Feces	29	6(20.7%)	2.63%
	Meat sample	28	7(25.0%)	3.07%

Caprine	Meat swab	28	-	-
	Skin swab	28	-	-
	Feces	28	11(39.3%)	4.82%
Total		228	34	14.91%

Table 4: Prevalence of *Salmonella* in slaughtered sheep and goats on basis of sample source, species and sex categories

Variables	Categories	No Examined	No Positive (%)	X^2 (P- value)
Sample source	Meat sample	57	10 (17.54%)	17.836 (0.000)
	Meat swab	57	5 (8.77%)	
	Skin swab	57	2 (3.5%)	
	Cecum feces	57	17 (29.82%)	
Species	Ovine	116	16 (13.79%)	0.233 (0.629)
	Caprine	112	18 (16.1%)	
Sex	Male	152	27 (17.76%)	2.921 (0.087)
	Female	76	7 (9.21%)	
	Total	228	34 (14.91%)	

Antimicrobial Susceptibility test

All of the 34 *Salmonella* isolates were subjected to eight antimicrobials to test its *in vitro* sensitivity. The highest resistance level (100% resistance)was observed for both amoxicillin and chloramphenicol. The next most frequent resistance was encountered to ampicillin, tetracycline and erythromycin with 32 (94.1%), 24 (70.6%) and 18 (52.9%)isolates being resistant, respectively (see Figure 5). On the other hand, the isolates were 100%, 97.1% and 47% sensitive to gentamicin, kanamycin and nitrofurantoin respectively (see Table 5).All of the 34 isolated *Salmonella* exhibited resistance to three or more antimicrobials, 100% multi-drug resistance was encountered (see Table 6).

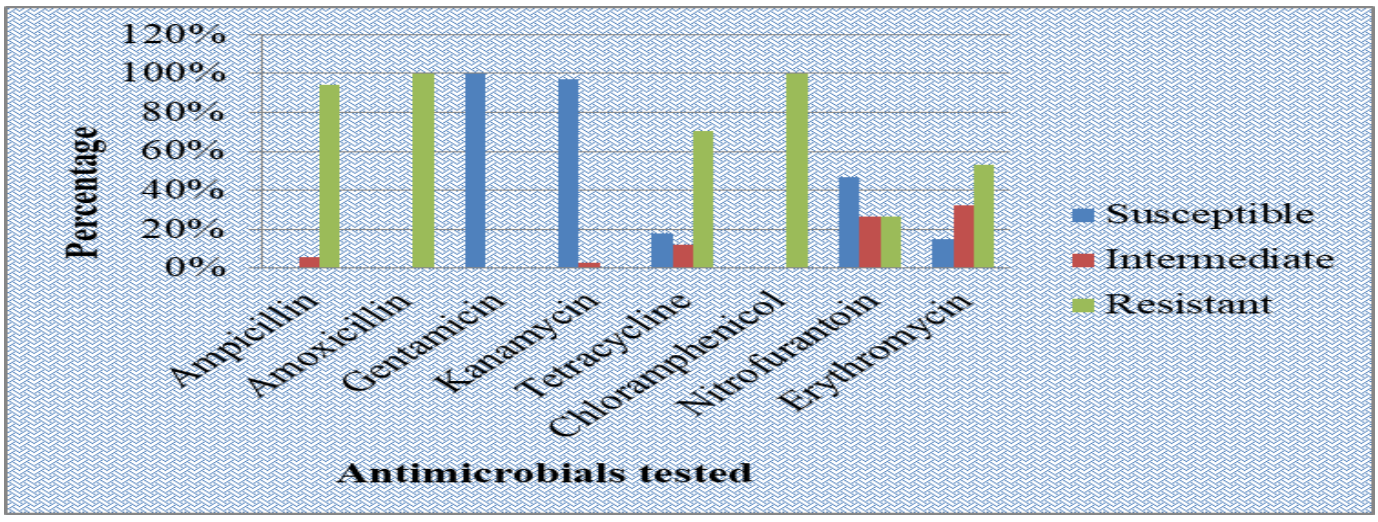


Figure 5: Percentage of activity of antimicrobials tested against isolated *Salmonella* from sheep and goats in Haramaya municipal abattoir.

Table 5: Antimicrobial drugs and *Salmonella* isolates recovered from slaughtered sheep and goats that subjected to antimicrobials

Antimicrobial drugs	Disc contents	Susceptible	Intermediate	Resistant
		Isolate (%)	Isolate (%)	Isolate (%)
Ampicillin (AMP)	10 µg	-	2 (5.9%)	32 (94.1%)
Amoxicillin (AML)	25 µg	-	-	34 (100%)
Gentamicin (GM)	10 µg	34 (100%)	-	-
Kanamycin (K)	30 µg	33 (97.1%)	1 (2.9%)	-
Tetracycline (TE)	30 µg	6 (17.6%)	4 (11.8%)	24 (70.6%)
Chloramphenicol (C)	30 µg	-	-	34 (100%)
Nitrofurantoin (F)	300 µg	16 (47%)	9 (26.5%)	9 (26.5%)
Erythromycin (E)	15 µg	5 (14.7%)	11 (32.4%)	18 (52.9%)

Table 6: Multiple antimicrobial resistances of *Salmonella* isolated from slaughtered sheep and goats in Haramaya municipal abattoir

Number of antimicrobial resistance	Antimicrobial resistance pattern (number of isolates)	Number ofresistantisolates (%)
Zero/One/Two	None	0 (0%)
Three	AML, AMP, C (3)	5 (14.7%)
	AML, TE, C (1)	
	AML, C, E (1)	
Four	AML, TE, AMP, C (10)	15 (44.12%)
	AML, AMP, F, C (1)	
	AML, AMP, C, E (4)	
Five	AML, AMP, F, C, E (1)	8 (23.53%)
	AML, TE, AMP, F, C (1)	
	AML, TE, AMP, C, E (6)	
Six	AML, TE, AMP, F, C, E (6)	6 (17.65%)

Key to Abbreviations: AMP= Ampicillin, AML= Amoxicillin, TE= Tetracycline, C= Chloramphenicol, E= Erythromycin, F= Nitrofurantoin.

DISCUSSION

Since *Salmonella* is zoonotic pathogen and several antibiotic classes of the same family are used to treat salmonellosis in both veterinary and human medicine, surveillance on prevalence and antimicrobial resistance of *Salmonella* is essential to resolve and hinder the

problem that will arise. In this study, the prevalence of *Salmonella* was 14.91%.The finding of this study is higher than that of studies conducted in Bishoftu (7.5%) by Woldemariam*et al.*(2005),Modjo (8.3%) byAkafete and Haileleul, (2011) and Addis Ababa (4.64%) byAbe *et al.*(2016). The Bishoftu, Modjo and Addis Ababa abattoirs are export standard abattoirs and the current Haramaya municipal abattoir has poor sanitation and hygienic standard in comparison with export abattoirs. Therefore, the difference in prevalence of *Salmonella* could be due to the difference in sanitary and hygienic practices employed in the abattoirs, cross-contamination of carcasses with intestinal tract contents during slaughtering, water used and the hygiene of environment up on which the animals are slaughtered are important factors(Rahimi, 2012; Kagambega*et al.*, 2013; Wondimu*et al.*, 2017). And might be because of variation in animal feeding habits, types of feed provided, housing condition(Addis*et al.*, 2011), sampling methods and culturing techniques (Li *et al.*, 2013).

However, the finding of this study was parallel with the studies conducted in Tigray region (16.4%) by Abebe *et al.*(2014),WolaitaSodo (12.5%) by Wondimu *et al.*(2017),Dire Dawa (17.7%) byBeshatu(2014)and Elfora and Luna export abattoirs (17.21%) by Feyisa *et al.*(2017). In this study, out of 228 samples collected and processed, 34 (14.91%) was found to be positive for *Salmonella*. Higher prevalence was observed in goats (7.89%) than in sheep (7.02%), which was in contrary with finding of study conducted by Sime(2021)in which higher prevalence was in sheep (4.08%) than goats (0.85%). This difference may be due to stressing factors, animal management differences within and between study areas and also variation in study population of two species. However, this finding is supported by study of Tadesse and Tesfaye,(2014) in which prevalence was higher in goats (9.01%) than sheep (8.41%).

Out of 34 *Salmonella* isolates, 17 (7.45%) were found on feces, 10 (4.39%) on meat sample, 5 (2.19%) on meat swab and 2 (0.88%) on skin swab. *Salmonella* is carried in intestinal tract of animals and excreted in their feces especially during stresses such as transportation(Aftab*et al.*, 2012). This study revealshigher prevalence of *Salmonella* in feces (7.45%) in comparison to other sample sources and it is in line with finding of Feyisa *et al.*(2017) in which *Salmonella* isolates are higher (5.73%) in cecum content. Hence in the abattoir, feces could be potential source of *Salmonella* for meat and environmental contamination and risk for abattoir workers. In the current study, *Salmonella* contamination was present on meat sample and swab at a level of 4.39% and 2.19%, respectively. This level of meat contamination is lower as compared to the 12.5% and 17.7% prevalence in study conducted by Wondimu*et al.*(2017) and Beshatu(2014), respectively. However, a report of Lidya *et al.*(2018) indicated 2.5% prevalence of *Salmonella*on carcass swab, which is in line with result of this study. This carcass contamination is public health issue for a country like Ethiopia, where there is a culture of eating raw and/or undercooked meat.

As compared to other enteric microbes, *Salmonella* most frequently present on animals body coat (Yan*et al.*, 2003).In current study, the occurrence of *Salmonella* on skin of sheep in abattoir was 0.88%. The proportion of *Salmonella*on skin was lower compared to a study by Aftab*et al.*(2012), Sibhat*et al.*(2011) and Fanta*et al.*(2021) who reported 8% at farm and 25% prevalence in slaughterhouse in Pakistan, 31% and 7.1% prevalence on hide in Ethiopia, respectively.This difference could be due to

overcrowding of animal in lairage and hair coat contamination during transportation. Thus, presence of *Salmonella* on animal body coat (skin) can be a source of infection for individuals in contact with infected animals.

Antimicrobial resistance (AMR) is an emerging problem and the most significant animal and public health challenge of this century globally(APVMA, 2017; Lambertini*et al.,* 2019).In the present study, all of the 34 *Salmonella* isolates were subjected to eight antimicrobials and each isolates were resistant to at least three antimicrobials, 100% multiple drug resistance (MDR) was detected. High percentage of multi-drug resistant *Salmonella* isolates to commonly used antimicrobials observed in this study could pose challenge to both public health and longer use of effective antimicrobials. The finding of this study regarding 100% multiple drug resistance was higher than thestudy by Zewdu and Cornelius, (2009) who reported 23.5%,Alemuand Zewde, (2012) 36.4%, and Abunna*et al.*(2017)53.84%.However, the occurrence of 100% multiple drug resistant *Salmonella* isolates in this study is consistent with study by Wondimu*et al.*(2017) and Alam*et al.*(2020)in wich both of them reported 100% multi-drug resistant *Salmonella*in Ethiopia and Bangladesh, respectively.

Ayalu*et al.*(2011)reported that *Salmonella* isolates from stool samples in Harar were resistant to commonly used antimicrobials including ampicillin, amoxicillin, tetracycline and chloramphenicol. The result of the current study also indicated resistance of *Salmonella* isolates to commonly used antimicrobials including amoxicillin, ampicillin, chloramphenicol, tetracycline, erythromycin and nitrofurantoin with resistance rate of 100%, 94.1%, 100%, 70.6%, 52.9% and 26.5%, respectively.All the isolated *Salmonella*, in present study, were exhibited 100% resistance to amoxicillin and chloramphenicol. Resistance to amoxicillin in 100%rate in this study was higher than that of study byWondimu*et al.*(2017)in which 42.9% resistance were reported, however, the current study is consistent with finding of study by Ayalu*et al.*(2011) who reported 100% resistance. High resistance percentage (100%) to chloramphenicol in this study also higher than 69.23% reported by Abunna*et al.*(2017)and 51.8% by Wondimu*et al.*(2017), however, it is supported by study of Tizazu*et al.*(2011)who reported 100% resistance of*Salmonella*isolate to chloramphenicol.

The resistance of isolated *Salmonella* to ampicillin in this study was 94.1%, which is in line with 100% resistance reported by Addis*et al.*(2011) but disagree with reports from Holeta by Abunna*et al.*(2017) and Wolaita Sodo by Wondimu*et al.*(2017) in which 38.46% and 46.4% resistance reported respectively. This difference of resistance could be due to frequent and inappropriate utilization of antimicrobials both in humans and animals, which favors selection pressure that increase resistance genes in bacteria(McGeer, 1998; Mathew*et al.*, 2007).

Gentamicin and kanamycin showed a good antimicrobial activity against isolated *Salmonella*. The sensitivity of all 34 isolates to gentamicin in this study is comparable with findings of study by Ayalu*et al.*(2011) andAbe*et al.*(2016) in Ethiopia and by Mutai*et al.*(2018)in Kenya who reported 92.8%, 92.3% and 97% susceptibilityrespectively, but contradict with the study conducted in Jimma University specialized hospitalby Tizazu*et al.*(2011) and in Hossana by Abebe*et al.*(2018)in which both study reported 100% resistance against gentamicin. The high level (97.1%) of susceptibility of isolated *Salmonella* to kanamycin in this study is in agreement with study by Abebe*et al.*(2018) who reported 100%

susceptibility but higher than the findings of Addis*et al.*(2011), Kemal*et al.*(2016) and Wondimu*et al.*(2017) who reported 41.7%, 25% and 17.9% susceptibility rate, respectively.The highest antimicrobial activity of gentamicin and kanamycin against isolated *Salmonella* in the present study maybe due to limited access and usage in veterinary and public health sectors compared to other antimicrobials in different parts of Ethiopia.

CONCLUSION AND RECOMMENDATIONS

Salmonellosis is the main foodborne zoonotic and animal husbandry problem throughout the world.This study detected 14.91% overall prevalence of *Salmonella* in samples collected from slaughtered sheep and goats, which can significantly be a potential source of human salmonellosis. In this study, *Salmonella* was isolated from feces with 7.45% and 0.88%proportion inskin swab (body coat), which suggests *Salmonella* from feces and exterior of animal body coat can contaminate meat in abattoirduring slaughtering process. The contamination of meat that observed with 4.39% in this study is also risk for consumers.All of the isolated *Salmonella* were exhibited 100% multi-drug resistance to antimicrobials that are used commonly in veterinary and human medicine, this pose risk to human and animals health. Generally, the level of antimicrobial resistance observed in this study gives us a cues on how we have to use antibiotics.Thus, a judicioususe of antibiotics in healthcare and animal health sectoris essential to slow the emergence of resistance and extend the useful lifetime of effective antibiotics.

Therefore, depending up on the findings of this study, the following recommendations are forwarded in order to reduce the emergence and impacts of antimicrobial resistance:

It is advisable to avoid cross contact of carcass with gastrointestinal contents in abattoir which will minimize the contamination of *Salmonella*from intestinal contents

The floor of abattoir up on which the animals are slaughtered should be cleaned, personal hygiene should be improved and potable water should also be used for washing purpose

It will be better if principle of antimicrobial stewardship applied and unregulated use of antibiotics avoided both in humans and animals

It is better if other studies concerning sources of *Salmonella* contamination in the abattoir is performed.

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ANNEXES

Annex 8.1: Data collection and laboratory test results record format.

SAMPLE COLLECTION AND LAB TEST RECORD FORMAT													
			Sample source and its code				Lab tests and its results						
Sample No	Animal spp.	Sex	Meat sample	Meat swab	Skin swab	Ceacalfeaces	XLD Result	TSI test	Citrate test	Urease test	Indole test	Methyl red test	Voges-Proskauer
1													
2													
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Annex 8.2: Performance standards for antimicrobial susceptibility testing of *Salmonella*.

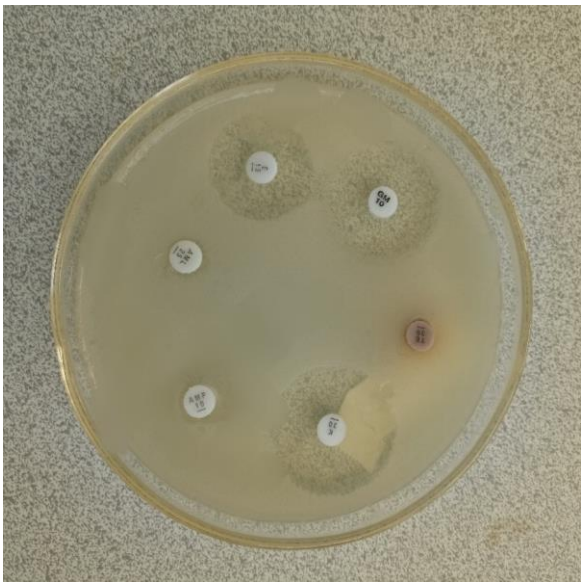
Antimicrobial drugs	Disc content	Zone diameter breakpoints, in millimeter		
		Susceptible	Intermediate	Resistant
Ampicillin (AMP)	10 µg	≥17	14-16	≤13
Amoxicillin(AML)	25 µg	≥18	14-17	≤13
Gentamicin (GM)	10 µg	≥15	13-14	≤12
Kanamycin (K)	30 µg	≥18	14-17	≤13
Tetracycline (TE)	30 µg	≥15	12-14	≤11
Erythromycin (E)	15 µg	≥23	14-22	≤13
Chloramphenicol (C)	30 µg	≥18	13-17	≤12
Nitrofurantoin (F)	300 µg	≥17	15-16	≤14

Source: Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplement M100. Clinical and Laboratory Standards Institute (CLSI, 2021).

Annex 8.3: Picture showing uninoculatedand inoculated XLD plates.

A.Uninoculated XLD plate.B.Black colony at top of XLD plate.

Annex 8.4: Picture showing Mueller-Hinton agar plate and antibiotic discs.



A.Mueller-Hinton agar plate + antibiotic discs.

B. Measuring zone of inhibition with caliper.

Annex 8.5: Picture showing slaughtered sheep and goat, and its transportation way.

A.Slaughtered sheep and goat on floor in slaughter house.

B.Carcass going to be transported tobutcher shopsusing handcart.

Annex 8.6: Composition and preparation of used culture media.

Buffered peptone water

Composition (g/L):

Peptone..... 10.0 g

Sodium Chloride..... 5.0 g

Disodium Phosphate..... 3.5 g

Monopotassium Phosphate..... 1.5 g

Preparation: Dissolve 15gof powder in 1L of purified water, mix thoroughly and autoclave at 121°C for 15 minutes.

Nutrient agar

Composition (g/L):

Beef Extract..... 3.0 g

Peptone..... 5.0 g

Agar..... 15.0 g

Preparation: Suspend 28g of the powder in 1L of purified water, mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121°C for 15 minutes.

Xylose Lysine Desoxycholate Agar (XLD)

Composition (g/L):

Xylose..... 3.5 g

L-Lysine..... 5.0 g

Lactose..... 7.5 g

Saccharose..... 7.5 g

Sodium Chloride..... 5.0 g

Yeast Extract..... 3.0 g

Phenol Red..... 0.08 g

Sodium Desoxycholate..... 2.5 g

Ferric Ammonium Citrate..... 0.8 g

Sodium Thiosulfate..... 6.8 g

Agar..... 13.5 g

Preparation: Suspend 55g of the powder in 1L of purified water, mix thoroughly. Heat with agitation just until the medium boils. DO NOT OVERHEAT.Cool to 45-50°C in a water bath and use immediately. Overheating causes precipitation.

Rappaport Vassiliadis*Salmonella* (RVS) Soy Broth

Composition (g/L):

Soy Peptone..... 4.5 g

Magnesium Chloride (anhydrous)..... 13.5 g

Sodium Chloride..... 9.0 g

Dipotassium Phosphate..... 0.03 g

Potassium Dihydrogen Phosphate..... 1.45 g

Malachite Green..... 36.0 mg

Preparation: Suspend 26.6g of the powder in 1L of purified water. Mix thoroughly.Warm slightly tocompletely dissolve the powder.Dispense 10mL amounts into suitable containers.Autoclave at 115°C (10 psi pressure) for 15 minutes.

Triple Sugar Iron Agar (TSI Agar)

Composition (g/L):

Beef Extract..... 3.0 g
Yeast Extract..... 3.0 g
Pancreatic Digest of Casein..... 15.0 g
Proteose Peptone No. 3..... 5.0 g
Dextrose..... 1.0 g
Lactose..... 10.0 g
Sucrose..... 10.0 g
Ferrous Sulfate..... 0.2 g
Sodium Chloride..... 5.0 g
Sodium Thiosulfate..... 0.3 g
Agar..... 12.0 g
Phenol Red..... 24.0 mg

Preparation: Suspend 59.4g of the powder in 1L of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Dispense into tubes and autoclave at 121°C for 15 minutes.Cool in a slanted position so that deep butts are formed.

Simmons Citrate Agar

Composition (g/L):

Ammonium Dihydrogen Phosphate..... 1.0 g
Dipotassium Phosphate..... 1.0 g
Sodium Chloride..... 5.0 g
Sodium Citrate..... 2.0 g
Magnesium Sulfate..... 0.2 g
Agar..... 15.0 g
Bromthymol Blue..... 0.08 g

Preparation: Suspend 24.2g of the powder in 1L of purified water. Mix thoroughly.Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.Dispense and autoclave at 121°C for 15 minutes.Allow to cool in a slanted position for use as slants.

Mueller Hinton Agar

Composition (g/L):

Beef Extract Powder..... 2.0 g
Acid Digest of Casein..... 17.5 g
Starch..... 1.5 g
Agar..... 17.0 g

Preparation: Suspend 38g of the powder in 1L of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.Autoclave at 121°C for 15 minutes. DO NOT OVERHEAT.Pour

cooled Mueller Hinton agar into sterile Petri dishes on a level, horizontal surface to give a uniform depth of about 4 mm (60-70 mL of medium for 150 mm plates and 25-30 mL for 100 mm plates) and cool to room temperature.