JIMMA UNIVERSITY

COLLEGE OF AGRICULTURE AND VETERINARY MEDICINE

SCHOOL OF VETERINARY MEDICINE

ISOLATION, ANTIMICROBIAL USE INTENSITY AND RESISTANCE PROFILE OF *ESCHERICHIA COLI* AND *SALMONELLA* IN DAIRY FARMS IN SELECTED DISTRICTS OF WESTERN OROMIA, ETHIOPIA

MSC THESIS

BY

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FEBRUARY, 2020

JIMMA, ETHIOPIA

Jimma University

College of Agriculture and Veterinary Medicine

School of Veterinary Medicine

Isolation, Antimicrobial Use Intensity and Resistance Profile of *Escherichia coli*and *Salmonella*in Dairy Farms in Selected Districts of Western Oromia, Ethiopia

MSc Thesis

By

Diriba Oljira

A Thesis Submitted to the School of Veterinary Medicine, Jimma University College of Agriculture and Veterinary Medicine in Partial Fulfillment of the requirements for the Degree of Master of Science in Veterinary Public Health

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February, 2020

Jimma, Ethiopia

APPROVAL SHEET

JIMMA UNIVERSITY COLLEGE OF AGRICULITURE AND VETERINARY MEDICINE

Thesis Submission Request Form (F-08)

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Program of Study: Degree of Masters of Science (MSc) in Veterinary Public Health

Title: **Isolation, Antimicrobial Use Intensity and Resistance Profile of *Escherichia coli* and *Salmonella* in Dairy Farms in Selected Districts of Western Oromia, Ethiopia**

I have completedmy thesis research work asper the approved proposaland it has beenevaluated and accepted my advisors. Hence, I hereby kindly request the department to allow me to present the findings of my workand submit the thesis.

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DEDICATION

I dedicate this piece of work to my whole familywho committed their lives with strong prayers for the betterment, patience, love and success of my life and with memory of my beloved father Oljira Feyisa.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my real work and that all sources of materials used for this thesis have been duly acknowledged. It has been submitted in partial fulfillment of the requirements for MSc degree at Jimma University, College of Agriculture and Veterinary Medicine and deposited at the university 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.

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BIOGRAPHICAL SKETCH

Hewas born in 1990 G.C from his father Oljira Feyisa and his mother Maritu Erana in Amuru district, Horo Guduru Wollega zone, Oromia Region, Ethiopia. He started his primary elementary education in Ejere Gormoti in the year 1994 G.C. And he joined in Amuru primary and secondary school in 1997 to 2001 G.C received regional school leaving examination grade 8. Also, he attended his high school and preparatory school education in 2002 and completed grade 12 in 2005 G.C in same school respectively.After he passed Ethiopian School leaving certificate examination, he joined University of Gondar in 2006and graduated with Doctor of Veterinary Medicine on 16thJune, 2011 G.C. After his graduation he was employed in Horo Guduru Wollega Zone, Horo district and served in position of as team leader of animal health. After four years work experience he had given the opportunity to pursue master's degree program in 2017 G.C. He joined Jimma University College of Agriculture and Veterinary Medicine, School of Veterinary Medicine to study his Master of Science inVeterinary Public Health from October 2017 to date.

ACKNOWLEDGEMENTS

First and above all, I would like to provide thanksmy heavenly father God who give me all what I have. I could never have done this without the faith and helped supplying strengthto me and my familiesin all ups and downs regardless of situations.

I take it an extreme privilege to express my heartfelt thanks and sincere gratitude to my advisors Dr. Motuma Debelo and Dr. Abdu Mohammed for their overall guidance, unreserved encouragement, suggestions, advice in proof reading and constructivecriticism my thesis. I offer my special thanks toMr. Takele Beyene, Gelana Urgesa and Misgana Teferawho supportedme much, and who responded to my questions.

I have deep sense of respect for all staff members of Jimma University College of Agriculture and School of Veterinary Medicine, particular School of Veterinary Medicine all my teacher who taught me various courses and for all their support research work. Much of the acknowledgement go to all Bedele Regional Veterinary laboratory workers especially to Dr. Fekadu Bakala, Dasale Mardasa, Dinke Jalata, Mame Ahmed and Eshetu Gezahgen for their valuable assistances they gave me in doing this research. All communities around the study areas for individual cooperation and involvement to successful allowedtaken sample from his or her farms animals and providing me with relevant information.I have no words to express my gratitude to Horo district livestock development and fishery resources for inspired favorable environments and securingmy salary to study successful in such manner.

No phrase or words in any language can ever express my deep sense of love and gratitude to my belovedMother, Maro, aunts, sisters, brothers and my entire family members for being always with methroughoutin thick and thin conditions.That have cultivated and brought me up with delight and strong both in moral and financial support throughout in my life.

Lastly but not least, I want to acknowledge every person who has come into my life directly or indirectly and inspired me through their presence.

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LIST OF ABBREVIATIONS AND ACRNOMYS

ABs	Antibiotics
AHS	African Horse Sickness
AMs	Antimicrobials
AMR	Antimicrobial Resistance
AR	Antibiotic Resistance
AST	Antimicrobial Susceptibility Test
BARC	Bako Agricultural Research Center
BPW	Buffered Peptone Water
BRVL	Bedele Regional Veterinary Laboratory
CBPP	Contagious Bovine Pleuropneumonia
CDC	Center of Disease Control and Prevention
CSA	Central Statistical Authority
E. coli	*Escherichia coli*
EAggEC	Entero-aggregative *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EMB	Eosin Methylene Blue
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Entertoxigenic *Escherichia coli*
EU	European Union
DACA	Drug Administration and Control Authority of Ethiopia
FDA	Food and Drug Administration
HHs	House holds
IMIs	Intramammary infections
ISO	International Organizations for Standardization
Masl	Mean above sea level
MDR	Multidrug Resistance
MIC	Minimum inhibitory concentration
MOA	Ministry of Agriculture
NCD	Newcastle Disease
OIE	Office of International Des Epizootics
Pexp	Expected prevalence
Qnr	Quinolone resistance
RVS	Rappaport Vassiliadis Soy broth
STEC	Shiga Toxin producing *Escherichia coli*
Spp	Species
TSA	Tryptic Soy Agar
TSI	Triple Sugar Iron
VTEC	Verocytotoxin *Escherichia coli*
WHO	World Health Organization
XLD	Xylose lysine Deoxycholate

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ABSTRACT

Escherichia coli and *Salmonella* species that were distributed widely contribute to risein resistance to various antibiotics used commercially with problem to health as uses of antimicrobials have critical importance for emergence of resistance bacteria.A cross-sectional study was conducted from November 2018 to December 2019 to assess prevalence and antibiotic resistance profile of *Escherichia coli*and *Salmonella*species from samples in dairy farms with survey on perception antimicrobial use, resistance and public health aspect in selected districts of western Oromia, Ethiopia.Of 60 respondents were interviewed and subsequently, 384 samples collected consisting cow milk, fecal, bucket milk and bucket swab for isolation and identificationby biochemical tests, SPSS analyzed andChi-square used to assess association of bacteria in samples.Isolated *bacteria*subjected susceptibility test using 8 antibiotics by using disk diffusion test.From overall prevalence observed 63(16.4%) for *Escherichia coli* and 31(8.1%) *Salmonella.*Significantly high proportion of *Escherichia coli* (19.2%) and *Salmonella* species (10.5%) obtained in udder milk indicated (p= 0.016, 0.004); 5% and 5% low proportion in bucket swab and bucket milk, respectively. High prevalence of *Escherichia coli* (15.7%) and *Salmonella* species (6.4%)was observed in feces of cattle. *Escherichia coli* and *Salmonella* isolate result of disk diffusion was significantly resistant to cefoxitin (71.4%, 77.4%), tetracycline (65.8%, 67.8%), streptomycin (55.6%, 61.3%), for ciprofloxacin and gentamycin greater than 50%. Isolates, 90.5% *Escherichia coli* and 87.1% *Salmonella* species isolate resistant to multiple antibioticswere used. About, (84.9%, 83.3%), and (100%, 90.9%) *Escherichia coli* and *Salmonella* species isolates from milk and feces respectively were multi-drug resistant. Study bacteriawere isolates susceptible to nitrofurantoin, nalidixic acid, ceftriaxone and ciprofloxacin. Of respondents, yet worryingly 86.7% knew what antibiotics and 88.3% believed waste management not causes antibiotic resistance. Samples harbored targeted study bacteria and antibiotic resistant profile of isolated*Escherichia coli* and *Salmonella* that have health hazard. Therefore, strengthening of communitiesto minimize malpractice of antibiotic useswas importance to limit associated health risk and occurrence of antimicrobial resistance in study areas.

Key words: *Antimicrobial resistance,Dairy farms/holders, Escherichia coli, prevalence, Salmonella*

1. INTRODUCTION

Bacteria are an integral part of the world and ubiquitous to every habitat on earth (Alexandra, 2007). They found in body systems of both vertebrate and invertebrate hosts as well as in external environments, food, soil, water and air. Commensal exists, some forming the normal intestinal bacteria of animals and human as non-pathogenic. Under certain circumstance, may become pathogenic by acquiring resistance (Khalif *et al*., 2018; Peterson and Kaur, 2018).

Food-borne diseases are important public health and economic burden (WHO, 2015). Milk is a high risk food as it is highly nutritious and serves as an ideal medium for progression of bacteria (Mohamed and Gihan, 2014).*Escherichia coli* and*Salmonella*sppcan exist in food products at any point along the production chain, farm environment (Sobur *et al*., 2019) and act as an important vehicle for transfers of AMRfactor to human very efficiently (Pant *et al*., 2013). Presences of pathogenic bacteria in food often emerge as a major public health concern since early days of dairy industry (Torkar and Teger, 2008). Drugs that increaserate of weight gain; improve feed productivity, chemotherapeutics agent in food-producing animals critically desirable to the challenge of providing adequate amounts of food for population.Still major problems are observed in different countries as bacteria found resistant are threat to mankind (Khalif *et al*., 2018).

Pathogenic *E. coli* and *Salmonella*strains are food borne zoonotic pathogens that can transmit to humans by direct or indirectly (Sobur *et al*., 2019). Recently,AMR pathogens emerged in food-production chain: extended beta-lactamase producing *Salmonella* and *E. coli*, quinolone resistance in *Salmonella* and *E. coli*,which transmit and cause infections in humans.Cattle in dairy farms could be potential sources for contamination of the farm environments and product by AMRof *E. coli* and *Salmonella*that leadsto health problems (Xia *et al*., 2010; Hao *etal*., 2014). Food contaminatedwith AMR bacteria act as vehicle for transmission of resistance strainsand reliable indicator of contamination by pathogenic bacteriaget access to dairy product (Abdel-Salam, 2010).Andsituation further complicated by possible resistant bacteria to transfer their resistance gene to resident of the human microflora (Aarestrup *et al*., 2008; Olatoye *et al.*, 2012).

Prevalence of antibiotics resistance bacteria present in animal product (milk) is common (Farzan *et al*., 2012). Fufa*et al.*(2018) reported food borne diseases occur commonly in developing countries particularly in Africa as prevailing poor food handling and sanitation, inadequate food safety, lack of financial resourceand awareness for food-handlers.AMR bacteria are circulating in environments and considerably risen humans health problems initiating emerging pathogen (Isibor *et al*., 2013). Uncritical use of antibiotics and insight the most factorsfor rises and spread of AMR bacteria in setting ecosystem (Sobur *et al*., 2019).

Livestock a vital role in improving well-being of people in developing countries from health, nutrition and socioeconomics angles (Abdul, 2014).Like Ethiopia, constitutes urban, peri-urban and rural dairying as important subsector of agricultural production system(Girma, 2017).Regardless oftheir importance also expose human to zoonoses and food borne pathogens (WHO, 2007), contributes resistance in human (Abdul, 2014). These conditions occur without being reported in developing countries, no exclusion for Ethiopia (Fufa *et al*., 2017).Countries where foodborne illness wasexamined the relative importance of pathogens like *E. coli*and *Salmonella* recorded as a major causeof problem (Mohammed *et al*., 2014; WHO, 2015).In African countries excessive antibioticsclasses use human and animal diseases treatment:beta lactams, macrolides, aminoglycosides, tetracycline and nitrofurans (Darwish *et al*., 2013; Haftay *et al*., 2018). Increasing AMR of *Salmonella* and *E. coli* isolate in human and animalhealth (Zelalem *et al.,* 2015). Even though it needs understanding of antibiotics usepractice inanimal and human that favor selection pressure maintaining resistance genes (Addis, 2015).At national level food shortage and inappropriate food safetyare problem that obstacleto country economic and public health (WHO, 2007; Ayelaw *et al*., 2013).

Generally, relationship between humans and animals needs be considered to better understand and manage obstacles (Durski, *et al*., 2014).Major concern to human health the issue of AMR due to use of antibiotics in livestockand human diseases treating conditions. There is a need to generate from dairy animals (milk, feacal,bucket milk and swabssamplesto realize AMR profile of *E. coli* and*Salmonella*and perception on AMU and public health impact relatedAMRin study areas.

1.1.Statement of Problem

Antibiotics have saved millions of lives, and their use has contributed significantly improving human and animal health (Oliver *et al*., 2011). However, Increasing clinical incidence global health issue (Ferri *et al*., 2017), condition aggravated in developing countries due to use or misuse of antimicrobial, drug consumer, spread of resistant bacteria and limited surveillance to AMR (Berendonk *et al*., 2015). Indiscriminately, use of AMs in animal or human a key contributor to AMR worldwide (Nguyen *et al*., 2018), which is increasing threat to health security, threatens economic, social and puts an extra burden on resource-poor countries. Ethiopia an agricultural society with over 80% of population situated in rural areas living in close proximity to domestic and wild animals in ecosystem. Small holders' dairy productions are somewhat ubiquitous and serve as sources ofmilk(FAO, 2016). Milk-borne pathogencausegastrointestinal disturbances, life threatening (Zdolec *et al.*, 2016), losses in dairy productionand impair socioeconomic progress.Level of awareness farmers on public health importance of zoonotic diseases in these countries is lowand further stifles efforts to control diseases (Munyeme *et al*., 2010). This may help as better ideas of potential public health risks associated with the dispersion of AMRClearly evidence of AR bacteria cause infection in human and animal origin as foodborne pathogen "nearly against antibiotics in animals complicated by use of the same agents in human are equally to upsurge to resistance" (Chang *et al.*, 2015).

Escherichia coli and *Salmonella* assumed to be present in all ecological niches particularly commensals in human, animal and environment and transfer resistance. AMR bacteria were found in farm animals where AMs are used, in associated food products, contaminated environment and farm worker.Antimicrobials resistancebacteria in animal critical challenge to human and animal health also threat to food security.Also, the public health is inseparably linked to animal health and production that intense contact can lead to serious risk to public health.Information on prevalence ofstudy bacteriaand AMRas well as perception on AMUmade available to public important for designing appropriatemeasuresand prudent use of antimicrobials.Use of antibiotics for animals implicated source of human infection with AMR *Salmonella* spp and *E coli*through contact and consumption of raw milk, meat and others. Study areas selected because of absence research done previously and no reported on AMsuses and resistance of *E. coli* and *Salmonella* in dairy farms. It is important to know and

identify AMs use and assess drug resistance for those bacteria from sample in study districts that practical control strategies for future.

1.2.Objectives

The general objective of studyto assess prevalence*E. coli* and *Salmonella*, AMR in samples and perception on AMUof dairy farm/holders in selected districts of western areas ofOromia.

Specific objectives:

- To isolatethe prevalence of *Escherichia coli* and*Salmonella* sppfromsamples collected dairy farms/holders.
- To assess the AMR profile of*E. coli* and *Salmonella*sppisolatedin the samples.
- To observe dairy animal holderperception on AMU and its resistance associated with public health aspects.

2. LITERATURE REVIEWON *E.COLI* AND *SALMONELLA*

2.1. A general Overview of *E. coli* and *Salmonella*

Escherichia coli originally called "*Bacterium coli*" were first isolated from the stool of a 2-3 days old new-born baby and subsequently from young calves in 1885 by a German pediatrician, Theodore Escherich (Khan and Steiner, 2002).The name of bacteria was later changed to honor its discoverer. *Escherichia coli* a gram negative, rod shaped, highly mobile and non-sporulating bacteria. They are motile, and those from extra intestinal infections may produce polysaccharide capsule. They are often classified under *Enterobacteriaceae,* known to be normal inhabitant of the gastrointestinal tract of animals and human beings (Oliver *et al*., 2011),but only some strains of *E. coli* become highly adapted to causes diarrhea and range of extra-intestinal diseases.*Escherichia coli* are a facultative anaerobe can grow from 7ºC to 50ºC with an optimum temperature of 37ºC, although some ETEC strains growing at temperatures as low 4ºC (Xia*et al*., 2010*).* A near neutral pH is optimal for its growth but growth is possible under optimal conditionsto pH 4.4 (Adams and Moss, 2008).

Among *E.coli* areenterohemorrhagic *E. coli* strains, especially serotype O157:H7. *E. coli* O157:H7 has become apathogen of major concern in both food and dairy industries, and to the public, because of its abilityto cause severe illness, haemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic Purpura. All shiga-toxin producing *E. coli* including serotype O157:H7 have the same morphology (Reuben *et al.,* 2013)

The genus *Salmonella* was named after Daniel Elmer Salmon, American veterinary pathologist and Theobald Smith was the actual discoverer of the type bacterium (*Salmonella enterica* var. *choleraesuis*) in 1885, Salmon was the administrator of the USDA research program, and thus the organism was named after him (Rao, 2004; FDA, 2008). *Salmonella* are Gram negative rod-shaped, which can grow facultative anaerobic belonging to the family *Enterobacteriaceae* that grow well between 35 and 37 °C andabout PH 6.5-7.5 (Ricke *et al*., 2013).*Salmonella*have large number of serotypes affecting human beings as well as mammals that antigenically related to one another (Lamas *et al*., 2018). They are able to growthon a wide range of relatively simple media. They can be distinguished from other members of the family by their biochemical characteristics and antigenic structure (Adams and Moss, 2008).

There are two species of *Salmonella*: *Salmonella enterica* and *Salmonella bongori*. Serotyping differentiates strains and *Salmonella enterica* is further classified into 6 subspecies (*Salmonella enterica* subspecies *enterica*, *S. enterica* Subspecies *salmae*, *S. enterica* subspecies *arizonae*, *S. enterica* subspecies *diarizonae*, *S. enterica* subspecies *hautenae*and *S. enterica* subspecies *indica*). Most of the *Salmonella* serotypes are part of *S. enterica* subspecies *enterica,* and over 99 % of human and animal infections are caused by serotypes subspecies. Non-typhoidal Salmonellosis results from infection by *Salmonella* serovars such as *S. typhimurium*, *S. dublin* and *S. newport*in cattle and others *Salmonella* serovars in animals (Majowicz *et al*., 2010).

2.1.1.Biochemical properties

*Escherichia coli*might be differentiated from other members of the *Enterobacteriaceae* on the basis of biochemical tests. Classically an important group of tests used for this purpose are known by the acronym IMViC. These tested for the ability to produce: indole from tryptophan (I); sufficient acid to reduce the medium pH below 4.4, the break point of the indicator methyl red (M); acetoin (acetylmethyl carbinol) (V); and ability to utilise citrate (C) (Adams and Moss, 2008). Despite *E. coli* identified with a variety of biochemical reactions, indole test remain the most useful to differentiate from other members of the *Enterobacteriaceae* lack of production of β- glucuronidase (Xia*et al.*, 2010). Furthermore, theygrow well on non-selective media and most strains ferment lactose producing large red colonies on MacConkey agar (Xia*et al*., 2010).

Salmonella are classified in 2,579 serotypes according to Kauffman-White scheme, considering differences in flagellar, capsular, and somatic antigens (Lamas *et al*., 2018). Additionally, *Salmonella* serotypes can be subdivided by molecular subtyping methods or by phage typing (Ricke *et al*., 2013). They are able to grow on a wide rangeof relatively simple media, pre-enrichment (BPW), selective enrichment (Rappaport-Vassiliadis Soya) broth and selective plate (XLD). They can be distinguished from other members of the family by their biochemical characteristics typical (TSI) and antigenic structure (Kabir, 2017).

2.1.2. Reservoir hosts

Both domestic and wild animals are sources of *E. coli* and *Salmonella enterica* ruminants primarily cattle, sheep,goats, and othersidentified as major reservoirs and source for human infection (Schikora*et al*., 2012; Chaudhuri *et al*., 2013).

Salmonella sppand*E. coli*enters dairy farm through new herd members; environmental media such as air, water, soil and or organic materials, like feed (Edrington *et al*., 2008; Mohammad *et al*., 2011). The dynamics and routes of introduction, colonization and persistence in both animals and the farm environment are not well characterized (Fairbrother and Nadeau, 2006; Klevin *et al*., 2018).*Salmonella* are ability of these microorganisms to survive under adverse conditions presents a formidable, challenge to the agriculture and food processing industries in marketing safe products (Eng *et al*., 2015). *Escherichia coli* one of the most common commensals bacterial flora of animal and human gut, however these bacteria can be pathogenic and cause infections in both animals and humans (Peterson and Kaur, 2018). Cattle are generally regarded as the main natural reservoir of EHEC.All ages of cattle are susceptible to colonization with EHEC (Hussein and Sakuma, 2005; Joris *et al*., 2012). Also, *E.coli* are indicator bacteria serve that reservoirs of antimicrobial resistance genes as easily develop resistance which are transferrable to pathogenic bacteria of animals and humans (Varga *et al.*, 2008).

2.2. Food borne Bacterial Pathogens

Milk is one of the greatest blessings that are given to humans by nature and are considered as a nutritive food from the prehistoric period. According to the (FAO, 2012) of the United Nations, world milk production reached 754 million tons resulting in an enormous consumption and trade in dairy products. The presence of food borne pathogens in milk may be due to direct contact with contaminated sources in the dairy farm environment and excretion from the udder of an infected animal (Mohamed and Gihan, 2014). Worldwide, food-borne diseases are a major health burden leading to high morbidity and mortality (CDC, 2009). Foodborne disease is any illness that results from consumption of contaminated food, pathogenic microorganisms (WHO, 2007). Foods act as vehicles for transfer of antimicrobial resistant bacteria and resistant gene to humans. Food-borne diseases are serious threat to people in Africa, responsible for 33-90% cases of mortality in children. Pathogenic bacteria

contaminants pose serious threat to human health, and constitute to about 90% of all dairy related diseases (Ruth and Ishaleku, 2015).

In Ethiopia milk and milk product are important role in serving the rural and urban communities owing to its high nutritional value. Consumption of raw milk is common in Ethiopia (Mebrate *et al*., 2019), which is not safe from consumer health point of view. It is a cash crop in milk shed area that enables families to buy other foodstuffs and significantly contributing to the household food security (Reta *et al*., 2016). Microbes gain entry into milk directly from dairy cows, farm environment particularly from water, utensils used for the storage of milk, and or pathogenic bacteria may be present in raw milk as consequence of udder disease (Benkerroum *et al*., 2004). The most common bacterial milk borne pathogens are *Salmonella*spp, pathogenic *E. coli*and others (Tryness *et al*., 2012).

Escherichia coli strains are acknowledged as main pathogensof colibacillosis in food animal particularly ruminant and poultry, some strain causes severe human diseases like hemorrhagic colitis and hemolytic uremic syndrome (Ferens and Hovde, 2011).*Escherichia coli* transmitted by food or water directly from one person to another also occasionally through occupational exposure (Gyles, 2007). As illnesses caused by this bacterium often requires treatmentwith antimicrobial, which is increasing level of antimicrobial resistance (Mooljunteee*t al*., 2010). The rapid spread of resistance genes, facilitated by mobile genetic elements such as plasmids and transposons, hasled to the emergence of MDR strains of many clinically important speciesthat now frequently leave clinicians out of therapeutic options (Hawkey and Jones, 2009).

*Salmonella*sppusually contracted from animals and associated with infections in humans is non-typhoidal *Salmonella* and contracted from sources such as milk,milk products, meat, fruits and vegetables processed inappropriately. Foodborne diseases caused by non-typhoidal *Salmonella* representan important public health problem worldwide (Yang *et al*., 2015). The other type of*Salmonella*spp, which is carried only by humans and usually tight through direct contact with the fecal matter of an infected person, is named as typhoidal *Salmonella*, whichmainly occurs in less developed countries, where unsanitary conditions are more likely tooccur. *Salmonella* spp of animal origin acquires their resistance in animals before being transmitted to human through food chain (Iovine and Blaser, 2004).

Over the years, bacterial pathogens including *Salmonella* developed resistance to various antibiotics. Antimicrobial resistance in *Salmonella*sppthat has led to failure of treatment in salmonellosis and other bacterial infectionsin has been the concern of individual patients and public health (Chao *et al*., 2007).

2.3. Use of Antimicrobial Drugs in Veterinary Medicine

Many drugs used in veterinary medicine have identical analogs that are used in humanmedicine (Smith, 2005). Ultimately, extensive and improper use of antibiotic drugs in food producing animal can establish reservoirs of resistant bacteria, greatly impacting public health (Marshall and Levy, 2011). Animal derived antimicrobial-resistant bacteria can colonize the intestinal flora of humans. Use of antibiotics in food-producing animals resulted in healthier, more productive by improving feed utilization and production of abundant quantities of nutritious; prophylactic use to prevent infection and lower disease incidence (Mathew *et al.*, 2007; Abdul, 2014).

Animal health services in developing countries have been sub optimal with an increased tendency for animal owners to stock drugs in their houses and engaging unskilled people, such as farmers themselves and animal attendants to treat animals (Katakweba *et al*., 2012). This AMR is driven by both appropriate and inappropriate use of anti-infective medicines for human and animal health practice with inadequate measures to control the spread of infections (WHO, 2012).

In human medicine, antimicrobials are approved for disease treatment and prevention (Kagashea *et al*., 2010). According to WHO (2010), stated more than half of all medicines in developing countries are prescribed, dispensed or sold inappropriately and that half of all patients fail to take them correctly. This complied with use of antibiotics in animals have resulted selection of antibiotic-resistant bacteria that contaminate animal food products and the environment (Abdul, 2014).The potential threat to human health resulting from these significant, as pathogenic-resistantmicroorganisms propagated in these livestock are poised to enter the food supply and widely disseminates zoonotic pathogens(Addo *et al*., 2011; Pal, 2012).These explain low education, poorer knowledge, over the counter use and misuse of antimicrobials and zoonotic infections significantly related to higher rate ofantimicrobial resistant and zoonotic infections (Eltayb *et al*., 2012; Katakweba *et al*., 2012).

2.4. Antimicrobial and Bacterial Resistance

An antibiotic is a chemical substance produced by a micro-organism, originally referred to as a natural compound produced by a fungus or other microorganisms that suppress the growth of other microorganism and may kill(Peterson and Kaur, 2018).Whereas antimicrobial is a broader than antibiotic, it refers to any substance of natural, or synthetic origin that used to kill or inhibit growth of microorganisms (Brunton *et al*., 2013). Availabilities of agentsfor treating bacterial diseases significantly improved health and life expectancy of humans as well as health and welfare of animals (Syit, 2008; Woolhouse *et al*., 2015). However, bacteria change that they can protect themselves from the deleterious effect of the medicines (Abdul, 2014), lead into AMR.

There are many different mechanisms by which this agent inhibits multiplication and growth, and the destruction of bacteria. Among these include 1) Inhibition of cell wall synthesis such as beta lactams, 2) Disruption of cell-membrane function, 3) Inhibition of protein synthesis (both 50S and 30S) 4) Inhibition of nucleic acid synthesis both the DNA synthesis and RNA synthesis and 5) inhibition of intermediary metabolic pathways/action as antimetabolites (Kohanski *et al*., 2010; Brunton *et al.,* 2013).

Antimicrobial resistance is the ability of microorganism to resist the growth inhibitory or killing activities of antimicrobials (Mathur and Singh, 2005). The rampant and indiscriminate uses of antimicrobials among the livestock keepers increase possibility of AMR bacteria that may be transferred from animals to humans and leads to various chronic diseases to the users of milk and milk products (Katakweba *et al*., 2012; Abdul, 2014). Antibiotic-resistant bacteria can simply transfer their resistance character to unrelated bacteria once inside the human body (Kaur and Peterson, 2018). Antibiotic resistant bacteria transmitted from animals to humans and vice versa, and food-borne transmission through food chain of animal origin is a recognized risk. The second way is through working with animals. Resistant bacteria may be picked up by workers in the livestock industry through handling animals, feed, and manure (Davies and Davies, 2010).

Antibiotic use sometimes occur in response to several challenges that face the livestock owners that include high level of stress, diseases, poor management, poor nutrition and drought (Mellau *et al*., 2010).Because of limited extension services and poor animal health

delivery systems, the farmers buy veterinary drugs and treat by themselves. Katakweba *et al*. (2012) reported that a lot of drugs such as oxytetracycline, streptomycin and others are used abusively to treat and protect animals against various diseases.

2.5. Mechanism of Antimicrobials Resistance Emergence

Once antimicrobials are used, pressure of antimicrobials drives occurrence of resistant strain (Carlos, 2010), which exclusively associated with use and misuse of antibiotics in animalsand humans(O'Neill, 2015). The dissemination of bacteria with resistancestrains(Aminov, 2010) and that favour the survival of resistant organisms over susceptible strainand dispersed among bacterial populations (Mathew *et al*., 2007). Antimicrobial use practice that seems to be a critical driving factor in resistance development is the use of broad spectrum antibiotics over narrow spectrum. Consequently, allowing selection pressure to increase the advantage of maintaining resistant gene in diverse groups of bacteria (Carlos, 2010). This is based on altering target molecules through mutational events and selection of mutants. Majority of AR are likely cases of acquired resistance, through the lateral transfer of AR genes from other ecologically and taxonomically distant bacteria (Aminov, 2010).

2.6.Antimicrobial Resistance in Food-borne Pathogens

According to Zelalem *et al.* (2015) over the years, bacterial pathogens have developed resistance to various antibiotics. Antibiotic resistance occurs naturally, but misuse of antibiotics in humans and animals is accelerating dissemination of resistant bacteria and resistant genes (Lukasova and Sustackova, 2003). The antimicrobials used in animal care are important, not only in increasing resistant in animal pathogens, but also in bacteria transmitted from animals to humans *(*Aarestrup *et al.*, 2008). Frequently exposed bacteria as the use of antibiotics, (Zelalem *et al*., 2015),gut flora microbes there is a possible development of resistance in the pathogenic and commensal bacteria (Bonomo and Rossolini, 2008).

In developing countries, household subsistence farming is common, which means that a large proportion of population has close contact with food animals; therefore, if resistant organisms are common in animals, the chance that they transmits to human beings is more likely (Okeke *etal*., 2007). Food-producing animals served a possible reservoir of antimicrobial-resistant bacteria and food borne pathogens transferred to humans either directly via the food chain or

indirectly as a result of spread of animal waste on cropland (Oliver *et al*., 2011) as indicated in (figure 1)The commensal bacterial or enteric bacteria flora may play role as acceptors and/or donors of transmit AMR genes (Bonomo and Rossolini, 2008). Bacteria commonly found in the intestinal tract of humans and animals and are also implicated in human and animal infectious disease (Oliver *et al*., 2011).

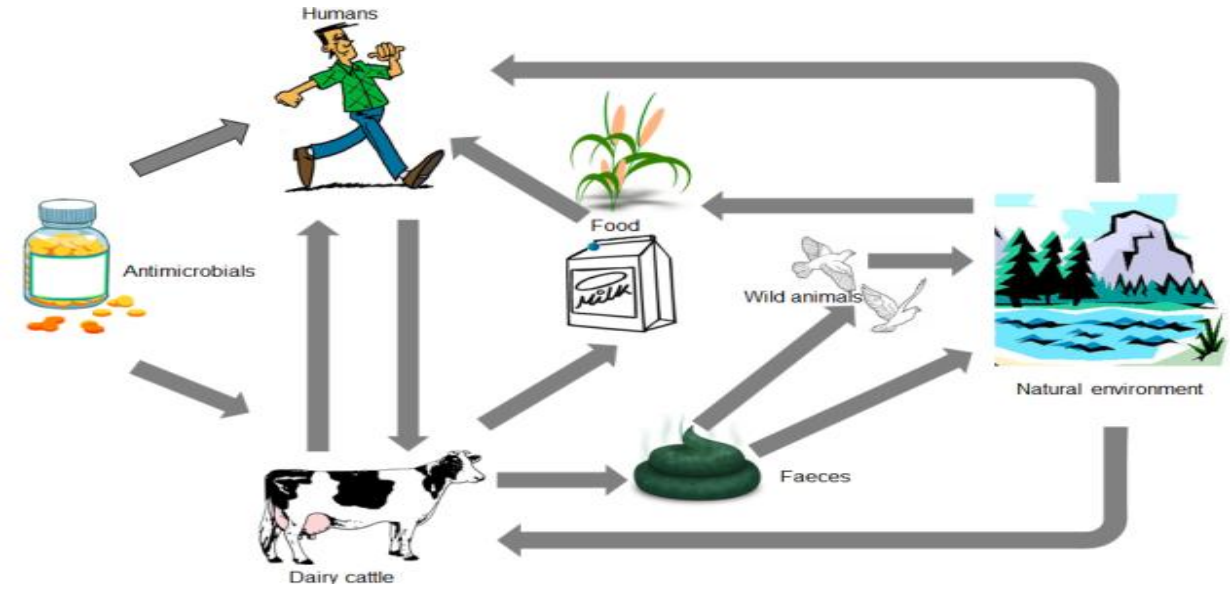

Figure 1: Potential transmission pathways for the spread of AMR (Adapted from Woolhouse and Ward, 2013)

2.7. Transmission of AMR between Animals and Humans

In both veterinary and human medicine practices, the same conditions lead to the selection and spread of resistant bacteria. Antimicrobial agents are heavily prescribed, mostly based on the risk of infection, rather than on the documented presence of the infection it and the natural microflora in humans and animals are colonizedby many antimicrobials from different classes (Silbergeld *et al*., 2008). In animal and human populations resistant bacteria are by consequence present and result in antimicrobial resistance transfer (Woolhouse and Ward, 2013). Two major resistance pathways support this process: transmission of entire bacteria harboring the resistance genes and the specific transmission of the concerned resistance genes. Transmission due to direct pathways the result of direct contact between animal and human (Peterson and Kaur, 2018). Transmission via indirect pathways takes place through contact of humans to food, biological, environmental, water and others (Colville and Berryhill,

2007).Direct contact between animal and human well recognized with farmers that live in close relation with their animals. That assiststo transfer ofcommensals or zoonoses from colonized or infected animals (Founou*et al*., 2016).

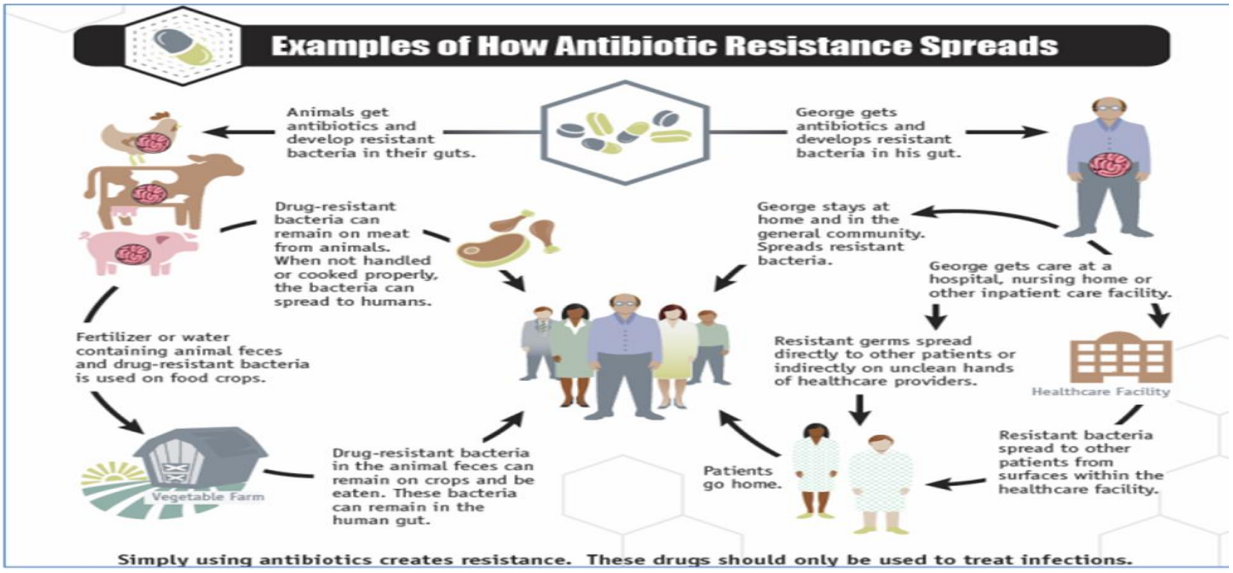

Figure 2: Examples of how antibiotic resistance spreads and circulating (CDC, 2013)

2.8. Bacterial Resistance Strategies

Microbial resistance is a naturalbiological reaction of microbes to selective pressure, such as weather conditions, food, oxygen, or the presence of an antimicrobial drug (Walsh and Duffy, 2013). When new class of antibioticintroduced, it is effective at first, but will eventually select for survival of the small fraction ofbacteria populations that have intrinsic or acquired resistance mechanism (Nikaido, 2009). Ability of the bacteria to evolve mechanisms to resists by agents recognizedafter widespread development of first antibiotics (Gargouri*et al.*, 2009).

The increasingprevalence of resistance in many pathogensover years in world particularly in developing countries (Byarugaba *et al*., 2011). Exacerbating the problem, pharmaceutical companiesare developing fewer new antibiotics to replace those that are no longer effective (Silbergeld *etal*., 2008). Call for new antibiotics therapies have issues, but there is continuing decline inthe number of approved antimicrobial agents (Cassir *et al.*, 2014). In General, the bacteria use 3 main strategies to become resistant to different antibiotics: inactivating the antibiotic, preventing the drug from reaching its target and altering the target (Robicsek *et al*., 2006; Nikaido, 2009).

2.8.1. Inactivating the antibiotic

Occurrences of antimicrobial resistant bacteria are inevitable to most every new drug and recognized major problem in the treatment of bacterial infections (Cassir *et al.*, 2014). Certain bacteria produce modifying enzymes that live within or near the cell surface that selectivelytarget and inactivate the agents. Of the target sites for antimicrobial agents, theirvital role in microbial growths and survival (Lambert, 2005). Enzymatic inactivation by hydrolysis or modification is a major mechanism ofresistance to natural antibiotics in pathogenic bacteria. These resistance determinants are mostprobably acquired by pathogenic bacteria from a pool of resistance genes in other microbial or antibiotic producing organisms (Robicsek *et al*., 2006).

2.8.2. Preventing the drug from reaching its target

Increasing the efflux plays a role, especially with hydrophobic compounds that presumably enterthe cell viadiffusion (Nikaido, 2009). At the same speed where these antimicrobials are enteringthe cell, efflux mechanisms are pumping them out again, before they reach their targetby ATP hydrolysis. The mutation results in overexpression of multidrug efflux pump lead to resistance to wide variety of structurally unrelated antimicrobials. Multidrug resistance proteins(MDRs) or multidrug efflux pumps are widespread in bacteria (Langton*et al*., 2005).

2.8.3. Altering the target

The presence of genes affording resistance to self-produced antibiotics, the outer membrane ofGram-negative bacteria, absence of an uptake transport system for the antimicrobial or generalabsence of the target or reaction hit by the antimicrobial (Wright, 2017).

2.9. Factors Contributing to Antimicrobial Resistance

Drug resistance emerges only when the two components come together in an environment or host, which can lead to a clinical problem. Selected resistance genes and their hosts spread and propagate under continued antimicrobial selection to amplify and extend problem to other hosts and geographic locations (Williams *et al*., 2016). Millions of kilograms of the antimicrobials are used each year in prophylaxis and treatment of people, animals and agriculture globally (Marshall and Levy, 2011), driving the resistance problem by killing susceptible strains and selecting those are being resistant. A number of important factors that include microbial characteristics, environmental or humanreservoirs in which resistant geneor

resistant organisms can persist, patterns of antimicrobial use, and societal and technologic changes that affect the transmission of organisms(Baharoglu *et al*., 2013).

2.9.1. Behavioral factors

Larson (2007) categorized behavioral and environmental factors that are involved in developing resistance.Improper use of drugs for prophylaxis in animal husbandry increases antibiotics resistant in the environment. Behavioral factors include inappropriate use of antibiotics, such as prescribing for nonbacterial infections and community member self-prescribing of antimicrobial (James *et al*., 2017).Antimicrobial use in animals in unsanitary and crowded conditions clear associated with risk of transmission of antibiotic-resistant bacteria resulting in harm to humans.In Africa, people traditionally keep livestock in close proximity to the homestead or inside the domicile (Abdul, 2014). Moreover,contact including animals, crowding, contaminated items, compromised skin integrity, cleanliness and failure to vaccinate for vaccine-preventable diseases (WHO, 2010).Abdul (2014) reportedairlines now carry more than two billion passengers annually vastly increasing the opportunities of infectious agents worldwide including antibiotic resistant bacteria.

These situations increase the risks of pathogen transmission through direct or faecal-oral route (Abdul Ahmed, 2014). The long term use sub-therapeutic doses regarded as one of the major factors responsible for development of resistance. The emergence of antibiotic resistance is further complicated by the fact that bacteria and their resistance genes are travelling faster and further (Grundmann *et al*., 2006).

2.9.2.Environmental factors

Environmental and policy factors include the continued use of antibiotics in agriculture and the lack of new drug development. Antimicrobials used in agriculture for growth promotion and major source of environmental contamination (Larson, 2007). Adverse climatic condition such as high temperatures and humidity may affect the overall qualities of antimicrobials agents during storage (Byarugaba, 2005). Specific environmental conditions generate different evolutionary selection pressures and environmental pollution is potential selective pressure favoring the evolution of resistance in bacteria (Abdul, 2014). The lack of appropriate regulations in the sales of antimicrobialagents also a driving factor in the access and misuse of antimicrobials (Okeke *et al*., 2007).Apart from the irrational use of

antimicrobials, unique environmental conditions such as crowding and poor sanitation also contribute in the circulation and spread of resistant microorganisms (Smith *et al*., 2005). According to Food and Drug Administration (FDA, 2012) estimates that 10% of drugs worldwide are fake and in many parts of Africa it is as high as 30%.

2.10. Diagnostic methods

Microorganisms, mostly bacteria are present in gut and skin in human as normal flora, which are harmless and helpful in many important functions of the body. Gastrointestinal tract is one of the routes through which pathogens enter the human body and cause many foodborne diseases such *Salmonella*spp and *E. coli* (Bajaj*et al*., 2016). It is important to detect the presence of pathogens in food that enters the body to cause a serious outbreak. The major requirements of detections are in public health, water and food industry, pharmaceutical industry and environment (Khan *et al*., 2010). Bajaj*et al.* (2016) stated culture based methods the oldest in detecting microorganisms, the pathogenic strains, which a confirm presence of a particular bacterial pathogen. Polymerase chain reaction, Enzyme linked immunosorbent assay, agar gel electrophorese and others.

2.10.1. Bacterial isolation

Bacteriological method for detecting pathogens typically involves culturing the organism in selective media and identifying isolates according to their morphological, biochemical, and/or immunological characteristics. This method is sensitive and permits the specific detection ofbacteria of interest in complex environments such as foods and certain clinical samples. However, this method is time consuming and usually requires 5-11 days (Riyaz *et al.*, 2004).

2.10.2. Antimicrobial susceptibility test

Antimicrobials susceptibility testing methods manipulate that pathogen are isolated from samples by culture methods. In separate test, isolated bacteria then exposed to different concentrations of antimicrobial agents under specified growth conditions, and the ability of these antimicrobials to inhibit growth is observed. Methods that are frequently used for cultured bacteria include disk diffusion, broth dilution, agar dilution and gradient diffusion. The sensitivity of the bacterial isolates to each antimicrobial agent is measured and the result is interpreted in accordance with criteria provided by (CLIS, 2015).

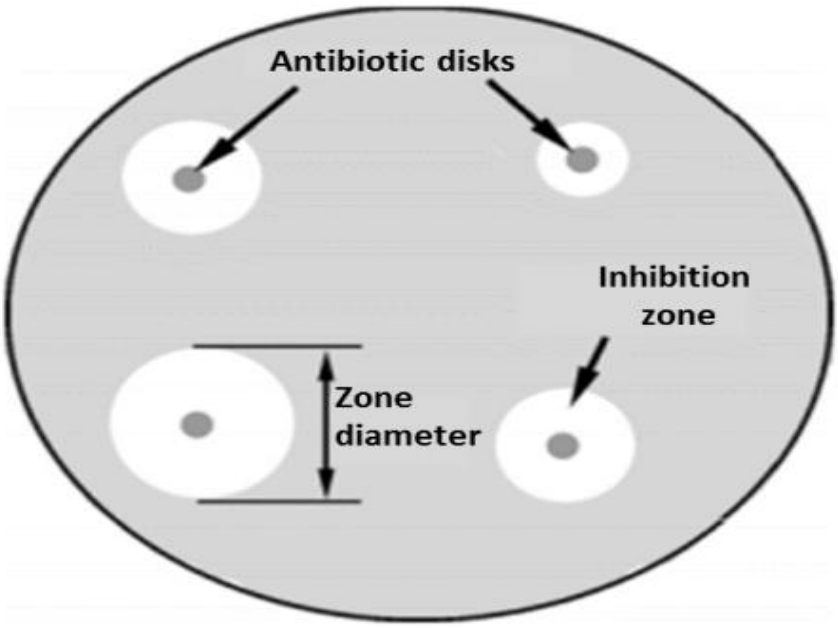

Figure 3: Kirby - Bauer antibiotic sensitivity tests

Source:adopted from Yohannes, 2016.

2.11. Public and Economic Importance

In developing countries, household subsistence farming is common. Which means that a largeproportion of populations close contact with animals.Infections that were previously easy to treat are becoming increasingly difficult to manage (WHO, 2012). Resistant microorganisms are common in animals; the chance that will be transmitted to human beings is more likely (Okeke *et al.,* 2005).Resistant infections in livestock result in reduced productivity due to prolonged treatment periods and withdrawal periods; and in worse scenarios, increased mortalities due to treatment failure (WHO, 2012). Measurements cost and economic impact program to minimize antimicrobial-drugs resistanceare imprecise and incomplete.AMR pathogenic bacteria may be ingested by consumers and present an immediate risk for public health. Increased consumption of antimicrobial agents and inappropriate use are among factors which further accelerated this phenomenon (Vander and Pitou, 2012). The consequence of these states of matters includes increases mortality, morbidity, costs of treatment, and loss of production in animals (DACA,2009). Not only these zoonotic potential and the ability to elaborate toxins by many of the microbes causing fatal intoxication are sufficient to understand the seriousness of the situation (Dhama *et al*., 2013).

Resistant bacteria from animals can infect humans by direct contact as well as via food products of animal origin (Szmolka and Nagy, 2013) and AR in animal pathogens can lead to therapy failure with direct negative effect on animal health and welfare (Bjorn and Christina, 2014). Antimicrobial resistance is causing problems through three different mechanisms in both veterinary and human medicine: infections caused by AR bacteria cause a longer duration of illness and higher rates of mortality. Secondly, treatment costs of resistant infections are rising and at thirdly, procedures relying on effective AM agents to prevent infections cannot be carried out without creating an increased risk for infection (Kevin*et al*., 2010; Klevin *et al*., 2018).

2.12. Control and Prevention

The preventive and control strategies may be approached based on the major site in the cycle of transmission or acquisition, and following the source of infection, environment and host (Negga *et al*., 2005). One Health approaches are the opportunity to implement control programmes that reduce multiple impacts of zoonoses in human and animal (Yohannes, 2016).Most of the food-borne illnesses can be outlined to infected food handlers, which is important strict personal hygiene measures adopted during food preparation (Marcus, 2008).

The improvement of farming conditions, creating awareness among consumers, water supply and sanitation infrastructure in health facilities offer significant co-benefits for combatting AMR and proper sewage disposal are other intervention strategies (Pal, 2007; Pozio, 2008). Hygienic measures are required throughout the continuum from “farm to fork”.
Further research also required to explore pathways of the food-borne illness and to assess the vehicles of the greatest importance (Unicomb, 2009).

Identified social solutions involved an increase in education and infrastructure. Emphasis on hygienic condition, vaccination programs, as well as educating professional, veterinarians, and farmers about appropriate prescribing, misuse, and reducing the use of antibiotic drugs were all identified as potential solutions to antibiotic resistance (Orzech and Nichter, 2008). Detection of food borne bacterial appropriately and laboratory based surveillance for early investigation of pathogen and dairy farmer also informed about the hygienic method of handling food (Abebe *et al*., 2014; Biruke and Shimeles, 2015).Overcome the threating of AMR via three-pillar approach advocated: optimize the use of existing antimicrobial agents;

prevent the transmission of drug-resistant bacteria through infection control and improve environmental decontamination (Carlet, 2012).

2.12.1. Alternative therapies of AMR

Theemergence of MDR bacteria wereretains the antibiotic activity against these pathogens and amplified by dearth of novel classes of antibiotics. However, rising of these consequence has forced scientiststo search alternative therapies (Haq *et al*., 2012), such as phage therapy, antimicrobial peptide therapy and combinations of two or more antibiotics (Fjell *et al.*, 2012; Haq *et al.*, 2012). Whichare highly specific and very effective in lysing bacteria, safe as several clinical studies and readily modify to fight the emergence to new multiresistant bacteriastrain.Characterizing lytic phages specific for different *E. coli* strains demonstrating their potential therapeutic value (Maura *et al*., 2012; Sillankorva *et al*., 2012).

2.13.Status of Antimicrobial Resistance in Ethiopia

In different animal foodand humans antimicrobial drugs are used for the treatment and control of diseases as well as for growth promotion of animals(Alexander *et al*., 2009; Bruno and Carolissen-Mackay, 2012). As well asin world, there are indications of the misuse of antibiotics by health care providers, unskilled practitioners, and drug consumers(DACA, 2009). Result of antibiotics used inappropriately, AMR pathogen increasing (Alexander *et al*., 2009) and severely hampered therapeutics option in both public health and veterinary practices (Thaker *et al*., 2012).Antimicrobial resistance in *E. coli and Salmonellaspp* have reported worldwideand increasing rates of resistance in both *E coli* and *Salmonella* is growing concern in developed and developing countries (Erb *et al.*, 2007).

In Ethiopia, various studies have done on the prevalence and antimicrobial resistance patterns of *E. coli* from various clinical sources (Gebre-Sealsssie, 2007). As Taye *et al.* (2013) reported on the drug resistance of *E. coli* isolates from animal-derived food products. Also study indicated drug resistance of *E.coli* due to high AMU in dairy farms, fruit juices, and individual cows to treat various diseases affecting the dairy sector (Haftay *et al*., 2018). Similarly, several studies have indicated that *E. coli* isolated high resistance to erythromycin, streptomycin, tetracycline, and ampicillin (Kindu, 2015) in Ethiopia. Accordingly, the resistance of *E.coli* was tetracycline (90%), streptomycin (78%), and ciprofloxacin (38%)

with 92.3% of the isolates tested showed MDR(Tesfaheywet *et al*., 2013). On the other hand, 14.4% resistance isolates to drugs of ampicillin, vancomycin, streptomycin and tetracycline have reported from milk and milk product samples collected from open market and supermarket sources in Bishoftu town (Bedasa *et al*., 2018).

Various studies conducted in Ethiopia on *Salmonella* suggest increase in the antimicrobial resistance of bacteriato commonly used antimicrobial in both the public health and veterinary sectors (Zelalem *et al*., 2011; Teshome and Anbessa, 2012; Hailu *et al*., 2015; Fufa *et al*., 2017). According to Fufa *et al.* (2017) observed multiple drug resistance*Salmonella*species isolated in the dairy farms from different samples to nalidixic acid, cefoxitin, streptomycin, tetracycline, ampicillin, amoxicillin, kanamycin and others.

Zelalem *et al.* (2015) reported that food consumers in developing countries including Ethiopia suffer from food-borne bacterial illnesses especially from those *Salmonella*spp and others. Bacteria are commonly found in soil, water, plants and animals (including humans). Here, "food sources" is broadly defined to include all of sources of exposure to pathogens in the food chain, between exposures at the farm or production level to exposure at the food consumption level. Linscott (2011), stated more than 250 different food-borne illnesses are caused by various pathogens or toxins (WHO, 2011) stated that food-borne illnesses result from consumption of food containing pathogens such as bacteria, viruses, parasites.

3. MATERIAL AND METHODS

3.1.Description of Study Areas

The study was conducted in selected districtsof zones; Horo Guduru Wollega (Horo), West Shoa (Bako Tibe) and East Wollega zone (Gobu Seyo) of Western Oromia, Ethiopia. Study areas were selected purposively based onthe accessibility of dairy farm/ holder, access for data collection and scant of information on prevalence and AMR of targeted bacteria as well as perception of dairy holders on AMU and AMR associated public health aspects. Horo, Bako Tibe and Gobu Seyo districts are located a distance of 310, 265 and 288 km respectively from Addis Ababa(CSA, 2007). Horo is situated in 09º29' North latitude and 37º26' East longitudes with altitude of2296 masl; it has mean annual temperature of 17.2ºC and annual rainfall ranges 1200-1800 mm.The altitude range of Bako Tibe and Gobu Seyo districts are 1650 masl and their latitude and longitude are 09°06' North and of 37º 09' East respectively. Bako Tibe district have average rain fall of 886.5mm and with mean annual temperature of 21.2°C.Annual rainfall of Gobu Seyo district ranges from maximum 1658 mm to minimum 830 mm and it has temperature that range from 10°C to 30°C.Main rainfall seasons for thethree district are from June to October and dry season being from December to April (BARC, 2014).

All the areas share similar farming systems which are predominantly practiced as the mixed type with crop and livestock productions.According to information from districts Agricultural office, majority of the livestock keepers and production systems are of small-holder dairying and mainly keep indigenous cattle and some of them have cross breed.Animals are managed in extensive and semi-intensive farming type. The cattle populations of Horo, Bako Tibe and Gobu Seyo district are 213, 924, 137,343 and 76,791 respectively (HWOARD, 2011; GSWOARD, 2011; BARC, 2014). Also other animals kept by these livestock keepers are goats, sheep, poultry and other (CSA, 2007; BARC, 2014). Farmers practice in dairy farming with improved breeds especially in urban and peri-urban areas. Ayantu *et al.* (2012) and Dereje *et al.* (2016) stated major constraints of animals as feed shortage and diseases ranked as priorities in areas. These usages of antimicrobial agentsforcattle implicated as a source of human infection with AMRbacteriathrough direct contact with livestock and consumption of raw milk, meat and contaminated materials (Reta *et al*., 2016; Fufa *et al*., 2017).Primary dairy

product (milk) produced by individual farmer from lactating coware consumed by many families in their home whereas the dairy farm owners brought the milk to local consumers and surrounding areas(Ayantu *et al.,* 2012).

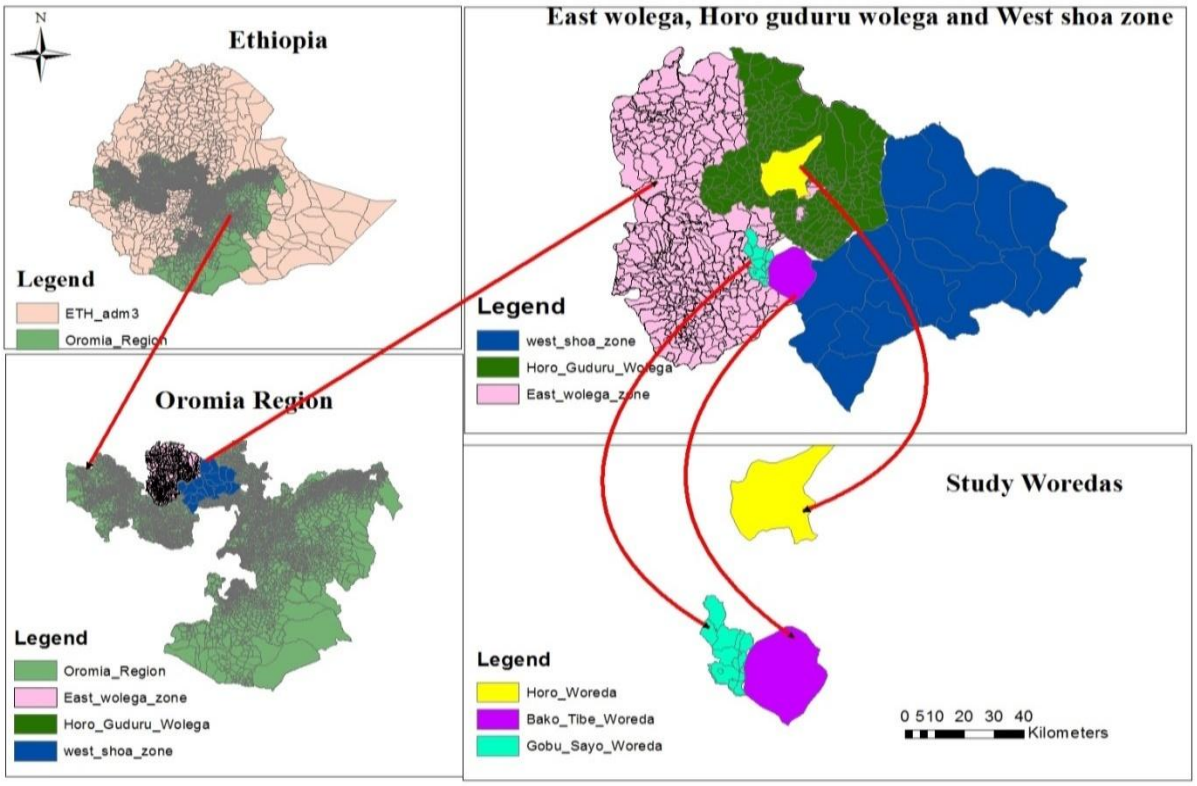

Figure4: Map of Study areas

3.2. Study Animals and Population

Study population werehealthylactating cows both localand cross breed apparently healthy dairy cowsinselected districts underextensive and or semi-intensivemanagement large, medium and small dairy holders.Populations of lactating cows present were 689 in selected kebeles owned sites of Horo; Bako Tibe and Gobu Seyo districts; 331, 198 and 160 lactating cows respectively. Using sampleof 60 dairy owners28, 16, and 16 were selected from Horo, Bako Tibe and Gobu Seyo districts, respectively. All of smallholder farmers own dairy cattle and Kebeleswereconsidered as sampling frame.

Table 1: Targeted lactating cows selected population at districts level

Districts	No Kebeles	Selected	Selected dairy holders	Extensive	Semi-intensive
Horo	24	12	28	17	11
Bako Tibe	18	6	16	11	5
Gobu Seyo	9	6	16	13	3
Total	51	24	60	41	19

No =Number

3.3.Inclusion and Exclusion Criteria

The study inclusion criteria were farms/smallholder of dairy animalsthat willing to participate in study, ready to give required information through questionnaires and availability of time at samplingor data collection. All animal fulfill the inclusion criteria (apparently health dairyanimals) visual observed and dairy animal holders selected purposively.The exclusion criteria were those are not voluntary to participate in the study and unable to give information. Also those male animals within farm including calves were excluded for this study.

4.4. Study Design

A cross-sectional study was carried out from November 2018 to December 2019 to isolate and assess AMR profile of *E.coli* and *Salmonella*from samplesextensive or semi-intensive dairyfarm/holders.Samples collected consists of udder milk, feces, considering swab sample at farms level including bucket milk and bucket swab of milk container This study comprised; bacterial isolation from samples and antimicrobialssusceptibility analysis of isolated bacteriaand also questionnaire supported on AMU intensity.

3.5.Sampling Techniques

The study was includes representative sites in major dairy farms/holders in selected districts. The three districts selected purposively while kebeles randomly with consent and willingness taken into consideration based on access of data collection dairy holders. Prior to sample collection, all dairy holders that had small, medium and large farm/holders in selected kebeles were identified and recorded. Accordingly, 12, 6 and 6 kebeles were obtained from Horo, Bako Tibe and Gobu Seyo districts respectively. Numbers of samples from kebeles weredetermined by proportion of dairy animal within farm/holders. In this sample60 dairy

holders owningboth local (N=128) and crossbreed (N=44)lactating cowswere selectedsystemic random sampling in study sites.Also, 172 non lactating dairy animalswere targeted to be sampled for fecal. Accordingly, 232 and 152 sampleswerecollected from extensive and semi-intensive management respectively of dairy farms or holders.

Dairy holders were grouped into three; small (having 1-10 dairy cows), medium (having 6 to 10) and large (having >10 dairy cows) using categories made by Sefinew*et al.* (2018) for collection of samples.Thecategories were made as small, medium and large scale dairy farms/holders. With population of lactating cows present 253, 347 and 89in small, medium and large scale dairy farm/holders in selected areas respectively.

The numbers of lactating cows ranged 1 to 24 on the visited dairy farms/holders. The districts have 4 large, 30 medium and 26 small scale dairy farms/holders with data obtained from the respective of livestock and fishery office of districts.Systematic random sampling was used to allocate number of samples to be collected for individual animals in selected farm/holders.

The number of lactating cows in large, medium and small holder was 22, 87 and 63 respectively selected. Accordingly, 23, 12, 9, cross breed and 59, 38, and 31 local lactating cows were selected in Horo, Bako Tibe and Gobu Seyo respectively. Of these 82 lactating cows selected(10 from large, 42 from medium and 30 from small), 50 (6 from large, 24 from medium and 20 from small) and 40 (7from large, 20 from medium and 13 from small) in Horo, Bako Tibe and Gobu Seyo districts dairy farms/holders respectively.Likewise, 60 dairy farm or holders were then interviewed using a semi structured questionnaire.

Table 2: Population of selected animals based on scale of dairy farms in study areas

Districts	Small	Medium	Large	Total
Horo	30	42	10	82
Bako Tibe	20	24	6	50
Gobu Seyo	13	20	7	40
Total	63	86	23	172

3.6. Sample Size Determination

The sample size was calculated formula given by Thrusfield (2007). The concerns were 95% confidence interval and 5% desired absolute precision.Since, there was no previous study on prevalence and status of AMR profile of *E. coli* and *Salmonella*in the study areas.

$N= \frac{(z^2 \times P_{exp} (1-P_{exp})}{d^2} = \frac{(1.96^2 \times 0.05 (1-0.05)}{(0.05)^2} = 384$

Where,N = sample size, z = Confidence interval

Pexp = Expected prevalence, d =Desired absolute precision

Therefore, expected prevalence set 50%, required sample size was calculated 384. However, these sample sizes (N=384) were processed for both *E.coli* and*Salmonella*isolation. Thus, 172lactating cow and 172 non lactating cows in 60 dairy farm/holders were selected for samples. The total sample size then became 384 (172 udder milk, 172feces sample, 20bucket milkand bucket swabs(N=20) were sampled.

3.7. Sample and data collection

Prior to sample collection, cooperation letter was sent to each districtof Livestock and Fishery resource development officefor sampling in each dairy farm. From the list provided, individual dairy animals/ samples in farm fromkebeleswere selected for sample collection. Samples collected early in the morning (12:00-3:00 AM) and/or afternoon around (10:00-12:00 PM) local time. The samples were stored in refrigerator at 4 °C until transported toBedele Regional Veterinary Laboratory (BRVL)on the next day for laboratory analysis.Sample were coded with random numbers for identification purpose and stored in ice box with ice packs during fieldwork.All collected samples were labeledand transported to BRVL using ice box in cold chain.Up on arrival, the samples were stored at + 4°C being processed for isolation and identification as described by (Quinn *et al*., 2004).All samples were process for the detection of *E. coli* and *Salmonella*species.

3.7.1. Faecal and milk sample collection

Fecal and milk samples were collected fromnon-lactating and lactating cows through rectal palpation and directly from all quarters of selected animals respectively. Approximately10g fecal and 10ml of udder milksamplewere collectedfrom each selected animal using

insterileuniversal bottle and stored in ice box with ice pack. Accordingly, the near teats were sampled first and then followed by the far ones (Quinn *et al*., 2004).Milk (N=172*10ml) and feces (N=172*10gm) samples were collected from animalandprocessed for study bacteria isolation (Quinn *et al*., 2004).

Farm bucket/tank milk (N=20*5ml) was sampled after milking process completed and milk from all cow collected in one container. Before sampling from milking bucket container, the milk was thoroughly mixed/agitate and sampled from the top of bucket by sterile syringe from each around 5ml collected in universal bottle (Richardson, 1985). The universal bottle labeled with permanent marker after sampling and samples were transported and incubated at 37^{o}C processed the following day**.**

3.7.2. Swabs sample

From farm visited, N=20bucket/tank swab of milk container samples were collected. The swabswererotated and rubbed against sampled surface several times. After completion of swabbing, the swabs were put inside into a sterile test tube containing 4mmpre-enrichment media (BPW) to be moistened. Bucket/tank swab was taken before milking using sterile wooden cotton and put in pre-enrichment media (BPW)24 hrs at 37^{o}C. All samples were processed for isolation of *E. coli* and *Salmonella*sppwith antibiotic susceptibility.

3.7.3. Questionnaire

Semi-structured questionnaire was prepared both closed and open-ended questions included in questionnaires (Annex 9). Verbal consent was obtained and objectives of study explained to the respondents. Questionnaire was intended to the owners of dairy farms/holders to obtain information related on AMU, and associated public health through face to face conversion. Drug usage practice and data on antimicrobial commonly used instudy area were collected from veterinary clinics case recordand personal communication.The questionnaire was pre-tested and adjusted as requiredtranslated into local language (Afan Oromo) for interviewees. A total of 60 respondents were 16, 16 and 28 from Bako Tibe, Gobu Seyo and Horo districtsselected, respectively. Information collected wasethical respected and owners interviewed from kebeles were proportionally selected from each site.

Table 3: Details types of sample collected in the study areas

Type of samples	Districts of sampling			
	Horo	Bako Tibe	Gobu Seyo	Total
Feces	82	50	40	172
Udder milk	82	50	40	172
Bucket/tank milk	13	5	2	20
Bucket/tank swab	13	5	2	20
Total	190	110	84	384

3.8.Bacteriological Isolation and Identification

The isolation and identification of *Escherichia coli* and *Salmonella*were examined in respective of samples at BRVL following standard procedures and guidelines recommended (ISO-6579, 2002; Quinn *et al*., 2004). *Escherichia coli*isolates wereincubated primarily on nutrient agar at 37◦C for 24hrsand transferred on MacConkey agar aerobically for 18 to 24 hrs at 37°C with respect to samples. *Escherichia coli*isolate revealed characteristics colonies morphology such as smooth, circular, white to grayish colonies in nutrient agar and pinkish color appearance on MacConkey agar. Further, obtaining pure colonies with typical color and appearance of *E. coli* were picked and streaked on Eosin Methylene Blue (EMB) agar (Oxoid, England) incubated at 37^{o}C.Colonies characteristics metallic green sheen on EMB agar considered as *E. coli*. Bacteria confirmed on the basis of coloniescharacteristics and further biochemical test, namely Indole test, TSI, methyl red test *Enterobacteriaceae* (Xia, 2010).

Isolation of *Salmonella* was done using pre-enriched in Buffered Peptone Water (BPW) followed by selective enrichment in selenite cysteine and Rappaport-Vassiliadis Soya broth (Himedia, India). After incubation, a loop-full of selective enrichment was transferred and streaked onto the surface of Xylose lysine Deoxycholate (XLD) agar (Oxoid, England) and incubated at 37°C for 24 hrs. All typical suspected colonies of lightly transparent zone of reddish color with/without black color at the center were picked and streaked onto nutrient agar and incubated at 37°C for 24 hrs. Colonies of bacteria were taken from the nutrient agar (Oxoid, Basingstoke, England) and inoculated intofollowing biochemical test tubes for

identification tryptone soya broth(Oxoid, England), Triple Sugar Iron (Oxoid, Basingstoke, England) agar, Indole and urease test using urea broth (Himedia,India), and incubated for 24 to 48 hrs at 37^{o}C(Quin *et al*., 2004).

3.9. Antimicrobial Susceptibility Test (AST)

Antimicrobial susceptibility test of all isolates were conducted by using the Kirby–Bauer discdiffusion on Mueller–Hinton agar (Oxoid, England) according to the guidelines by (CLIS, 2015).Criteria to selectantimicrobialsused were based on availability and chemotherapeutic agents for *Enterobacteriaceae* infectiontreatment in human and animal (CLSI, 2015) suggest guideline for AST (annex 8). Also, information obtained from personal communication on antibiotics that are most commonly used to treat bacterial infection like salmonellosis in the country and selected areas.

Pure colonies of bacteria, 2-4 on nutrient agar were obtained by sterile wire loop and transferred into tube containing 5 ml normal saline and mixed. Colonies of bacteria emulsified in the tube containing 5ml of normal saline matching with 0.5McFarland turbidity tube. After the broth culture was incubated at 37^{0} C for 24hrs. Each swab were separately immersed into suspension and streaked uniformly on surface of Mueller-Hinton agar media at least three times. The antibioticsdisk with concentration obtained from (Oxoid, UK, England) company includes: cefoxitin (30µg), ceftriaxone (5µg), ciprofloxacin (5µg), gentamycin (10µg), nalidixic acid (30µg), nitrofurantoin (300µg), streptomycin (10µg) and tetracycline (30µg) (Table 4).After streaking the antibiotic disks placed on top of agar plates using a sterile forceps; and the inoculated plates were incubated aerobically at 37^{0} C for 24hrs. Finally, diameters of zone of growth inhibition produced around disc were measured to the nearest millimeter for each using transparent ruler. Then the results of clear zone diameters were interpreted as susceptible, intermediate or resistant according to the guideline provide by the Clinical Laboratory Standard Institute (CLSI, 2015).

Table 4: Antimicrobial Susceptibility test interpretative criteria for *Enterobacteriaceae*

Antimicrobial Agent	Disk concentration	Zone diameter: interpretive criteria (nearest whole millimeter)		
		I	S	R
Cefoxitin (CXT)	(30 µg)	15-17	≥18	≤14
Ceftriaxone (CTX)	(5 µg)	20-22	≥23	≤19
Ciprofloxacin (CIP)	(5 µg)	21-30	≥31	≤20
Gentamycin (GEN)	(10 µg)	13-14	≥15	≤12
Nalidixic acid (NAL)	(30 µg)	14-18	≥19	≤13
Nitrofurantoin (NIT)	(300 µg)	15-16	≥17	≤14
Streptomycin (S)	(10 µg)	12-14	≥15	≤11
Tetracycline (TET)	(30 µg)	12-14	≥15	≤11

Key: I, Intermediate; S, susceptible; R-Resistance [Source: (CLIS, 2015)]

3.10.Data Management and Analysis

Data collected from questionnaire survey and laboratory result were entered into Microsoft excel spread sheet (Microsoft excel 2010). The data coded and entered to excel spreadsheet were transferred to software SPSS (version 23) and processed for analysis. Descriptive statistics such frequency used to assess prevalenceand distribution of *E.coli* and *Salmonella*species isolatedpositive from samples.Chi-square (X^2)wasutilized to observe significant relationshipsbetween in presenceand distribution of AMR profileof *E.coli* and *Salmonella*speciesisolated withinsamples.

Also percentage of AMR of isolates*E. coli* and *Salmonella*species were state as susceptible, intermediate and resistance obtained (CLSI, 2015) interpretive criteria for *Enterobacteriaceae*(table 4). In all the analyses, 95% confidence interval and $P<0.05$ is set for significance and not significant as $P> 0.05$. Descriptive statistics such as frequencies also used to present the findings of questionnaires on antimicrobial usage, its resistanceand public health aspects.

4. RESULTS

4.1. Prevalence of *Escherichia coli* and *Salmonella* Species

A total 12.2% (94/768)of targeted study bacteria were isolated and identified from samples. Of this 63 (16.4%, 95% CI: 0.1304, 0.2044) *E. coli* and 31 (8.1%, 95% CI: 0.0535, 0.1080) *Salmonella* spp were isolates through culture and biochemical test conducted.

Distributions of targeted bacteria were observed in the study areas. Accordingly, 384 samples were examined for both *E. coli* and *Salmonella*spp. Of sample examined positive result of *E.coli* and *Salmonella* spp 63 and 31 respectively.Relatively higher prevalence of *E. coli* was isolated from Horo 33 (17.4%) when compared with Bako Tibe 17 (15.5%) and Gobu Seyo 13(15.5%) districts. On other hand, higher proportion of *Salmonella*spp obtained 10 (12%) in Gobu Seyo as compared with samples collected in Horo 17(9%) and 4 (3.64%) Bako Tibe. Results show no significant variation in existence of both *E. coli* and *Salmonella*among study areas (P >0.05) (Table 5).

Table 5: Proportion of *E. coli* and *Salmonella* occurrence among three districts (N=768)

Districts	*E. coli*(N=384)	X^2 (PV)	*Salmonella*(N=384)	X^2 (PV)
Horo (N=190)	33 (17.4)*		17 (9)*	
Bako Tibe (N=110)	17 (15.5)	2.033 (0.362)	4 (3.64)	3.521 (0.073)
Gobu Seyo (N=84)	13 (15.5)		10 (12)	
Total (N=384)	63 (16.4)		31 (8.1)	

PV=P-value, X^2=Chi-square,*values in parenthesis are percentage

These 384 sampleswere processed for each target study bacteria isolated. Correspondingly, prevalence of *E.coli* and *Salmonella* sppwere assessedamong sample types. Of these positive cases, isolation of *E.coli* was the highest in udder milk 33 (19.2%, 13.30, 25.07), followed by 27 (15.7%, 10.26, 21.13) in feces, 2 (10%, 3.15, 23.15) in bucket milk and 1 (5%, 4.55-14.55) from swab of milk container. Likewise, as described in table 6 the overall prevalence of *Salmonella*spp isolate, highest in udder milk 18 (10.5%, 0.0589, 0.1504) when compared with feces 11(6.4%, 2.74, 10.05), from bucket milk 1(5%, 4.55, 14.55) and 1(5%, 4.55, 14.55) bucket swabs.

Table 6: Proportion of *E. coli* and *Salmonella* spp isolated from samples

Samples Type	No of examined	No of *E. coli* positive	Prevalence (95% CI)	*Salmonella* positive	Prevalence (95% CI)
Rectal feces	(N=172)	27	15.7 (10.26-21.13)	11	6.4 (2.74- 10.05)
Udder milk	(N=172)	33	19.2 (13.30-25.07)	18	10.5 (5.89-15.04)
Bucket milk	(N=20)	2	10 (3.15-23.15)	1	5 (4.55-14.55)
Bucket swab	(N=20)	1	5 (45.5-14.55)	1	5 (4.55-14.55)
Total	(N=384	63	16.4 (12.70-20.11)	31	8.1 (5.35-10.80)

Proportion of *E. coli* and *Salmonella*isolate were statistically significant among the samples milk with (X^2= 12.148, 15.667) and P-value (0.026, 0.004) indicated below respectively. Test statistic in both *E.coli* and *Salmonella*sppisolatesin udder milk of samples obtained that there were significance differences in prevalence P< 0.05 (Table 7).

Table 7: Prevalence and association of *E. coli* and *Salmonella* isolated among sample types

Samples type	No of *E. coli* Positive (%)	X^2 (Pv)	*Salmonella* spp positive (%)	X^2 (Pv)
Rectal feces (N=172)	27 (15.7)*		11 (6.4)*	
Udder milk (N=172)	33 (19.2)	12.148 (0.026)	18 (10.5)	15.667 (0.004)
Bucket milk (N=20)	2 (10)		1 (5)	
Bucket swab (N=20)	1 (5)		1 (5	
Sub-total (N=384)	63 (16.4)		31 (8.1)	

Key:N=384*2=768 total sample, P-value (PV), Chi-square (X^2),*values in parenthesis are percentage

4.2. Antimicrobial Susceptibility Test

All isolates of *E. coli* and *Salmonella* spp were confirmed further subjected to AST against 8 commonly used antibiotics as indicated in table 4 by using disk diffusion methods.Isolates of targeted bacteria were resistance to at least one or more antibiotics used. It is indicated that the isolates of *E. coli* and *Salmonella*spp were highly resistance to cefoxitin (71.4%, 77.4%), tetracycline (65.8%, 67.8%) and streptomycin (55.6%, 61.3%) respectively. Likewise, *E. coli* of isolate were 50.8% resistant to ciprofloxacin and 54.8% of *Salmonella* spp isolates resistant

to gentamycin. On the other hand, *E. coli* isolates completely/highly susceptible to nitrofurantoin (100%), nalidixic acid *(*96.8%), ceftriaxone (88.9%) and gentamycin (55.6%). Similarly, *Salmonella*sppisolates found 100% susceptible to ceftriaxone, followed by 77.4% nalidixic acid, 74.2% nitrofurantoin and 70.9% ciprofloxacin.

In this finding, as much as intermediate pattern of antibiotic resistance concerned isolate were intermediate resistance to gentamycin (38.1%), streptomycin (14.3%), ciprofloxacin (4.8%), ceftriaxone (6.3%), nalidixic acid (3.2%) and tetracycline (1.6%). Regarding *Salmonella*spp isolates were 29.1%, 19.4%, 16.1%, 12.9% and 3.2% reflected intermediate resistance to ciprofloxacin, nalidixic acid, nitrofurantoin, streptomycin and tetracyclineas depicted in table 8.

Table 8: Overall antimicrobial susceptibility patterns of *E. coli* and *Salmonella* spp isolates

	Escherichia coli isolates (N=63)			*Salmonella* spp. isolates(N=31)		
Antibiotics	Susceptible N (%)	Intermediate N (%)	Resistance N (%)	Susceptible N (%)	Intermediate N (%)	Resistance N (%)
CXT	18 (28.6)	0 (0)	45 (71.4)	7 (22.6)*	0 (0)	24 (77.4)
CTX	56 (88.9)	4 (6.3)	3 (4.7)	31 (100)	0 (0)	0 (0)
CIP	28 (44.4)	3 (4.8)	32 (50.8)	22 (70.9)	9 (29.1)	0 (0
GEN	35 (55.6)	24 (38.1)	4 (6.3)	14 (45.2)	0 (0)	17 (54.8)
NAL	61 (96.8)	2 (3.2)	0 (0)	25 (80.65)	6 (19.4)	0 (0)
NIT	63 (100)	0 (0)	0 (0)	23 (74.2)	5 (16.1)	3 (9.8)
S	19 (30.2)	9 (14.3)	35 (55.6)	9 (29)	3 (12.9)	19 (61.3)
TET	21 (33.3)	1 (1.6)	41 (65.2)	9 (29)	1 (3.2)	21 (67.8)

Keys: *values in parenthesis are percentage, CXT (Cefoxitin 30µg), CTX (Ceftriaxone 5µg), CIP (Ciprofloxacin 5µg), GEN (Gentamycin 10µg), NAL (Nalidixic acid 30µg), NIT (Nitrofurantoin 300µg), S (Streptomycin 10µg), TET (Tetracycline 30µg)

Of isolated *E.coli* and *Salmonella* withrespect to the study areas or samplecollected assessed for resistant to antibiotics.With resistance isolates of *E.coli* (N=57) derived from all samples were subjected to antimicrobial susceptibility tested 50.9% (29/57), 29.8% (17/57) and 19.3% (11/57) in Horo, Bako Tibe and Gobu Seyo of farms sampled respectively. Accordingly, *Salmonella* sppisolated with resistance was 52% (14/27) in Horo, 33% (9/27) from Gobu Seyo and Bako Tibe 15% (4/27)in sampled areas. Antimicrobial susceptibility test revealed

the isolates obtained from Horo, Bako Tibe and Gobu Seyo was found resistant to cefoxitin (89.7%) and 82.4% and tetracycline (100%) respectively. Regarding of *E. coli* isolated, 6.4% (4/63) and 3.1% (2/63) were susceptible to antibiotics used in samples collectedfrom Horo and Gobu Seyo respectively. Also, isolate*Salmonella*spp 9.7% (3/31) from Horo and 3.2% (1/31) Gobu Seyo sampled were susceptible to antibiotics used. Significant variation was observed in *Salmonella*sppisolated resistance with respect to study areas (P <0.05) in table 9.

Table 9: Proportion of resistant *E. coli* and *Salmonella* isolates among study districts

Antibiotics	No of isolatesof *Escherichia coli* resistant (N=57)			
	Horo (N=29)	Bako Tibe (N=17)	Gobu Seyo (N=11)	X^2P-value
Gentamycin (10µg	0(0)	3 (17.65%)	1 (9.1%)	
Ciprofloxacin (10µg)	15 (51.7%)	9 (53%)	8 (72.7%)	
Cefoxitin (5µg)	26 (89.7%)	14 (82.4%)	5 (45.5%)	
Ceftriaxone (5µg)	2 (6.9%)	0 (0)	1 (5.9%)	1.113, 0.573
Streptomycin (10µg)	18 (62.1%)	8 (47.1%)	9 (82%)	
Tetracycline (30µg)	17 (58.6%)	13 (76.5%)	11 (100%)	
Antibiotics	No of isolates of *Salmonella* spp. resistant (N=27)			
	Horo (N=14)	Bako Tibe (N=4)	Gobu Seyo (N=9)	X^2P-value
Gentamycin (10µg)	6 (42.9%)	3 (75%)	8 (88.9%)	
Cefoxitin (5µg)	13 (92.9%)	3 (75%)	8 (88.9%)	
Nitrofurantoin (300µg)	1 (7.1%)	0 (0%)	2 (22.2%)	6.345, 0.017
Streptomycin (10µg)	11 (78.6%)	2 (50%)	6 (66.7%)	
Tetracycline (30µg)	11 (78.6%)	3 (75%)	7 (77.8%)	

Among total *Escherichia coli* and *Salmonella*species isolatefive to six antibiotics were resistant.Resistance to cefoxitin, tetracycline and streptomycin were found 89.3%, 67.9%, 60.7% in milk and 74.1%, 77% and 62.9% in feces of cows respectively in *E.coli*. Also in this result 100% isolated resistant of *E. coli* to streptomycin and tetracycline was analyzed in bucket milk. Resistance to cefoxitin and gentamycin, tetracycline and streptomycin were obtained 90%, 70%, 45.5% and 86.6%, 86.7% 73.3% and 53.3% in feces and milk respectively for *Salmonella* isolates (Table 10).

Subsequently, 100% of *Salmonella spp.* was isolate from bucket milk sample for tetracycline and streptomycin resistance. When analyzed by samples, *E. coli and Salmonella*sppisolated from udder milk and feces of cows more resistant than those isolated from bucket milk and bucket swab. For individual antibiotics tested *E. coli and Salmonella* isolates from udder milk and feces revealed a high level of AMR more than 45% of isolates resistant to each antibiotics except for gentamycin, ceftriaxone and nitrofurantoin with P <0.05 as portrayed in table 10.

Table 10: Antimicrobial resistance of *E. coli* and *Salmonella* spp isolates from sample types

	Number of *Escherichia coli* isolates resistance (N=57)				
Antibiotics	U.milk(n=28)	Feces (N=27)	BM (n=1)	BS (n=1)	Total
CXT (N=45)	25 (89.3%)	20 (74.1%)	0 (0%)	0 (0%)	45 (78.9%)
CTX (N=3)	1 (3.45%)	0 (0%)	1 (100%)	1 (100%)	3 (5.3%)
CIP (N=30)	13 (46.4%)	17 (63%)	0 (0%)	0 (0%)	30 (52.6%)
GEN (N=4)	1 (3.6%)	3 (11.1%)	0 (0%)	0 (0%)	4 (7 %)
S (N=35)	17 (60.7%)	17 (62.9%)	1 (100%)	0 (0%)	35 (61.4%)
TET (N=41)	19 (67.9%)	21 (77%)	1 (100%)	0 (0%)	41 (72%)
X^2 (P-value)	35.063 (0.001)	51.571(0.000)	1.333 (0.514)	1.286 (0.257)	
	Number of *Salmonella* spp. isolates resistance (N=27				
Antibiotics	U.milk(n=28)	Feces (N=27)	BM (n=1)	BS (n=1)	Total
CXT (N=22)	13 (86.6%)	9 (90%)	0 (0%)	0 (0%)	22 (85.2%)
GEN (N=17)	8 (53.3%)	9 (20%)	0 (0%)	0 (0%)	17 (62.9%)
NIT (N=3)	1 (6.7%)	2 (90%)	0 (0%)	0 (0%)	3 (11.1%)
S (N=19)	11 (73.3%)	5 (45.5%)	1 (100%)	1 (100%)	19 (70.4%)
TET (N=21)	13 (86.7%)	7 (70%)	1 (100%)	0 (0%)	21 (78%)
X^2 (P-value)	7.258 (0.007)	20.161(0.002)	0.806 (0.369)	2.173 (0.144)	

Key: BM (Bucket milk),U.milk (udder milk), BS (Bucket swab), CXT (cefoxitin), CTX (ceftriaxone), CIP (ciprofloxacin), GEN (gentamycin), NAL (nalidixic acid), NIT (nitrofurantoin), S (streptomycin), TET (tetracycline)

With regards to distribution of multiple drug resistance*E. coli* and *Salmonella*sppisolated from samples presented in table 11. Up on this, among 63 (*E.coli*) and 31(*Salmonella*) isolates analyzed against eight antibiotics, only 57 (90.5%) and 27 (87.1%) of isolates were resistant.

Accordingly, 100% of feces dairy animals and bucket milk higher *E. coli* isolated resistant to antibiotics. Also in this data, 100% resistances of Salmonella spp isolates were detected from bucket milk and swab (Table 11).

Table 11: Distribution of multiple drug resistance in isolated *E. coli* and *Salmonella* samples

Antibiotics applied	Udder milk	Feces	Bucket milk	Bucket swab	Total
No of *E. coli* isolates (N=63)	33	27	2	1	63
Multi-drug resistant (N=57)	28	27	1	1	57
Overall prevalence (%)	(84.9)*	(100)	(50)	(100)	(90.5)
No of *Salmonella* isolate (N=31)	18	11	1	1	31
Multi-drug resistant (N=27)	15	10	1	1	27
Overall prevalence (%)	(83.3)	(90.9)	(100)	(100)	(87.1)

*Values in parenthesis are percentage, No,number

In this study, different patterns of multiple drug resistances were also observed at different proportion. Beside, this both cases of *E. coli* and *Salmonella* spp all of the isolates tested were resistant to at least one or more antibiotic (Table 12). Overall rate of multiple drug resistance were 75.4% (43/57) and only 6 (9.5%) of the isolates susceptible to eight antibiotics tested with respect to *E coli* isolated. Moreover, most common pattern found in multidrug-resistance isolates; cefoxitin, tetracycline, streptomycin, ciprofloxacin and gentamycin. Two isolates (3.51%) were found to be resistant to five antibiotics tested. Fourteen (14) of the isolates (24.6%) were resistant to three different antibiotics used followed by four (12.3%).

In addition, 81.5% (22/27) and 4 (14.8%) of *Salmonella*spp isolates showed multidrug resistance and susceptible to antibiotics respectively (Table 15). Correspondingly, *Salmonella*specieswereisolates indicated frequent pattern of multidrug resistance to antibiotic used for test that includes cefoxitin, tetracycline, streptomycin and gentamycin. Resistance alongside of four antibiotic were observed in 5 (18.5%) of the isolates of which, 3 (11.1%) were against cefoxitin, tetracycline, streptomycin and gentamycin. While, 1(3.7%) isolates were resistant to cefoxitin, tetracycline, streptomycin and nitrofurantoin including gentamycin. Of the isolates with resistance to at least three antibiotics, 63% (17/27) had a pattern of resistance including cefoxitin and tetracycline.

Table 12: Multiple drug resistance patterns of *Escherichia coli* and *Salmonella* isolates

Escherichia coli isolates (N=57)		*Salmonella* isolates(N=27)	
Drug types	N (%)	Drug types	N (%)
No of susceptible to all drugs	6 (10.5)*	No of susceptible to all drugs	4 (14.8)
Resistance to one drug	14 (24.6)	**Resistance to one drug**	5 (18.5)
Resistance to two drugs	**20 (35.1)**	**Resistance to two drugs**	**10 (37)**
CXT*TET	6 (10.5)	CXT*TET	2 (7.4)
CXT*S	5 (8.8)	CXT*S	1 (3.7)
CXT*CIP	3 (5.3)	CXT*GEN	1 (3.7)
TET*S	5 (8.8)	TET*S	3 (11.1)
TET*CIP	1 (1.8	S*GEN	2 (3.7)
		TET*GEN	1 (7.4)
Resistance to three drugs	**14 (24.6)**	**Resistance to three drugs**	**6 (22.2)**
CXT*TET*S	5 (8.8)	CXT*TET*S	3 (11.1)
CXT*TET*CIP	4 (7)	CXT*TET*GEN	1 (3.7)
CXT*S*CIP	5 (8.8)	TET*S*GEN	2 (7.4)
Resistance to four drugs	**7 (12.3)**	**Resistance to four drugs**	**5 (18.5)**
CXT*TET*S*CIP	4 (7)	CXT*TET*S*GEN	3 (11.1)
TET*S*CIP*CTX	2 (3.51)	CXT*TET*S*NIT	1 (3.7)
CXT*TET*S*CTX	1 (1.8	TET*S*GEN*NIT	1 (3.7
Resistance to five drugs	**2 (3.51)**	**Resistance to five drugs**	**1 (3.7)**
CXT*TET*CIP*S*GEN	2 (3.5)	CXT*TET*S*GEN*NIT	1 (3.7)
Total	57**(100)**	Total	**27 (100)**

*Values in parenthesis are percentage,CXT (cefoxitin), TET (tetracycline), CIP (ciprofloxacin), S (streptomycin), GEN (gentamycin), NIT (nitrofurantoin)

4.3. Questionnaire Survey

4.3.1.Sociodemographic information of respondents

The respondents who participated in questionnaire survey (n=60) were 45%,30% and 25% from Horo, Bako Tibe and Gobu Seyo districts respectively with 60% of farmer.From 60 dairy holders were interviewed questionnairemale to female ratio of 2.5:1(71.7%) male. Most of respondents age ranges between 36 and 44 years 28 (46.7%). Of respondents 24 (40%) attended primary level of education. With respectto respondentparticipated32 (53.3%) were inthe rural residence.Respondents 33(55%) showed that they had kept dairy cattle with 1-5 years. Dairy animalholder'sperspectives of respondents(58.3%) were medium,followed by small (36.7%) and large (5%) holders. Of 60 dairy farm or animal holders (95%) were private farm. All of participated respondents were cattle owners. Besides cattle, farmers owned sheep (78.3%), goats (58.3%) and poultry and equine species (75%) were kept.

Table 13: Sociodemographic characteristic of dairy holders interviewed for questionnaires

Characteristics	Category	N (%)	Characteristics	Category	N (%)
	18-35 years	21 (35)*		Illiterate	17 (28.3)
Age	36-44 years	28 (46.7)	Level	Primary level	24 (40)
	>45 years	11 (18.3)	of Education	Secondary level	11 (18.3)
Sex	Female	17 (28.3)		Diploma/degree	6 (10)
	Male	43 (71.7)		Others**	2 (3/3)
Residence	Rural	32 (53.3)	Ownership	Government	3 (5)
	Urban	28 (46.7)	of farm	Privative	57 (95)
Farming	1-5 years	33 (55)	Farm size	Small	26 (43.3)
Experience	6-10 years	16 (26.7)		Medium	30 (50)
	10 years	11 (18.3		Large	4 (6.7)
Animals	Cattle	60 (100)	Occupation	Farmer	36 (60)
Ownership	Sheep	47 (78.3)		Animal science	2 (3.3)
	Goat	35 (58.3)		Animal health	4 (6.7)
	Others***	45 (75)		Others	18 (30)

*values in parenthesis are percentage, others** certificate, *** donkey, horse, mule, poultry, ****Management, merchants, teachers.

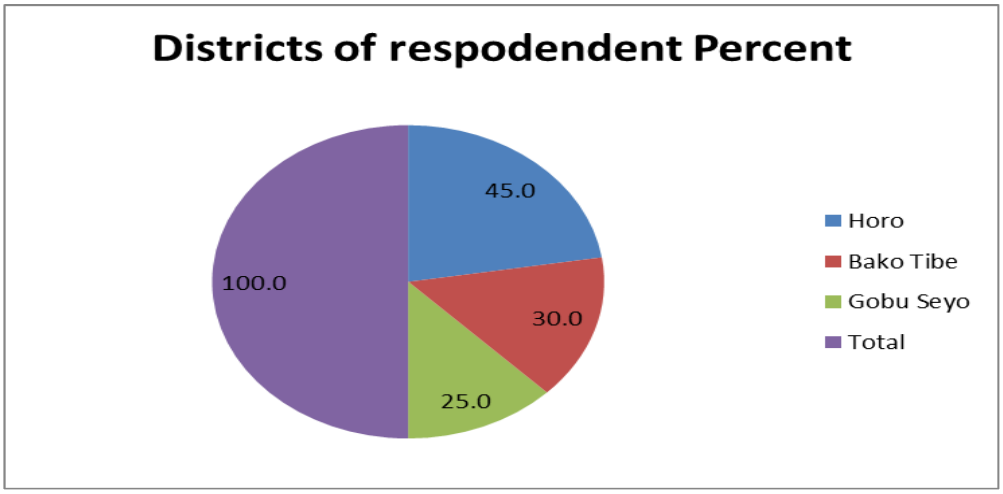

Figure 5: General distribution of the respondents selected for interviewed in study areas

4.1.2. Perception of dairy owner'srespondents on AMU and its resistance

In this survey all of respondents across study areas were aware and or know (100%) livestock diseases in the farm or in study areas.Respondents were asked about diseases as ranked 100% (anthrax, black leg, pasteurellosis, ghandi and diarrhea like symptoms), 95% (bloat, cough, mastitis, NCD and Fasciolosis.Similarly, 61.7% of dairy owners in the districts indicated diseases: lameness, abortion and AHS were reported in different animals.Almost 50% of dairy owners were obtain services from government vet clinic in the study areas; only 35% and 15% get by contact private vet pharmacy and human pharmacy respectively. Accordingly; 62.5%, 46.4% and 43.75% respondents in Bako Tibe, Horo and Gobu Seyo districts contacted veterinary clinicsof government during animals diseased, respectively. Likewise, 48.3%, 43.3% and 8.3% of the respondent obtain different drugs from vet pharmacy, government veterinary clinic and market respectively used as sources.About 43.7%, 31.3% and 31.1% of dairy owners were practice self-prescribed antimicrobials for their animal in Gobu Seyo, Bako Tibe and Horo, respectively.In this survey, 81.6%, 11.7% and 6.7% respondentswere choice drugs based on animal health prescriptions, experience and other health service respectively. Consequently, Bako Tibe 56.3%, 78.6% in Horo and 81.3% Gobu Seyo respondents were used expired AM drugs for their animal. In this study (83.3%) of dairy owner in area were not aware of ABR in animalsand86.7% of observed having used antimicrobial yet upsettingly near all of them not know what an antibiotics are the concept (table 14).

Table 14: Perception of respondents on antimicrobial usage in the study areas

Questions	Districts of dairy owners			Total
	Horo (n=28)	Bako Tibe (n=16)	Gobu Seyo (n=16)	(N=60)
Know or aware on existence of livestock diseases				
Yes	28 (100)	16 (100)	16 (100)	60 (100)
No	0 (0)	0 (0)	0 (0)	0 (0)
Common livestock aware or know D+s in your farm or Kebeles'				
Anthrax, Black leg, Pasteurellosis, Ghandi, Diarrhea	28 (100	16 (100)	16 (100)	60 (100)
Bloat, Cough, Mastitis, NCD, Fasciolosis	28 (100)	16 (100)	13 (81.3)	57 (95)
Others*	21 (75)	9 (56.3)	7 (47.8)	37 (61.7)
Animals encounter with such D+s or symptoms				
Call for A/H professional employed government	13 (46.4)	10 (62.5)	7 (43.75)	30 (50)
Goes to nearby vet pharmacy	10 (35.7)	5 (31.3)	6 (37.5)	21 (35)
Others**	5 (17.9)	1 (6.3)	3 (18.75)	9 (15)
Do you know an antibiotics or antimicrobials drugs are?				
Yes	4 (14.3)	3 (18.7)	1 (6.25)	8 (13.3
No	24 (85.7)	13 (81.3)	15 (93.75)	52 (86.7)
Mostly sources of antimicrobials/antibiotics you used				
From vet government clinic	13 (46.4)	6 (37.5)	7 (43.8)	26 (43.3)
From veterinary pharmacy	12 (42.9)	9 (56.2)	8 (50)	29 (48.3)
Others***	3 (10.7)	1 (6.3)	1 (6.3)	5 (8.3)
Any drugs/ABs commonly you used for your animals in your house				
Pencillin, oxytetracycline and Penstrip	10 (35.7)	4 (25)	6 (37.5)	20 (33.3)
Others****	18 (64.3)	12 (75)	10 (63)	40 (66.7)

Without or self-prescription of AMs for your dairy animals good				
Yes	9 (31.1)	5 (31.3)	7 (43.75)	21 (35)
No	19 (68.9)	11 (68.7)	9 (56.25)	39 (65)
Heard about health/medicines use records of treated animal				
Yes	5 (17.9)	3 (18.8)	1 (6.3)	9 (15
No	23 (82.1)	13 (81.2)	15 (93.7)	51 (85)
Mostly choice of Abs/drugs for Rx of your dairy cows/animals				
Based on vet prescription	24 (85.7)	14 (87.5)	11 (68.8)	49 (81.6)
based on own experience	3 (10.7)	1 (6.3	3 (18.8)	7 (11.7)
Others *****	1 (3.6)	1 (6.3)	2 (12.5)	4 (6.7)
Antibiotics on your hands bought are expired				
Will use them when needed	22 (78.6)	9 (56.3)	13 (81.3)	44 (73.3)
Throw away	5 (17.9)	5 (31.3)	2 (12.5)	12 (20)
Other******	1 (3.6)	2 (12.5)	1 (6.3)	4 (6.7)
Know or heard about drug resistance particularly antibiotic in animal				
Yes	5 (17.9)	3 (18.8)	2 (12.5)	10 (16.7)
No	23 (82.1)	13 (81.2)	14 (87.5)	50 (83.3)

*values in parenthesis are percentage, others**; abortion, CBPP, Orf, AHS, Lameness; ***open market buy medicine, villagers that sell drugs; *****no response,****** share with animals finished, consult the professional, ABs-antibiotics

4.1. 2. Public health aspects

In the study area, about 31 (51.7%) interviewed dairy ownerswere unaware ofrisk associated with consumption of raw milk. As well as 88.3% of respondents considers improper farm wastemanagement notcause antimicrobial resistance. The result also showed that, 53.6%, 43.75% and 62.5% of respondents in Horo, Bako Tibe and Gobu Seyo were misunderstood consumptions of raw milk having health impact, respectively. Only 48.3% of respondents were heard about consumption of raw milk expose consumers to diseases causing pathogens. About 25% of interviewers proper conclude that consumption of primary dairy product in follow of treated animals risk for public health.But, 75% of respondent were not considered possibilities of transmission of resistant bacteria cause effect on consumers. While 47 (78.3%) and 53 (88.3%) of respondentsmisunderstand antibiotics resistant in animals can transmitted to humans causes public health and economic impact respectively. Also, 83.3%of respondents were not understandcorrectly use of antimicrobials in animals cause resistance pathogens in human.

However, 88.3% of the respondents in study areas received AMR have economic important.On further questioning, only 23.3% of the respondents were aware of the public health importance of antimicrobial resistance transmitted to public via consumption milk.While, 76.7% highlightof respondents stress to rigorouslyexpose to AMR with the dairy product. Directly when asked to highlight on expired antibiotic use practices of respondents that 73.3% were used whenneeded in any animal. Dairy animals owners disposed of expired drugsin various ways including throw away into surrounding (garbage) (20%), and (6.7%) returning the drug where they buy or they shared with animals treating finished also un response observed (table 14).Also, 88.3% of them considers as improper waste management did not cause AMR (table 14). About 36 (60%) of interviewed were aware of inappropriately use of antibiotics in animal and human impacts (table 15).

T**able 15**: Public health aspects related questionnaire interviewed respondents

Questions	Districts of dairy owners			Total (N=60)
	Horo (n=28)	B/Tibe (n=16)	G/Seyo (n=16)	
Awareness as raw milk consumption may have health impact				
Yes	13 (46.4)	9 (56.25)	7 (37.5)	21(48.3)
No	15 (53.6)	7 (43.75)	9 (62.5)	31 (51.7)
Consumption of milk of cows treated with ABs have health effects				
Yes	8 (28.6)	5 (31.2)	5 (31.2)	15 (25)
No	20 (71.4)	11 (68.8)	11 (68.8)	45 (75)
Improper farm waste management can cause AMR				
Yes	4 (21.4)	2 (18.8)	1 (6.3)	7 (11.7)
No	24 (78.6)	14 (81.2)	15 (93.7)	53 (88.3)
Aware of withdrawal of antibiotics after the treatment of cows				
Yes	9 (32.1)	6 (37.5)	5 (31.3)	20 (33.3)
No	19 (67.9)	10 (62.5)	11 (68.8)	40 (67.7)
ABR bacteria in animals can be transmit to human via consumption of milk				
Yes	6 (21.4)	4 (25)	3 (18.75)	13 (21.7)
No	22 (78.6)	12 (75)	13 (81.2)	47 (78.3)
Incorrectly uses of ABs in animals can cause resistance in human				
Yes	5 (17.9)	3 (18.75)	2 (12.5)	10 (6.7)
No	23 (82.1)	13 (81.25)	14 (87.5)	50 (83.3)
In your opinion, antimicrobial resistance have economic impacts				
Yes	25 (89.3)	13 (81.25)	15 (93.75)	53 (88.3)
No	3 (10.7)	3 (18.75)	1 (6.25)	7 (11.7)
Visits and explains the effect of incorrect use of ABs in animal and human				
Yes	10 (35.7)	8 (50)	6 (37.5)	24 (40)
No	18 (64.3)	8 (50)	10 (62.5)	36 (60)

Parenthesis N (%)* number of frequency in percent, B/Tibe (Bako Tibe); G/Seyo (Gobu Seyo)

5. DISCUSSIONS

The existences of *E.coli* and *Salmonella*species in milk, fecal and equipmentof milk buckets were assessed. In this study of samples 384 collected for isolation of two targeted bacteria 94 (12.2%) isolated in study areas.This can be potentials source for the contamination of dairy farms and products by antibiotic resistance of *E. coli* and *Salmonella* their resistance elements can transmitted to human directly or indirectly cause serious public health impacts. These bacteria involved incausing food borne disease due to consumption of contaminated food of animal origin and resistance bacteria are circulated in host and non-host (Fredrick *et al*., 2016). The variation of *E.coli* and *Salmonella*sppprevalence among samples isolated bacteria survive in stressful by entering viable, but non-culturable state (Mollie and Eduardo, 2003).

The proportion of *E. coli* isolatedfrom Horo district (17.4%) was higher than prevalence of two Bako Tibe (15.5%) and Gobu Seyo (15.5%) districtin dairy farm/holderssamples collected with no significant variations (P=0.362). Accordingly, proportion of *Salmonella spp*isolated in Gobu Seyo(12%) was higher than isolated in Horo (9%) and Bako Tibe (3.64%) samples examined with (P=0.068). Existence of those bacteria implies contamination of milk that might be predisposing the public to food borne diseases.Isolate of bacteria even if not pathogenic crucial to health since they may act as reservoirs for resistance and disseminating to the environment (Marshall and Levy, 2011).

This finding was (16.4%) of *E.coli* among samples in line with various studies reported 17.44% from India and 15.89% from meat samples in ELFORA and Municipal abattoir (Das and Joseph, 2005; Ousman *et al*., 2014) respectively. From this result significantly high proportion(19.2%) of *E. coli*isolatedfrom udder milk. But, this is far lower when compared to results of Javeed *et al.* (2013), Haftay *et al.* (2018) and Yohannes (2018) who reported 25.36%, 44.57% and 25% in abroad and country respectively, from different food samples. Moreover, this finding also lower than report of Ali and Abdelgadir (2011) 63% from Khartoum and Fadaei (2014) 69% in Iran who have observed prevalence of *E.coli*. In the result of present study was comparable with reported 20% by Bedasa *et al.* (2018), 23.7% by Mekuria *et al* (2014) in Ethiopia and Elbagory *et al*. (2016) in Egypt (21.7%) from food of dairy animal. This result was high compared with different studies in the world observed as

11.6% by Sori *et al.* (2005) and 7.1% Ayano *et al.* (2012) and 2.5% Bitew *et al.* (2010) from Ethiopia, 8.75% Lye *et al.* (2013) from Malaysia and 11.2% by Addo *et al.* (2011) in Ghana.

But, this was far lower when compared with the reports from country and abroad by Bedasa *et al*.(2018) in Bishoftu town (32%), Mohammed *et al*. (2017) in Bangladesh (76%), Reta *et al.* (2016) in Jigjiga city (30%), Ombarak *et al*. (2016) in Egypt (76.4%), Shunda *et al.* (2013) in Mekelle town (44%), Thaker *et al.* (2012) in India (38%) and Farzan *et al*. (2012) in Slovenian (41.5%). *Escherichia coli* reside in the hindgut of animals and shed in feces, which assist as source of contamination milk and environment for infection of human (Ferens and Hovde, 2011; Yohannes, 2016). Milk services as excellent medium for growth of bacteria as compared to other samples. The disagreement observed due to the methods used and source of sample, transportation, season, geographical location and others in which studies are done.

Up on this study, 15.7% prevalence of *E. coli* was obtained from fecal sample of dairyanimals. However, this result waslower85% reported by (Jessica *et al*., 2008) in United States dairy products. Relatively, the present finding from bucket milk was (10%) high comparable with the records from abroad by Solomakos *et al.* (2009) in Greece from milk samples. When compared with the finding of Gwida and Gohary (2013) 20% in Egypt, Reta *et al.* (2016) 53.3% and 33.3% Haftay *et al.* (2018) 63.95% in Ethiopia from milk shop, higher prevalence was observed. These variations were realized in prevalence among different studies might be attributed to hygienic condition and others.

*Salmonella*speciesinfections in dairy cattle persist as major problem worldwide. Substantial economic losses through mortality and poor growth of animals. Also, the risk of transmission of infection to humansvia food chain in both developing and developed countries represents considerable burden (Majowicz *et al*., 2010; Fufa *et al*., 2017). Busani *et al*. (2006) stated *Salmonella*accounted as one of the most common causes of food born disease in the world.On the other hand, this finding (8.1%) was higher than who have reported 4% in Northern Thailand (Bywater *et al*., 2004), 3% from England (Padungtod and Kaneene, 2006) and from dairy farm in Asella 4.4% (Takele *et al*., 2016). When compared with higher results were reported by Teshome and Anbessa (2012), Zelalem *et al*. (2011) and Fufa *et al*. (2017), who reported prevalence of *Salmonella* 20% in Kersa district, 10.75% in Addis Ababa and 10.5% Modjo town, respectively from lactating cows and humans in dairy farms. The current study

was lower with studies prevalence of *Salmonella* 28.6% conducted in Asella by Takele *et al*. (2016) in Ethiopia. Difference may be due to the source of samples and hygienic status of abattoir. In agreement with this, relatively higher prevalence reported 27% in Cameroon among cattle (Akoachere *et al*., 2009) and 10.5% in and around Modjo town dairy farm and 14.3% reported from milkers hand swab by Fufa *et al*. (2017) respectively.

In this study, significantly *Salmonella* isolated was relatively highest in udder milk (10.5%) among samples with (P <0.05). Different studies in various countries revealed that very low isolations of *Salmonella* were 3.3% Reta *et al.* (2016), 4% Forough *et al*. (2012), 2.17% Junaidu *et al.* (2011) and 1.43%, Sanaa *et al.* (2005) from milk. However, it is relatively parallel with 12.1% reported by Fufa *et al*. (2017) in dairy farms from milk Modjo, Ethiopia. Likewise, 5% of *Salmonella*spp was isolate from each bucket milk and bucket swab sample. Similarly, higher result was reported by (Fufa *et al*., 2017) who found the prevalence of *Salmonella* (19%) in tank milk and 9.5% in bucket swab from dairy farms. Moreover, this finding indicated that *Salmonell*a spp isolate 5% from bucket milk which is in agreement with Fufa *et al.* (2017) who reported isolation of 4.8% in tank swab. But, El-Baz *et al*. (2017) observed 24% in raw bulk milk was higher in this finding. Ubiquitous nature of *Salmonell*a is persistent contamination hazard in raw milk (Carrasco *et al.*, 2012) contributing for potential sources of *Salmonella*spp infection in large communities.

In the present study prevalence 6.4% of *Salmonella* sppin feces of cows relatively lower than the proportion7.3% in USA and 7.7% reported by Blau *et al*. (2005), Zelalem *et al*. (2011) and Fufa *et al*. (2017) respectively, in feces of dairy cattle in Ethiopia. But, compared to the current findings, lower prevalence of 1.56% and 2.3% were also reported by Mohamed *et al.* (2011) and Eguale *et al*. (2016) in Egypt and central Ethiopia respectively. Higher isolation of *Salmonella* species found in California 44% (Heider *et al*., 2009) from dairy cattle feces. In addition, this current observed was lower than report of 8.6% by Fufa*et al*. (2018) in Adama and Hailu *et al*. (2015) 12.5% in Gondar from faecal and milk of dairy animals respectively. Fecal contamination is inevitable and consequently milk at risk for contamination with any pathogen that present in the feces or farm environment (Kevin *et al*., 2010).

The variation in existence and occurrence of *Salmonella*speciesisolate and previous reports attributed to numerous factors such as the source of sample and types, geographical location,

season of sampling, media and differences in detection methodologies used (Hui, 2015; Fufa *et al*., 2017). Nevertheless, in spite of variations all of these studies evidenced obviously milk significant vehicle for pathogens (Nagal *et al*., 2006; Guesh *et al*., 2017).

The present study antimicrobial sensitivity test of *E. coli* and *Salmonella* reflected varying degree ofsusceptibility to antibiotics used.With these results showed high level of resistance to most of theantibiotics used in both isolated bacteria were found.Antibiotics have role on animals and humans as whole by drastically increasing of life time (Aarestrup *et al*., 2008). Despites, effectiveness of antibiotics to control diseases rigorously hindered and wide spread use laid enormous bacteria to revolve as resistance to multiple drugs(Byarugaba *et al*., 2011; Da Costa *et al*., 2013). Zoonotic and antibiotic resistant *E. coli* and *Salmonella*sppnow have global issues rock bottom the capacity of different prophylaxis (Sorbur *et al*., 2019).

As Magiorakos *et al.* (2012) definition for multi-drug resistance isolates were resistant to at least three different antibiotics. Up on this present finding, among the resistant *E. coli* (90.4%) and *Salmonella* (87.1%) isolates from samples were multidrug resistance that exhibited five or six different MDR patterns to 8 antibiotics. Since use and misuse of antibiotics in animal and humans have increasing extraordinary multidrug resistance patterns exhibited by bacteria (Tadesse *et al*., 2012; Frederik *et al*., 2016). As Love *et al.*(2011)stated*E. coli* and *Salmonella*isolate from animal and human had same antimicrobial resistance determinants.Development of resistance by bacteria to these drugsposes a major challenge since commonly used in thetreatment of human and animals. Particularly in zoonotic bacteria can easily transfer to human via food chain or in contacts to environment (Sobur *et al*., 2019).

In this study revealed that isolates *E.coli* were obtained resistant to cefoxitin (71.4%) followed by tetracycline (65.2%), streptomycin (55.6%) and ciprofloxacin (50.8%). Similarly, the current result was in agreement with the reports of Rangel and Marin (2009), Sheikh *et al.* (2013)and Armanullah *et al.* (2018) who have reported 82.2%, 96.76% and 41.1% resistance to cefoxitin respectively. Li*et al.* (2018) in Central California has reported 96.6%, 89.8% and 69.5% susceptibility of *E. coli* isolate to cefoxitin, streptomycin and tetracycline respectively, which was dissimilar to this present finding. Likewise, Hiko *et al.* (2008), Bekele *et al*. (2014) and Bedasa *et al.* (2018) and Magwira *et al.* (2005) in Ethiopia and Botswana who have revealed the resistance of *E. coli* to streptomycin. The present study reflected that isolates

E.coli resistant to tetracycline (65.2%) was supported by various studies (Bekele *et al*., 2014; Mude *et al*., 2017). On the other hand, this finding was higher when compared with reports of (Yohannes, 2016) 40.5% who reported *E.coli* isolates resistant to tetracycline. Unrelated to this finding, Sekhar *et al.* (2017) observed sensitivity of *E.coli* isolated 64.06% to tetracycline from dairy farm sewage. This up to date finding was reverse to the report of Bedasa *et al.* (2018), Haftay *et al.* (2018) and Reta *et al.* (2016) who have reported 97.5%, 60% and 65.7% *E. coli* isolates susceptible to tetracycline and ciprofloxacin respectively. In Ghana Frederik *et al.* (2016) was observed 100% of *E.coli* isolates in milk susceptible to ciprofloxacin, which was disparate in this finding. This study revealed that those antibiotics displayed resistance were the most used antibiotics in study areas. Perhaps, antibiotics like cefoxitin, ciprofloxacin and others rarely used in veterinary practice due to human-animal interface via environmental cross transmission (Ungemach *et al.,* 2006; Juhasz-Kasanyitzky *et al*., 2007) could explain resistance.

In the present study, from isolates *E.coli* tested greater than or equal to 50% of sensitivity to the antimicrobial was observed to nitrofurantoin (100%), nalidixic acid (96.8%), ceftriaxone (88.9%) and gentamycin (55.6%). In agreement with this result, X Li *et al.* (2018) in Central California has indicated 96.6% and 93.2% sensitivity of *E. coli* isolated to nalidixic acid and ceftriaxone, respectively. In contrary to this observed, 88.3% and 62.5% of *E.coli* resistance to nalidixic acid and nitrofurantoin was reported by (Sheikh *et al*., 2013; Elmonir *et al*., 2018) in India and Egypt respectively. Similarly, Yohannes (2018) and Bagre *et al.* (2014) have reported *E. coli* isolated were 60% and 100% sensitive to gentamycin from Ethiopia and Burkina Faso respectively, which is higher than this observed. High susceptible to gentamycin (74.07%) and ceftriaxone (62.96%) was observed in Ghana by (Frederik *et al*., 2016) with covenant to this result. This outcome was in line with reports of Mohammed *et al.* (2017) in Bangladesh and Haftay *et al.* (2018) in Ethiopia who obtained 100%, 90%, 100% and 100% susceptibility of *E. coli* isolate to ciprofloxacin and gentamycin from food of animal origin and fruit respectively. This may indicate drugs are still useful in treatment of *E.coli* infections.

Antibiotic resistance of *E.coli* isolates from dairy farm/holders in study areas were presented in table 13.Besides, this finding overall multidrug resistance analysis exhibited that 90.5%, while only 9.5% of the isolate were sensitive to all antibiotic tested.Subsequently, *E. coli* that

were isolated from these samples have evidence of multi drug resistance, which is supported by previous studies. Likewise, higher proportion of multidrug resistance*E. coli* isolated udder milk 49.1% (28/57) and feacal 47.4% (27/57) with indicate significant P<0.05(Table13). Correspondingly, this result is coherent with findings of Frederik *et al*.(2016) and Bedasa *et al*. (2018). This high resistance in feces and milk might be correlated to greater highlighting given to the dairy production and contaminated by AMs used in study areas. Accordingly, increasing frequency of resistance to antibiotics has reported by various studies manipulated (Mohammed *et al*., 2017; Haftay *et al*., 2018; Bedasa *et al*., 2018).

Furthermore, the current study revealed the proportion of multidrug resistant isolates of *E. coli* was higher in milk (49.1%) to other samples. Similar lower result reported 28.4% and 28.13% by Bedasa *et al.* (2018)and Mohammed *et al.* (2017) MDR of *E. coli,*respectively. *Escherichia coli* isolates of udder milk sample were only 7.9% (5/63) sensitive to antibiotics used. Likewise, isolates from milk were highly resistant to cefoxitin (89.3%) followed by tetracycline (67.9%) and streptomycin (60.7%). This is in line with the findings of various studies, who reported multidrug resistance *E. coli* isolates (Uddi *et al*., 2011; Thaker *et al*., 2012; Yohannes, 2018). Similarly, the higher rate of multidrug resistance was observed for two drugs (35.1%) followed by three (24.6%), four (12.3%) and five (3.5%) drugs. Resistance of (45%) to two, (40%) to three and (75%) to four drugs reported by Bedasa *et al.* (2018) with comparable higher than in this indicated. Likewise, multiple resistance of *E. coli* isolated was observed 100% in feces and 3.5% from bucket milk and bucket swab. The isolate were highly resistant to tetracycline, cefoxitin, ciprofloxacin and streptomycin.

All of isolates *Salmonella* sppwere tested against 8 antibiotics to determine the susceptibility that ranges from 0 up to 100%, which is reflected that varying level of resistance. Meanwhile, this study revealed *Salmonella*isolates were resistant to cefoxitin, tetracycline, streptomycin, gentamicin and nitrofurantoin with rate 77.4%, 67.8%, 61.3%, 54.8% and 11.1% respectively. This finding in line with Tadesse *et al.* (2016) who has reported resistance to streptomycin (86.7%) and tetracycline (53.3%). Abuna *et al.* (2018) was observed 77.4% and 63.6% resistant to streptomycin and cefoxitin, respectively. Likewise, this identified high level of resistance to gentamycin 75.6% recorded from Gondar by (Daniel *et al*., 2008). Conversely, result of resistance to gentamycin was contradicted with the study indicated by (Takele *et al*.,

2016; Guesh, 2017). In accordance, Zelalem *et al.* (2011) showed that *Salmonella* isolate was resistant to streptomycin (66.7%) and tetracycline (33.3%) which was lower than observed in this result. Correspondingly, this resistant towards tetracycline 67.8% observed in present result, which is lower than findings 85.7% of Mohamed *et al*. (2011) in Alexandria, Egypt and 94.6% by Fufa *et al*. (2017) in Ethiopia.

In contrary 61.3% and 54.8% resistance of this finding, sensitive to cefoxitin and Gentamycin was reported by (Tadesse *et al*., 2016; Fufa *et al*., 2017). In addition, this finding was similar Chijioke and Christian (2013) due to tetracycline derived naturally exposed in nature outside any livestock use for diseases treatment or human. However, this obtainedisolatesof *Salmonella* resistance to tetracycline higher compared to results of (Blau *et al*., 2005) reported as 4.4% and 12.2% in America. Variations might uncontrolled availabilities of antimicrobial agents and handling utilizations, host specificity determined *Salmonella* strain andfrequent usage both in livestock and public health, which enhances sustaining resistant genes of bacteria (Karin et al., 2011; Tadesse *et al*., 2016; Fufa *et al*., 2017).

On the other hand, this finding showed all *Salmonella*isolated were completely and or highly susceptible to ceftriaxone (100%) followed by nalidixic acid (80.65%), nitrofurantoin (74.2%) and ciprofloxacin (70.9%). Likewise, this result linked with the report of (Tadesse *et al*., 2016; Fufa *et al*., 2018).In contrary to this finding, Forough *et al.* (2012) reported 78.75% resistance of *Salmonella*spp isolates to nalidixic acid.Up on thisstudy, susceptibility of *Salmonella*spp isolate to nitrofurantoin 74.2%, which was unlike to previous study reported as resistant (63.3%) by (Tadesse *et al*., 2016).Zelalem *et al.* (2011), Teshome and Anbessa, (2012) and Reta *et al*. (2016) who observed 83.3%, 75% and 65.7% of *Salmonella* spp isolate sensitive to ciprofloxacin respectively, which is similar in this finding. But, this finding higher than results of (Blau *et al*., 2005) reported as 4.4% and 12.2% and 27.3% (Fufa *et al*., 2018) *Salmonella*isolates sensitive to ciprofloxacin. Also, Fadlalla *et al.* (2012), and Fufa *et al.* (2017) reported 100% of *Salmonella*isolate sensitive to ciprofloxacin respectively, which is higher than this study.

Furthermore, multi-drug resistant *Salmonella* isolates were obtained from different samples with overall of 87.1% MDR in this study. Multi drug resistance *Salmonella* isolates supported by various studies reported 72.22%, 70% and 89.19% by (Guesh, 2017; Tadesse *et al*., 2016;

Mohammed *et al*., 2018) respectively. Besides, multiple resistances with respect to samples were 100%, 83.3% and 100% isolate*Salmonella* in feces, milk and bucket milk, respectively were resistant. In line with other studies, who reported multidrug resistant among isolates of *Salmonella* (Zelalem *et al.,* 2012; Fufa *et al.,* 2017). Antimicrobial-resistant of *Salmonella* in raw milk may be able to colonize the gut of consumers, making infections difficult to treat.

Isolates from fecal and udder milk samples, MDR to 5 antibiotics used; cefoxitin, gentamycin, tetracycline, streptomycin and nitrofurantoin. Whereas isolated from bucket milk together and swab resistance to two antibiotic used tetracycline and streptomycin. In addition, significantly high proportions of multidrug resistance *Salmonella* spp isolated udder milk 55.6% and feacal 37% with indicate significant $P<0.05$ (Table13). In agreement with result of other studies, who reported multidrug resistant*Salmonella* isolatesby Zelalem *et al.* (2012) and Fufa *et al.* (2017).In this study also, 10 (37%), 6(22.2%), 5 (18.5%) and 1(3.7%) of *Salmonella* sppisolates resistance to two, three, four and five antibiotics of 8 antibiotics used, respectively. Somewhat, proportion of multidrug-resistant *Salmonella*isolate in this study was observed higher than study conducted by (Guesh, 2017). In agreement with this resistance for two or more of antibiotics, which was assessed in this finding was lower than study observed in Addis Ababa, Ethiopia (Zelalem *et al.,* 2011).

Considerably, high level of resistance to most of antibiotics tested probably was an indication of extensive usage in both public health and veterinary practice. Resistance to these drugs poses challenge in both human and animal with bearing significant increasing zoonotic nature of *Salmonella*species. In this study, variation in resistance of *Salmonella* and *E. coli isolates* from the same samplesource may genes of bacteria as they are of different genera. Difference might be due to increasing inappropriate utilization of antimicrobials, which is the advantage of maintaining strains resistant genes (Reuben and Owuna,2013). Variations are probably due to differential clonal expressions and drug pressure in communities (Sekhar *et al*., 2017).

In general, AST revealed that ceftriaxone, nalidixic acid, nitrofurantoin and ciprofloxacin were antibiotics indicated as effective against *Salmonella spp*isolated from this study.Hence in therapeutic decision these drugs should be considered and only after antibiotic sensitivity testing. Antimicrobial resistance is global public health concern that impacted by both human andnon-human antimicrobial usage and spread of antimicrobialresistance (WHO, 2007). All

E. coli and *Salmonella*spp isolates in the present study exhibited resistance to at least two or more of eight antibioticagents tested.The results of the study indicated the need for increased educational and transfer of information from veterinarians to dairy owners in order to develop approaches for prudent antimicrobials use.

Antibiotics are prescribed for treatment of bacterial disease in human and animals (Frederik *et al*., 2016). Result of questionnaire in study site indicated dairy animal holders generally used antibiotics for different livestock disease treatment. Intensive use of antibiotics (Andersson and Hughes, 2010), globally increases frequency of resistance among clinical and commensal isolatebacteria. Also, most of dairy respondent treated their animals without prescription; use of expired antibiotic and inappropriate waste management that have implication on sources of pathogen and AMR in animals and humans. Meanwhile, such AMR bacteria circulating in dairy farm environments are also probably to be sources of human health associated risks.

In this current study, respondents in the study areas used antibiotics to treat their livestock they are being sick and control of diseases. In general, the socio-demographic characteristics of the respondents indicated that majority of dairy animal owners were male (71.7%), rural residence (51.7%) and age 36-44 years (46.7%) with 40% had attend primary level of education. In this assessment, 100% of respondents were aware or knew about diseases of animals that frequently observed (anthrax, black leg, diarrhea, mastitis, pasteurellosis and others in the area (Table 14). Diseases are major constraint to animals and associated with relatively poor awareness in these areas (Ayantu *et al.,* 2012; Dereje *et al.,* 2016). And certain antimicrobial agentto available for direct use by farmers without much restriction does create problems (FAO, 2016). Dairy cattle feces and milk are sources of zoonotic bacteria.The risks of acquiring foodborne diseases since 51.7% of respondents have habit of consuming raw milk. Resource constrained countries often lack information on distribution of zoonotic diseases (Zinsstag *et al*., 2007).

About 16.7% of respondents knew what antimicrobial agents had used for their animals diseases. In agreement with this indicated the low awareness observed in rural Peru among small dairy holders 0.6% of respondents knew antibiotics were used to relief bacterial infections (Redding *et al.*, 2014). However, 83.3% of the interviewers in this finding were lack to the term antibiotics specifically simple called as drugs. As observed in this result, most

of dairy holders bought their antibiotics 48.3% from private veterinary drug store and 8.3% from open market/shop. Positively observed the respondent 43.3% bought antimicrobial drugs from established veterinary government clinics. FAO (2016) stated antimicrobials drug in clinics are relatively quality secure and risk of occurrence of resistance due to use of poor quality agents significantly decreased.Parallel finding in the small scale farms in Zambia 91% indicated that they purchased their drugs from veterinary drug stores (Redding *et al.*, 2014). Furthermore, this result indicated that 33.3% (Pencillin, Penstrip and Oxytetracycline) were antibiotics observed on the hands/ house of 20 respondents. This finding related with survey conducted by (Amabile-Cuevas, 2010;Chauhan *et al*., 2018) in Malawi, Tanzania and India respectively stated as poor drug handling and storage conditions increase the risk of AMR. Ungemach *et al.* (2006) and FDA (2010) that stated prudent use of antibiotics in food producing animal as the cost effective and minimizes existence of AMR. This might be due to incorrect antibiotic treatment with poor awareness about antibiotic usage (Eltayb *et al*., 2012).

Almost, 35% and 75% of dairy animal holders were unaware of possible human health impacts associated with antimicrobials use without and or self-prescription and consumption of treated milk of cows respectively. Among respondents83.3% having used antibiotics yet worryingly practiceof them 86.7% neither know what antimicrobials nor realize concept of antimicrobial resistance. With this result,13.3% of dairy holdersindicated they heard about antimicrobial resistance. Of 66.7% respondents highlighted that they were not aware of importance of withdrawal period. In addition, responses were obtained 85% not heard of keeptreatment records and 88.3% misperceptionoccurrence of AMRdue to improper wastes management. According to Speksnijder *et al.* (2015) stated treatment records were supportive in tracking general drug use and prevention of diseasesoccurrenceon farm animals. This awareness relatively in line to that dairy farmer in rural South Carolina 40% reported by Kramer *et al.* (2017). However, 83.3% interviewed not highlighted about antimicrobial resistance in the area in line with 70% observedin livestock farmers by Katakweba *et al.* (2012) in Tanzania. The variations in response of respondentmight related to education level and perceptiongap in studyareas (Eltayb *et al.*, 2012). Understanding of respondents about antimicrobial resistance contributes in animals and human a substantial indicator for prudent use of antimicrobial drugs.

This present finding was corroborated with various studies those who reported imprudent use of antimicrobials and poor management systems in different livestock production that favors for development AMR (Carlos, 2010; Katakweba *et al*., 2012; Adesokan *et al*., 2015; Fufa *et al*., 2018) from Tanzania, Nigeria and Ethiopia respectively.

Up on this 78.3% and 83.3% respondents do not understand the chance of transmission of AMR consumption of dairy products (milk) and incorrect practices of antimicrobials with associated health impacts.Only, 16.7% of respondents believed that inappropriate uses of antibiotics in the animal may causes resistance to humans. Schneider and Garrett, (2009) reported resistant bacteria in animal can be transmitted to humans via consumption of food, close contact with animals or environment. In questionnaire study it was observed that thedairy animal owners werelack of information on effect of antibiotic use indiscriminately in animal or humans. Of respondentonly 40% were clear understandimpactsemanated from such practice of drugscontributing factor that frequently increases in AR bacteria. This might be due to socio-demographic, weak regulation of antibiotics and, absence of good services in most developing countries (Chenggang *et al*., 2011). In addition imprudent use of AMs either in animals or humans a substantial indicator for antimicrobial resistance bacteria circulatingwith consequent impacts on health in study areas.

6. CONCULSIONS AND RECOMMENDATIONS

In this study, considerable proportions of milk, fecal, bucket milk and bucket swab samples were obtain with *E. coli* and *Salmonella* species. Overall prevalence of *E. coli* (16.4%) and *Salmonella species* (8.1%) isolatedfrom samples by culturedon selective plates and further biochemical test method. Accordingly, isolate*E.coli*and *Salmonella*species were subjected to antimicrobial susceptibility test to antibiotics implies multidrug resistance high in samples observed that may have significant impact on health. Existence of such resistance targeted food borne bacteriain this finding evidently indicated use and misuse antibiotics positively initiate occurrence of resistance. Prominently, presence of such multiple resistance*E. coli* and *Salmonella spp* in this finding reflected of contaminated milk threat to public and animal health. Highlighted responses of the respondentsobserved in study almost all unaware of prudent AMU and its resistance. Take together worrying existence of multi-drug resistance of targeted bacteriaisolated in the samplesand understand of respondent on AMU practice with acquiring pathogenic bacteria as consumption of milk.This finding of study also compared with various aforementioned studies in country and abroad on the targeted study bacteria and AMR isolatefrom different samples. In general, dairy animal holders or farmers are somewhatever-present may serve as potential sources of antimicrobial resistance bacteriafrom surrounding environments.The complex nature on AMU and immense public health significance need for adoption of multiple measures at time to minimize this threat. This result provides baseline data on pattern of AMRof isolated bacteria and identifies perception gaps that can be used as reference in future in the study area.

Based on this finding of study the following recommendations were forwarded:

- It is advisable to increase extension and awareness creation in all aspect of dairy productions to minimize contamination of milk with pathogens of *E. coli* and S*almonella* spp which has public health importance.
- All-embracing AMR profiles of these *E.coli* and *Salmonella* isolated awareness creation should be conducted to the public on importance of the disease and perception of AMU and resistance risk associated to it.

- It is highly important to educate or give training for Para veterinariansand farmers on risk associate with practice of AMU and encouragesavoiding use of expired antibiotics due to prolonged storage and stocking in house.
- As a means toward reduction of AMR and awareness creation to encourage effecting behavioral changes in reduction of indiscriminately usage of antimicrobials.
- It is essential to assess human related antibiotics use practices to obtain more holistic image of antimicrobial resistance.
- Further study should be recommended to assess characterization of bacterial resistant genes of circulating *E.coli* and*Salmonella* species in the areas.

7. ETHICAL CONSIDERATIONS

Permission to conduct this research was obtained from the Research Ethical Committee Jimma University College of Agriculture and School of Veterinary Medicine. Before conducting this research, all the farmers/owners of cattle was informed about the purpose of study and also they should be well aware of the importance and benefit of the research in terms of immediate and future values. Informed oral consent was obtained from the animal owners (farms) at the time of sample collection. Besides, research is highly participatory in the sense that animal owners wereprovided their cow as research grounds. Furthermore, while collecting samples from all isolates of samples (milk, feces, bucket milkand bucket swabs), safe handling procedures followed.For notification, formal letterwas written and sent to the districts office of livestock and fishery development resource.

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9. ANNEXES

Annex 1: List of media, and materials used for isolation of *E.coli* and *Salmonella*

Distilled Water,Kovac‘s reagent, saline water, syringe, bottles, glove, test tube,buffered peptone water, peptone water,samples, Kit, Methyl Red, Discs,universal bottle, Brain Heart Infusion Broth,MacConkey Agar, Muller Hinton Agar, EMB Agar, XLD, nutrient media, icebox, alcohol,Rappaport Vassiliadis Soya broth, Refrigerator, cotton, swabs, forceps, marker, wire loop, autoclave, 0.5McFarland,Measuring cylinders, petridish, rack, slide, distilled water, and others.

Annex 2: Steps of isolation of targeted bacteria in study (*E. coli* and *Salmonella*)

Collectedof samples (feces, udder milk, bucket milk and bucket swabs)

Transportation of sample withinthe time to laboratory for processing

Preparation of culture media (non-selective or selective) based on specific bacterial

Primarily isolation of colonies on cultured media

Transferred of suspected colonies on selective medias/plates

Further biochemical tests for confirmation of specific colonies of bacteria

AST (antibiotic disc and Muller-Hinton agar) of isolated bacteria/colonies

Interpretations and result writing on *Escherichia coli* and *Salmonella*

Annex 3: Isolation and identification of *Salmonella* and *E. coli* from samples

Salmonella*:* The isolation and identification of *Salmonella* from faeces, udder milk, bulk bucket milk and swabswere performed at the BRVLby using techniques recommended by International Organizations for Standardization (ISO-6579, 2004).It involves three steps*:*

1. 5gm of fecal sample or 5ml of milk was pre-enriched with 45ml of BPW at a ratio of 1:9 and swabs taken from farm bucket swabwas also pre-enriched with 10ml BPW and incubated for 24 hrs at 37°C.
2. One ml of the pre-enriched culture in (BPW) 0.1 ml portion from each BPW tube (after incubation) was transferred into a 10 ml Rappaport Vassiliadis Soy broth and incubated at 37°C for 24 and 48 hours.
3. Finally, the RVS broth culture samples were streaked onto XyloseLysine Deoxycholate agar (Oxoid) plates andincubated overnight at 37 °C. Typical colonies were picked and further tested by standard biochemical methods.

Suspected colonies detected, sub cultivation of 3-4 colonies from XLD on to a non-selective nutrient agar media plates for confirmation by using biochemical tests including Triple sugar iron (TSI), Indole, ureaseand citrate test. Atypical biochemical reaction on TSI i.e. alkaline (red) slant, acidic (yellow) butt, H_2S and gas production, citrate utilization as a carbon source, Indole and urease (Hendriksen, 2003) was performed.

Escherichia coli:detection was carried out according to protocol of ISO-16654: 2001 standard. Approximately 1 ml of milk or 1g of feces (homogenized) wassuspended into 9 ml of sterilized BPW and incubated overnight at 37 °C for 24hrs. Samples swab were incubated overnight at 37 °C after being suspended into tryptone soya broth (Oxoid) at 1:9 ratios.

After incubation, the culture was streaked onto MacConkey agar for primary isolation of *E. coli* and incubated aerobically at 37 °C for 24 hours. The plates were observed for the growth of *E. coli* (pink colony; lactose fermenter). A single, isolated colony was picked and transferred on Eosin Methylene Blue (EMB) agar for formation of metallic sheen (Quinn *et al.*, 2004), biochemical tests wereperformed to confirm the *E. coli* (ISO 2003). The colonies obtained were transferred to nutrient agar slants in duplicate and incubated at 37 °C for 24 hrs for biochemical tests were performed to confirmthe *E. coli* using Indole test, triple sugar iron test, urease testandCitrate test.

Annex 4: Types and preparation of media used for isolation of suspected *E.coli* and *Salmonella* isolates

All the unused prepared media was stored under refrigeration temperature.

Buffered Peptone Water (BPW) (Oxoid, England, CM0509)

Composition: Buffer peptone water composed of 10 g/l Peptone, 5 g/l Sodium chloride, 5 g/l Di-sodium phosphate and 1.5 g/l Potassium di-hydrogen phosphate. The medium was Prepared suspend 20 gram of components in l liter of distilled water. Mix well and distribute into universal bottle appropriate capacity to obtain necessary portion for test and sterilize in autoclave at 121 ^{o}C for 15 minutes.

Nutrient agar

Composition (g/l):containing 5g/l of sodium chloride, 5 g/l peptone, 5g/l peptic digestion of animal tissue, 15g/l agar. It was prepared 28 g of the powder dissolved in 1 liter of distilled water according to the manufacturer's instructions. The solution was boiled to dissolve completely and sterilized by autoclaving at 121 ºC for 15 minutes. Before use, the media were cooled up to45 ºC and poured into sterile Petri dishes.

MacConkey Agar (Oxoid Ltd, Basingstoke, Hampshire, UK)

Composition (g/l): Peptone from casein 17.0; peptone from meat 3.0; sodium chloride 5.0; lactose 10.0; bile salt mixture 1.5; neutral red 0.031; crystal violet 0.001; agar-agar 13.5, PH 7.1+ 0.2.Suspend 80.0 gm in 1000 ml distilled water and sterilized by autoclaving at 15 pressures, 121°C for 20 minutes.

Eosin Methylene Blue (EMB) Agar(Dehydrated, HI Media, India)

Composition (g/l): Pancreatic digest of gelatin 10.0; Lactose 10.00; Dibasic potassium phosphate 2.00; Eosin 0.40; Methylene blue 0.065; Agar 15.00; final pH (at 25℃) 7.2. Prepared by suspend 37.00 gm in 1000 ml distilled water and sterilized by autoclaving at 15 pressure, 121℃ for 20 minutes.

Xylose Lysine Deoxycholate (**XLD) Agar**(Oxoid, England CM 0469)

Composition (g/l): yeast extract 3.0; lysine hydrochloric acid 5.0; xylose 3.75; lactose 7.5, sucrose 7.5; lysine hydrochloride 5.0, sodium chloride 5.0, Sodium thiosulphate 6.8; ferric ammonium citrate 0.8; phenol red 0.8; agar 15.0. Prepared by suspend 53 grams in 1000 ml distilled water. Heats with frequent agitation until the medium boil and do not autoclave or overheat. Transfer immediately to a water bath at 50℃. After cooling, pour into sterile Petri

dish /plates. It is advisable not to prepare large volumes that would require prolonged heating, thereby producing precipitate.

Mueller-Hinton (MH) Agar (Oxoid, England, CM0337)

Composition (g/l): Beef dehydrated infusion 300.0; Casein hydrolysate 17.5; Starch 1.5; agar 17.0. Prepared suspend 38 g of the powdered in 1 liter of distilled water, mixed well and brought to boil to dissolve the medium completely. Sterilized by autoclaving at 121℃ for 15 minutesand cooled to below 45℃ and poured into sterile petri dishes. The plates are left at room temperature for two hours for the media to solidify then put upside down in the incubator for 24 hours at 37℃ to check for sterility and to dry the condensed vapor on the plate cover.

Brain Heart Infusion agar (BM018, Sisco Research Laboratories Pvt. Ltd., Mumbai, India)

Composition (g/l): Calf brain infusion form 200; beef heart infusion 250; protease peptone 10; sodium chloride 5; dextrose 2; di-sodium phosphate 2.5; agar 15, and pH 7.4. Prepared according to the manufacturer's directions, 33 g of the powdered medium was added into one liter of distilled water, mixed well, gently heated and brought to boil to dissolve the medium completely. Then, it was sterilized by autoclaving at 121 ℃ for 15 minutes and cooled to 45-50 °C before use. Thereafter, the medium was poured into sterile test tubes and allowed to cool in a slant position. After that, it was stored in a refrigerator to ensure the shelf life.

Annex 5: Biochemical test procedures conducted

Indole Test: Sterile loops were used to pickfresh well-isolated colonies of bacteria and inoculated into a test tubes which contains 5 ml of the tryptophan medium (HI Media, India). Thereafter, the tubes were incubated at 37 ºC for 24-48 hours. After incubation period, 0.5 ml of Kovac's indole reagent (TR008, Titan Biotech Ltd., Rajasthan, India) was added to the inoculated test tubes. The tubes was subjected to gentle shaking and examined for red colour in the surface layer within 10 minutes (Cheesbrough, 2006). A red ring on top of the tube indicated indole positive reaction.

Triple Sugar Agar (Oxoid, England, CM0277)

Composition (g/l): meat extract 3.0; yeast extract 3.0; peptone 20.0; sodium chloride 5.0; lactose 10.0; sucrose 10.0; glucose 1.0; ferric citrate 0.3; sodium thiosulphate 0.3; phenol red

0.024; agar 12.0. Preparation: suspend 65 g in 1 liter of distilled water and bring to boiling dissolve completely. Mix well and distribute into container then sterilize by autoclaving at 121 ^{o}C for 15 minutes and dispense into test tubes. Allow the medium to set in sloped form with a butt 1 inch deep. Well isolated colonies are picked with a sterile wire.The slant is streaked and the butt is stubbed. Incubate inoculated tubes at 37 ^{o}C for 18 to 24 hours. Interpretations based on typical reaction of bacteria (*Salmonella*) were seen as red slant, yellow butt.

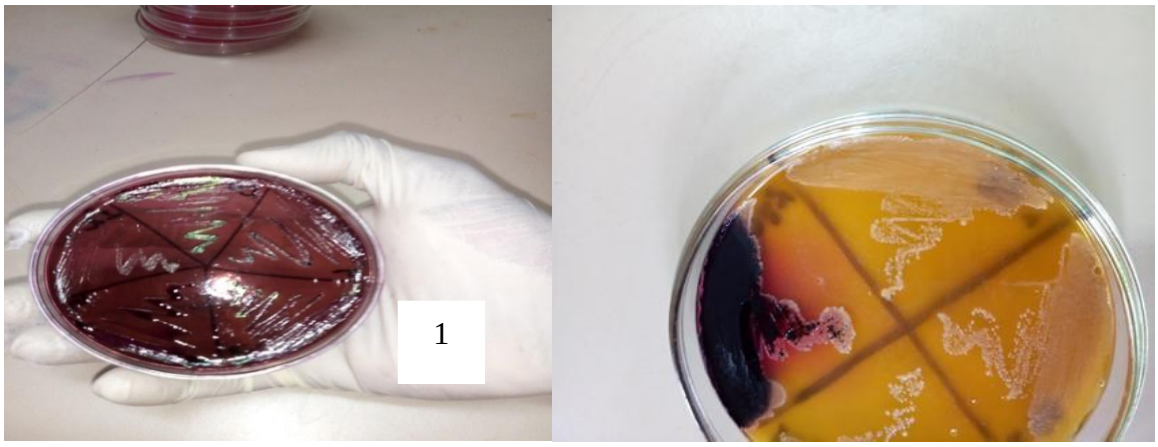
Urease test500g (Merck, Germany):

Composition (g/l): Yeast extract 0.1; potassium dihydrogen phosphate 9.1; disodium hydrogen sulphate 9.5; urea 20.0; phenol red 0.01. Preparation: Dissolve 38.5 g/l and sterilize in 5 minutes in a current of steam under mild condition. Dispense approximately 3 ml into test tubes and sterilize for 5 minutes, but do not autoclaved.Inoculate the medium massively with pure culture and Incubate up to 48 hrs. Interpretation: red = urea positive; Yellow = urea negative.

Simmon's Citrate (Oxoid**)**

Composition (g/l): Sodium chloride 5.0 g, Magnesium sulphate 0.2 g, Ammonium dihydrogen phosphate 1.0 g, Potassium dihydrogen phosphate 1.0 g, Sodium citrate 1.0 g, Bacto agar 20 g Distilled water 1000 ml, Bromothymol blue (0.2 %) 40 ml, pH adjusted to 6.8. Sterilized the media by autoclaving at 121 ℃ for 15 minutes at 15 pressures and cooled for slope formation.

Annexes 7**:** Portraits isolated of bacterial colonies growth on cultures media.



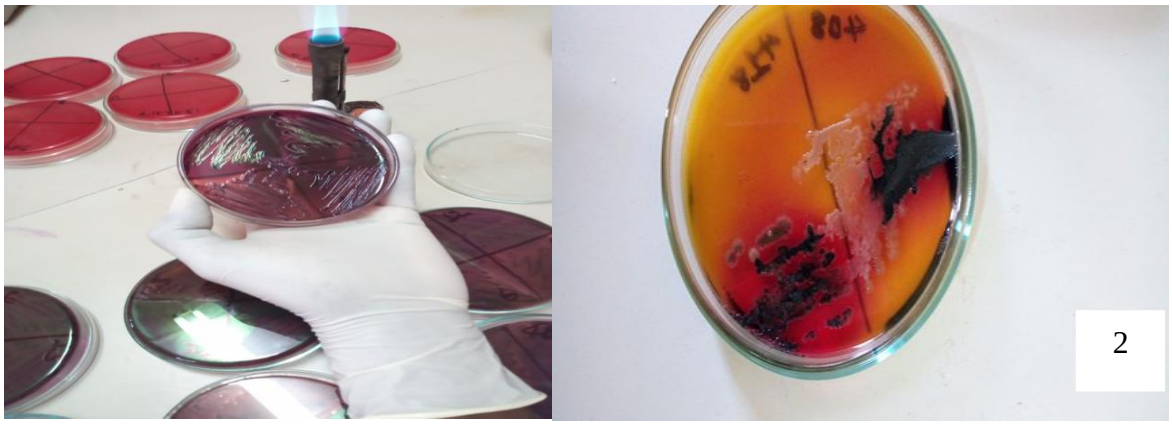


Figure 6: Characteristic colonial growths of *E. coli* and *Salmonella* as observed on EMB and XLD were displayed in Figures 1 and 2 respectively

Figure 7: Sketch biochemical characteristic of *Escherichia coli* and *Salmonella* spp

Annex 6: Antimicrobial Susceptibility test, the disc diffusion method

The isolated*E.coli*and *Salmonella* were tested for their susceptibility to 8 different antibiotics (table 4) using the disk diffusion method with incubation at 37°C overnight.

✍Three to five well-isolated colonies of the same morphological type were selected from the nutrient agar medium (Oxoid, England) (non-selective medium), from 18 to 24 hours agar plate was touched with the loop, and transferred into a tube containing 4 to 5 ml of sterile saline solution.

✍The inoculum was prepared by making direct colony suspension and was adjusted to match the 0.5 McFarland turbidity standard. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, asterile cotton swab was dipped into the adjusted suspension. The swab was rotated severaltimes and pressed firmly on the inside wall of the tube above the fluid level to removeexcess inoculum from the swab.

✍The dried surface of a Mueller-Hinton agar plate (Oxoid, England), already prepared media was inoculated by streaking the swab over the entire sterile agar surface. The procedure was repeated by streaking two more times, rotating the plate approximately 60º each time to ensure an even distribution of the inoculum. Finally, the border of the agar was swabbed.The top was left ajar or open for 3 to 5 minutes to allow for any excess surface moisture to be absorbed before applying antimicrobial discs.

✍Then, antimicrobial discs were placed onto the surface of the inoculated agar plate byusing sterile forceps, no closer than 24 mm from center to center. The discs were pressedgently down to ensure complete contact with the agar surface. The large Petridish accommodate 8 discs in outer ring and two in the center, where as no more than 4 should be placed in small plates (10cm plates).The plates were inverted and incubated at 35 ºC for 18 hours.After incubation, each plate was examined and the diameters of the zones of completeinhibition were measured, using sliding calipers and determined by naked eye (vernier caliper) on the back of inverted petridish.

Interpretation: The sizes of zones of inhibition, to the nearest whole millimeter were interpreted accordingto criteria (CLSI, 2015). The result was interpreted according to the table presented below (Table 15).

Annex 7: AST interpretive criteria for *Enterobacteriaceae*antibiotic used with expired date

Antibiotics	Disk concentration	Zone diameter: interpretive criteria (nearest whole millimeter)			
		I	S	R	Expired date
Cefoxitin (CXT)	(30 µg)	15-17	≥18	≤14	2019
Ceftriaxone (CTX)	(5 µg)	20-22	≥23	≤19	2019
Ciprofloxacin (CIP)	(5 µg)	21-30	≥31	≤20	2020
Gentamycin (GEN)	(10 µg)	13-14	≥15	≤12	2019
Nalidixic acid (NAL)	(30 µg)	14-18	≥19	≤13	2019
Nitrofurantoin (NIT)	(300 µg)	15-16	≥17	≤14	2019
Streptomycin (S)	(10 µg)	12-14	≥15	≤11	2020
Tetracycline (TET)	(30 µg)	12-14	≥15	≤11	2020

Abbreviations: I: Intermediate, R: Resistant, S: Susceptible

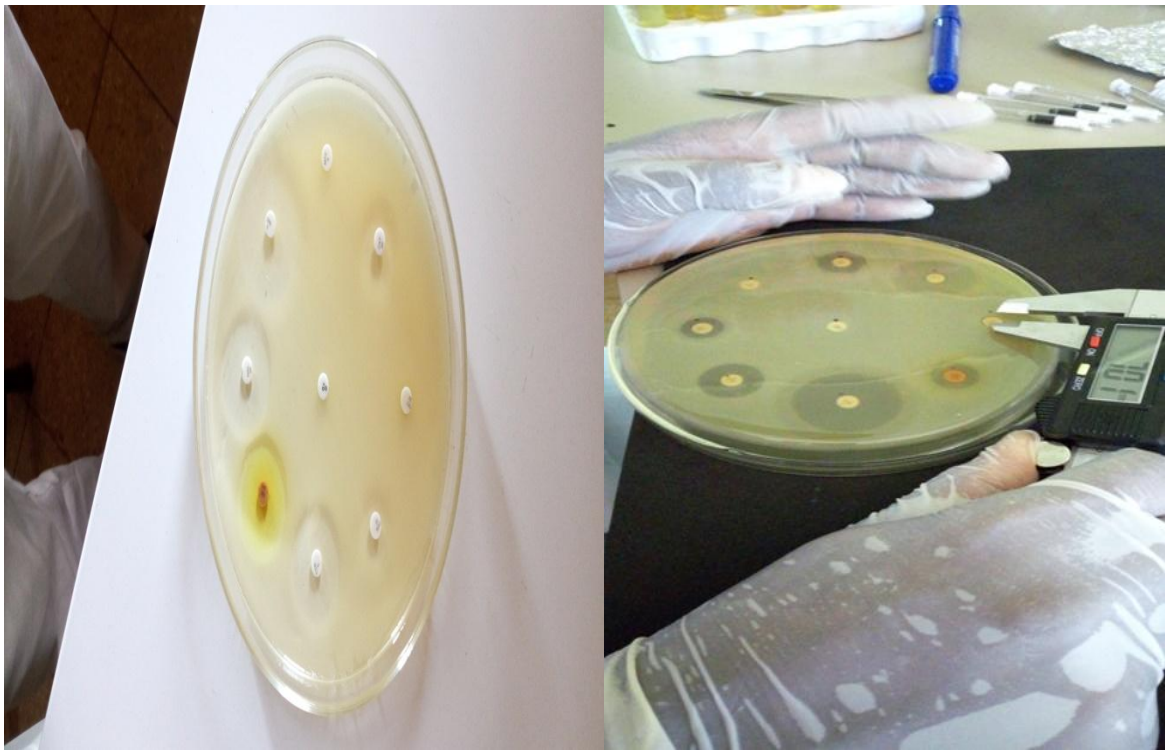


Figure 8: Antimicrobial sensitivity test showing different degrees of inhibition zones

Annex 8: Questionnaires intended to dairy animal owners (English version)

Assessment of dairy owners on AMU, its resistance and associated public health aspects

Number of interview: _____ Date_____

Name of respondent's _____farm name: _____phone:_____

Zones: _____District: _____kebeles: _____

A. Sociodemographic characteristic of dairy farm owners/respondents

1. Age (in year): (A) 18-35 years (B) 36-44 years (C) >45 years

2. Sex: (A) Male (B) Female

3. Level of education:

(A) illiterate (not able to read and write)

(B) Primary level (grade 1-8)

C) High school (grade 9-12)

D) diploma/degree

(E) Others (specify) _____

4. Residence: (A) Rural (B) Urban

5. Farm size: (A) small scale (B) medium scale (C) large scale

6: Farming experience: (A) 1-6 years (B) 6-10 years (C) > 10 years

7. Ownership of farm: A) Government B) Private

8. Occupation: A) Farmer

B) Animal science

C) Animal health D) others

B. Perception on antimicrobials/antibiotic Usage and Resistance

1. Have you ever aware or known livestock diseases?

A) Yes

B) No

2. If say yes Q1, what are common livestock diseases aware /know in your farm or Kebeles'?

List major diseases_____

_____

3. What did you do when animals encounter with such diseases or symptoms?

(A) Call for animal health employed in government clinic

(B) Goes to a nearby veterinary pharmacy

(C) Other (specify) _____

4. Do you know an antibiotics or antimicrobials drugs are?

A) Yes B) No

5. Mostly, where are the sources of antimicrobials/antibiotics you used or obtained?

(A) From veterinary government clinics

(B) From private veterinary pharmacies

(C) Others (specify) _____

6. Are there any drugs/antimicrobials commonly you used for your animals in your house/hands?

A/Yes (if yes) _____

B/No

7. In your opinion without or self-prescription of antimicrobials or drugs foranimals good?

(A) Yes (B) No

8. Have you heard about health medicines use records of each animal?

(A) Yes (B) No

9. Mostly, choice of AMs drugs/antibiotics for Rx your dairy cows or animals based on:

A/ Based on vet prescription

B/ based on own experience

C/Others (specify) _____

10. If the antimicrobials/drugs in your hands bought are expired, what do you do with them?

A) Use when needed

B) Throw away

C) Other (specify) _____

11. Improper farm waste management can cause antimicrobial resistance?

A/Yes

B/No

12. Have you ever aware or know about AMR particularly in food producing animals?

A/Yes

B/No

C) Public Health Aspects

1. Do you have awareness as raw milk consumption may have health impact?

A/ Yes B/No

2. In your opinion, consumption of milk of cows treated with antibiotic have health effects?

A/ YesB/No

3. Do you have aware of withdrawal of antimicrobials/antibiotics after treatment?

A/ Yes B/No

4. Inappropriately used antimicrobials in animals can lead to resistance in pathogens?

A/Yes B/ No

5. Incorrectly uses of antibiotics in animals can cause resistance in human?

A/ Yes B/No

6. Do you think antibiotics resistance in animals can transmit to human via consumption of milk?

A/ Yes B/No

7.Any veterinary or medical practitioner that visits you and explains the effect of use of antibiotic in animals and humans?A/ Yes B/No

Thank you very much for participation and cooperation in this study!!!

The outcome of the study was shared among stakeholders whenever available for the purpose of improving animal, public and environmental health.

Annex 9: Sample collection format and Lab result for *E.coli* and *Salmonella* from dairy farms

Zone_____Town or district: _____kebeles (PAs) _____

No	Sample types	Code	MacConkey agar	EMB agar	XLD agar	Indole	Methyl red	Urea agar	TSI	Citrate	Results		AST		
											+ *E.coli*	+ *Salm*	S	I	R
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															

Sample types and code: Feces (F), udder milk (UM), Tank/bucket milk (TbM), Bucket/tank swabs (BTs) + (positive*E.coli), Salmonella;* –ve (negative) result, AST (Antimicrobial susceptibility test), S-susceptible, I-intermediate, R-resistance