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COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE
DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND VETERINARY PUBLIC
HEALTH (MIVPH)**

DOCTORAL THESIS NO. _____ 2023



**AN IN-DEPTH ASSESSMENT OF KNOWLEDGE, ATTITUDE AND PRACTICES
ABOUT TRANSMISSION OF ZONOSSES, FOOD SAFETY, ANTIMICROBIAL
RESISTANCE AND DETECTION OF *E. COLI* O 157:H7 ALONG THE MEAT VALUE
CHAIN IN ETHIOPIA: ONE HEALTH APPROACH**

PHD DISSERTATION

BY

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**JUNE, 2023
BISHOFTU, ETHIOPIA**

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As members of the examining board for the final PhD open defense, we certify that we have read and assessed the dissertation prepared by: **Fufa Abunna Kurra** title: **An In-Depth Assessment of Knowledge, Attitude and Practices about Transmission of Zoonoses, Food Safety, Antimicrobial Resistance and the Detection of *E. coli* O157:H7 along the Meat Value Chain in Ethiopia: One Health approach** and urge that it be approved as meeting the dissertation requirement for the following Doctor of Philosophy degree: **PhD in Veterinary Public Health**

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DEDICATION

***‘To Those Who Lost Their Lives Due To
COVID-19, Conflicts and Other
Human-Made and Natural Disasters’!***

STATEMENT OF AUTHOR

I, the undersigned, hereby affirm that the work in this doctoral dissertation is wholly original and properly cited all pertinent sources. By signing this document, I genuinely affirm that I have not submitted this doctoral dissertation to any other institution, anywhere, in order to obtain any academic degree, or certificate.

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1. **Fufa Abunna**, Mirgissa Kaba, Siobhan Mor and Bekele Megersa, 2023. Assessment of Food Safety Knowledge, Attitudes, and Practices among Meat Handlers In Bishoftu City, Ethiopia, *Am. J. Trop. Med. Hyg.*, **108**(1), 200–205. doi:10.4269/ajtmh.22-0268
2. **Fufa Abunna**, Girma Gebresenbet, and Bekele Megersa, 2023. Knowledge, Attitude, and Practices (KAP) of University Students towards Antimicrobial Usage (AMU) and Antimicrobial Resistance (AMR) in Ethiopia. (Accepted: *Ethiop. Vet. J.*, **27** (2)).
3. **Fufa Abunna**, Gezahegne Mamo and Bekele Megersa, 2022. Review Article: One Health – A Holistic Solution for Sustainable Management of Globalization-Driven Public Health Challenges. *Ethiop. Vet. J.*, **26** (2), 107-131.
4. **Fufa Abunna**, Girma Gebresenbet and Bekele Megersa, Assessment of Knowledge, Attitude and Practices (KAP) of Smallholder Farmers about Transmission of Zoonoses in Ada’a District, Oromia, Ethiopia (Accepted with minor revision: *Heliyon Journal*)
5. **Fufa Abunna**, Muhaba Yimana, Hika Waktole, Tsedale Teshome, Takele Beyene, and Bekele Megersa: Detection and Antimicrobial Susceptibility Profile of *E. coli* O157:H7 from Slaughterhouses and Butcher Shops in Bishoftu City, Central Oromia, Ethiopia (Accepted with major revision: *Journal of Consumer Protection and Food Safety*)

LIST OF ACRONYMS

AMR	Antimicrobial Resistance
AMU	Antimicrobial Usage
AMS	Antimicrobial Stewardship
CDC	Centers for Disease Control and Prevention
CDDEP	Center for Disease Dynamics Economics and Policy
CFU	Colony-Forming Unit
CHS IRD	College of Health Sciences Institutional Review Board
CLSI	Clinical and Laboratory Standard Institute
COVID-19	Coronavirus disease-19
CSA	Central Statistical Agency
CT-SMAC	Sorbitol MacConkey Agar with Cefixime and Tellurite
DNA	Deoxyribonucleic Acid,
EHEC	Enterohemorrhagic <i>E. coli</i>
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FMAEH	Federal Ministries of Agriculture, Environment and Health
HORN-OH	One Health Regional Network for the Horn of Africa
HUS	Hemolytic uremic syndrome
IMS	Immunomagnetic separation
IMViC	Indole, Methyl Red, Voges Proskauer, and Citrate Utilization
IOS	International Organization for Standardization
KAP	Knowledge, Attitude and Practices
MDR	Multi Drug Resistance
MRSA	Methicillin-Resistant Staphylococcus Aureus
OIE	World Organization for Animal Health
PPE	Personal Protective Equipment
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
STEC	Shiga toxin-producing <i>E. coli</i>
UK	United Kingdom
WHO	World Health Organization

ACKNOWLEDGMENTS

Despite numerous challenges, including the COVID-19 pandemic, limitation of logistics and security concerns during this PhD study; I was able to overcome those difficulties with the glory of Almighty GOD.

First of all, I wish to express my sincere gratitude to my supervisor, Prof. Bekele Megersa, for his excellent guidance and continual support, as well as his vast knowledge and abundant experience, which have inspired me throughout my academic research and daily life. I am eternally thankful to Dr. Siobhan Mor for her unwavering counsel and assistance during my PhD study.

I gratefully acknowledge Asso. Prof. Hika Waktole for his continues encouragement and assistance. Prof. Gezahegne Mamo, Dr. Haileleul Nigussie, and Dr. Mirgissa Kaba deserve my heartfelt gratitude for their technical assistance throughout my study. My special appreciation also goes to Prof. Girma Gebresenbet for inviting me to visit the Swedish University of Agricultural Sciences (SLU) as part of PhD research visit. I would also want to express my gratitude to the HORN-OH project, Liverpool University, China-Ethiopia friendship scholarship program, SIDA and Addis Ababa University Research Directorate for their financial assistance.

I would like to extend my special appreciation to the following individuals for their unreserved support during my study: Mrs. Fitsum Limeneh and Mr. Miky (Addis Ababa University, College of Health Sciences (HORN-OH Project)); Dr. Fesseha Getachew, Dr. Amene Fekadu, Dr. Temesgen Magule, Dr. Belayhun Kibret, Dr. Mishamo Suleyman, and Dr. Gizachew Hailegreriel (Hawassa University); Dr. Zewdu Teshome and Dr. Haileyesus Dejene (Gondar University); Dr. Lemi Guta (Adama Science and Technology University); Dr. Bulako Chebo (Wolita Soddo University); Dr. Gemechu Berhanu (Dambi Dollo University); Dr. Oda Gizaw (Metu University); Dr. Getachew Bereta (Arbamich University); Dr. Zerihun Assefa, Associate Prof. Takele Beyene, Prof. Getachew Terefe, Mrs. Tsedale Teshome, and Mr. Abiy Shimelis (Addis Ababa University); Dr. Dinaol Belina (Haramaya University) and H.E. Dr. Fikru Regassa, Dr. Tesfaye Rufael and Prof. Alemayehu Regassa (Minsitry of Agriculture). It is also

my pleasure to thank CVMA, Oromia Health Bureau, College of Health Sciences Institutional Review Board, and University of Liverpool (UK) for granting me ethical review certificate.

I would like to express my gratitude to my family, Addee Birqi Gadafa, Addee Dashure Kumara, Sinan Fufa, Koolit Fufa and Labsi Fufa. Without their tremendous understanding and encouragement in the past few years, it would be impossible for me to complete my study before the stipulated time.

Last but not least, I would like to express my gratitude to my friends, colleagues, staff, for their generous assistance and support during my study.

Fufa Abunna

SUMMARY

Food-borne diseases associated with zoonotic pathogens can be transmitted to humans primarily, but not exclusively through animal source foods. Antimicrobial resistance in the food chain is also currently a subject of a major concern globally. The excessive use or rather misuse of antimicrobials coupled with a poor hygiene in the food production chain has led to a rise of drug resistant bacteria, commonly transmitted through food chain. A cross-sectional survey was undertaken by using a structured pretested questionnaire to investigate the level of knowledge, attitude and practices towards the transmission of Zoonoses among farmers in Ada'a district, food safety among meat handlers of Bishoftu city and antimicrobial use (AMU) and antimicrobial resistance (AMR) among 10 public University students in Ethiopia. Another study was also carried out to detect *E. coli* O157: H7 along the meat value chain in abattoirs and retail meat shops and of Bishoftu city and to assess the susceptibility profile of the isolates against 13 antibiotics using a standard disk diffusion method. Logistic regression was used to assess the associations between socio-demographic characteristics and knowledge, attitudes, and practices. Accordingly, farmers with secondary school or above education background (OR= 6.8, CI=2.4 - 18.0, $p < 0.05$), aged between 41 and 50 years (OR=3.0, 95% CI: 1.2–7.2, $p < 0.05$), and good knowledge (OR=2.1, CI=1.3 - 3.5, $p < 0.05$), and positive attitude (OR=7.8, CI=4.7 - 12.9, $p < 0.01$) had better practices that reduce the risk of exposure to zoonotic infections than their counterparts. Most meat handlers had a good knowledge level, a positive attitude and poor food safety practices. In multivariable models, good knowledge was significantly ($p < 0.05$) associated with older age; positive attitudes were associated with lower educational attainment and good knowledge; and good practices were associated with working experience in an abattoir and having received training on food safety. Students' birthplace was found to be significantly ($p < 0.01$) associated with knowledge of students on AMU and AMR. Students with good knowledge had 3.9 times more positive attitudes than those with poor knowledge (OR = 3.9, CI = 3.0 -5.2, $p < 0.01$). Students from Veterinary medicine had 1.6 times better attitude than students from Helath Science and Non-Helath Science (OR = 1.6, CI=1.2- 2.1, $p < 0.05$). The prevalence of *E. coli* O157:H7 was found to be 3.6%. There was a significant difference in the occurrences of the pathogen among the sources of samples ($p < 0.05$). The antimicrobial susceptibility test revealed that the isolates were found to be resistant to three commonly used drugs, tetracycline (100%), erythromycin (92.8%), and

ampicillin (64.3%). However, all isolates were susceptible to azithromycin, cefotaxime, and chloramphenicol. However, 85.8% of the isolates were found to be resistant to three or more classes of antimicrobials. In conclusion, this study revealed knowledge gaps, a low level of the desired attitude, and high-risk behavioral practices among farmers about the transmission of Zoonoses. The study revealed that there is an inadequate food safety practice among meat handlers; and *E. coli* O157:H7 was detected in abattoirs and butcher shops. Furthermore, there were critical gaps of knowledge, attitudes and practices among University students about antimicrobial usage and antimicrobial resistance. Therefore, the need for awareness creation about zoonotic disease transmission among farmers, regular hands-on training and enforcement of general and personal hygiene among meat handlers is recommended. Moreover, interventions to raise awareness about AMU and AMR should also target students majoring in fields other than health sciences.

Keywords: AMR, Beef, E. coli O157:H7, Food safety, KAP, Zoonoses, Ethiopia

1. CHAPTER I: GENERAL INTRODUCTION

Zoonoses have a significant impact on both animal and human health, especially in developing countries. Zoonotic diseases place a double burden on people's well-being by jeopardizing the health and productivity of their livestock; however, they are frequently overlooked by health-care managers and policymakers in both the developed and developing worlds (WHO, 2006). In particular, human-animal interactions in the environment are exacerbating the transmission of Zoonoses from farm animals to humans and vice versa (WHO, 2015). Almost 60% of currently known infectious diseases are of zoonotic origin, and up to 75% of emerging infectious agents are as well (Woolhouse & Gowtage-Sequeria, 2005).

Ethiopia has Africa's second-largest human population and the continent's largest livestock population (Tilahun & Schmidt, 2012). The country is especially vulnerable to the effects of zoonotic diseases because its economy is heavily reliant on agriculture (McDermott & Grace, 2012), and roughly 80% of households have direct contact with domestic animals, creating opportunities for infection and disease spread (Lindahl *et al.*, 2015). It had the second-highest zoonotic disease burden in Africa (Grace, *et al.*, 2012). Rabies, brucellosis, bovine tuberculosis, food borne illnesses and poisoning, echinococcosis, and other zoonotic diseases continue to have an impact on human and animal health in many countries, particularly in developing countries (Meslin, and Stohr, 2000). Previous research revealed the presence of several zoonotic diseases, including bovine tuberculosis (Sibhat *et al.*, 2017), brucellosis (Kebeta *et al.*, 2015), bovine cysticercosis and/or taeniosis (Abunna *et al.*, 2008; Terefe *et al.*, 2014; Emiru *et al.*, 2015) and common food-borne pathogens (Zelalem *et al.*, 2019) in Ethiopia.

Surveys of knowledge, attitudes, and practices (KAP) provide critical information for investigating risk factors and potential intervention strategies for disease management. As a result, farmers' behavior is heavily influenced by their knowledge and attitude (Dernburg *et al.*, 2007). Poor disease knowledge correlates with disease prevalence and can set off a vicious cycle of under-diagnosis/underreporting and awareness deficit (Govindaraj *et al.*, 2016). Different countries have highlighted the importance of zoonotic disease education and KAP surveys in understanding country-specific circumstances (John *et al.*, 2008).

Every year, approximately 600 million people become ill as a result of contaminated food, resulting in the loss of 27 million healthy life years (WHO, 2015). Foodborne illness is one of the leading causes of human disease globally with contaminated animal source foods playing a significant role in illness (Jacob *et al.*, 2010). Meat is a common source of foodborne illness because it provides an ideal environment for the growth of microorganisms such as pathogenic organisms and spoilage organisms (Scharff *et al.*, 2009). Because meat has a high potential to support the growth of pathogens, it must be handled carefully both at the point of slaughter and afterward to avoid contamination (Abdul-Mutalib *et al.*, 2012). Raw meat contamination and cross-contamination is a major cause of foodborne disease, particularly in developing countries (Adesokan & Raji, 2014). In many developing countries, strict meat hygiene and safety control is difficult, and meat for human consumption is approved primarily through visual inspection (Bakhtiary *et al.*, 2016).

Like in many underdeveloped nations, the bulk of foodborne infections in Ethiopia are caused by organisms that cross-contaminate carcasses during the slaughter process and are intermittently excreted in the feces of sick animals (Egualé *et al.*, 2018; Atnafie *et al.*, 2017). In Ethiopia, for example, studies of children with diarrhea reveal a high prevalence of pathogenic organisms such as *E. coli* 28.8% (Zeleele *et al.*, 2019) and Salmonella 3.1% (Zeleele *et al.*, 2019); 1.3% (Tosisa *et al.*, 2020). Salmonella prevalence was also found to be 7.2%, 4.7%, and 4.4% in human patients, poultry, and swine, respectively (Egualé *et al.*, 2018, Egualé *et al.*, 2015). Microbial contamination of animal-source foods has also been reported in Ethiopia along the value chain. A recent meta-analysis of studies on the prevalence of *E. coli* in milk and meat discovered an overall random pooled prevalence of 15% (95% CI = 13-17%) (Assefa & Bihon, 2018), while another review found *E. coli*, Salmonella, Listeria, and Campylobacter in beef sold in retail stores (Edget *et al.*, 2014).

Given the critical role that slaughterhouses and meat retailers play in food safety, understanding the level of food safety knowledge, attitudes, and practices (KAP) of meat handlers is useful for developing appropriate interventions. According to studies conducted in various parts of Ethiopia, the behavior of meat handlers is an important risk factor for food contamination and can reduce the quality of food served for human consumption. In one study conducted in eastern Ethiopia, for example, meat handlers lacked knowledge about foodborne pathogens, time-temperature control to prevent bacteria growth, cross-contamination, and the distinction between cleaning and sanitation (Tegegne *et al.*, 2017). Another study in northern Ethiopia discovered that 15.4% of abattoir

personnel lacked a health certificate and that the abattoir lacked a hot water, sterilizer, or cooling facility, while 11.3% of butchers did not wear protective clothing when handling meat (Haileselassie *et al.*, 2013). Food safety measures such as investigating food establishments and recalling food products are not well established in Ethiopia. To ensure safe foods are available to consumers, special attention must be paid to “upstream” factors such as the personal hygiene of meat handlers in slaughterhouses and retail shops. Inadequately trained employees who practice unsanitary meat handling can expose the public to meat-borne illnesses, which can be avoided through training and the implementation of quality control systems (Gutema *et al.*, 2021). While some studies on food safety KAP in food handlers in Ethiopian food establishments have been conducted (Adane *et al.*, 2018, Admasu & Kelbessa, 2018; Tesfaye & Tegene, 2019), only one study (Tegegne *et al.*, 2017) has conducted an in-depth investigation of food safety KAP of meat handlers in abattoirs and retail meat shops. Other studies in this context have primarily focused on food safety practices (Haileselassie *et al.*, 2013, Gutema *et al.*, 2021; Yenealem *et al.*, 2020).

Despite advances in technology and sanitary practices at all stages of food production, food-borne diseases continue to be major public health and livelihood concerns for individuals and countries worldwide (Moawad *et al.*, 2017). Food-borne infections, particularly in developing countries, cost billions of dollars in medical care and social costs (Havelaar *et al.*, 2015). It is frequently caused by the consumption of contaminated foodstuffs, particularly meat from infected animals or carcasses contaminated with pathogenic bacteria such as *Escherichia coli*. Infection with *E. coli* O157: H7 can cause mild diarrhea, severe bloody diarrhea, hemorrhagic colitis, or hemolytic uremic syndrome (HUS) leading to kidney failure, depending on the infected person’s immune status and general health, as well as the dose and virulence of the bacteria (Smith *et al.*, 2014). Cattle are the primary reservoirs of *E. coli* O157:H7, and beef and beef products have been identified as major sources of foodborne transmission (Money *et al.*, 2010). Carcass contamination occurs during the slaughter process at processing plants due to pathogen transfer from skin to carcass or fecal to carcass (Abdissa *et al.*, 2017). Microbial cross-contamination can also occur during processing and manipulation, such as dehiding, evisceration, storage, and distribution in slaughterhouses and butcher shops (Zweifel *et al.*, 2014).

The animal production sector in developing countries has routinely used antimicrobials for therapy, disease prevention, and growth to increase output (Robinson *et al.*, 2016). Antimicrobials are

widely used in cattle to prevent disease and as a growth promoter (Herago & Agonafir, 2017). Shecho *et al.*, (2017) reported a multidrug resistance among *E. coli* O157:H7 from food of animal origin in Ethiopia. *E. coli* O157:H7 can infect humans through contaminated food and water, direct contact between people, and contact with animals or their environment. Antimicrobial use in food animals result in the development of resistance to pathogenic *E. coli* O157:H7, which can reach humans via the food chain (Ma *et al.*, 2021). *E.coli* O157:H7 detection methods include culture-based, immunological-based, nucleic acid-based, and biosensors (Valderrama *et al.*, 2016). Laboratory trials and an outbreak of hemorrhagic colitis were used to determine the value of a latex agglutination test for the rapid presumptive detection of *E. coli* serotype O157:H7. The latex test was discovered to be a simple, effective, and dependable method for detecting *E. coli* O157:H7 with 100% sensitivity and specificity. It was also discovered that sorbitol-MacConkey agar cultures were not as effective in screening for *E. coli* O157:H7 in food samples as they were in fecal specimens, but the latex screen was particularly effective in this setting (March & Ratnam, 1989).

In Ethiopia, animals for local consumption are commonly slaughtered and dressed under unsanitary conditions in the open air or in sub-standard slaughterhouses, compromising the microbiological quality and safety of meat (Atnafie *et al.*, 2017). Lack of food-borne pathogen surveillance, a lack of education and training among slaughterhouse and butcher shop workers, and poor hygienic practices of food handlers are all major factors contributing to Ethiopians' high risk of exposure to food-borne pathogens like EHEC (Assefa, 2019). Furthermore, raw meat is widely consumed in the country, and if hygiene and adequate temperature control are not maintained, there is a potential risk of foodborne disease occurrence due to the country's widespread consumption of raw meat. To ensure that prevention and control strategies are appropriate, it is necessary to investigate potential sources of *E. coli* O157:H7 in the beef supply chain, quantify risk factors, and evaluate the hygienic performance of slaughterhouses and butcher shops. However, it has not yet been well handled in Ethiopia to evaluate the standards of slaughterhouses and their environment, which could serve as sources of *E. coli* O157: H7.

Similar to Zoonoses and food safety concerns, antimicrobial resistance (AMR) pathogens can transfer from farm animals to humans through consumption of contaminated food and water or and direct contact with animals (Dafale *et al.*, 2020). Globally, AMR is thus becoming one of the leading causes of death with estimated annual annual mortality of five million people (Murray *et*

al., 2019). Increased patient morbidity, mortality, health-care-related costs, and treatment failure are key repercussions of this situation (Vasudevan *et al.*, 2015). AMR jeopardizes the sustainability of effective public health responses to infectious diseases with resistant organisms (Smith & Coast, 2013). The significance of AMR is not only in pathogenic organisms, but also in commensals (Kaesbohrer *et al.*, 2012) that could transfer the resistance genes to pathogenic organisms such as *E. coli* or Salmonella strains and vice versa (Szmolka & Nagy, 2013). In particular, hospitalized patients colonizing MDR commensal bacteria can lead to nosocomial infections (Carlet, 2012).

Antimicrobial over- and under-prescription, as well as their improper uses both in humans and animals, result in the development of antimicrobial resistance (Ventola, 2015; Robinson *et al.*, 2016). In addition to other equally important social and cultural factors, the CDC stated that self-medication, incorrect prescription, inappropriate consumption, and excessive use of these antimicrobial agents could be key factors in the increase and spread of antimicrobial resistance. Self-medication has become a serious concern and a leading cause of antimicrobial resistance. It can be caused by a variety of factors, including a lack of public knowledge and attitude toward antibiotics, easy access to antibiotics in many locations, and a lack of awareness and policies on appropriate antibiotic usage (Jairoun *et al.*, 2019).

Numerous studies have found that different groups of people such as young individuals (e.g. University students') misuse antibiotics due to self-medication and a lack of adequate knowledge of therapeutic agents, with regards to their indications, pathogen specificity, and adherence to dosage regimens (Sunusi *et al.*, 2019). Antibiotics taken for too short period of time can lead to the development of drug resistance in already pathogenic bacteria. Stopping antibiotics too soon may cause the patient to relapse and increase the risk of the infected individual spreading drug-resistant pathogens to others. Some people who are prescribed antibiotics discontinue them early due to unpleasant side effects, because they feel better, or because they want to save the remaining antibiotics for later use or to share with others (Mallhi *et al.*, 2019). Morbidity and mortality ratios are rising in tandem with emerging antimicrobial resistance (Hofer, 2019). Higher morbidity and mortality rates associated with AMR increase the economic burden on the health-care sector, particularly in low-income countries. Not to mention the significant impact antibiotic resistance will have on global economies and healthcare management systems (Jairoun *et al.*, 2019).

There is sufficient evidence to support a positive relationship between inappropriate antibiotic use and the rate of resistance development. As a result, prudent antibiotic use in both animal and human health systems is vitally important in preventing development of drug resistance among the microorganisms (Hockenhull *et al.*, 2017). A notion that antimicrobial resistance is not a public health issue, in that AMR only affects those who take antibiotics frequently and not everyone else was connected to non-prescription antibiotic usage, a practice that causes emergence of AMR (Tegegne *et al.*, 2017). Investigating public knowledge, attitudes, and behaviors regarding antibiotic use aids in the development of effective AMR prevention campaigns. We can prevent the development of antibiotic resistance by analyzing antibiotic use practices and correcting those that are inappropriate. To the best of our knowledge, no study has been conducted in Ethiopia to highlight aspects of KAP among university students in general. The most recent Ethiopian studies on the knowledge, attitudes, and practices (KAPs) of antimicrobial use and development of drug resistance have concentrated on a single University students studying in a similar field.

1.1. Background

1.1.1. Zoonoses

A zoonosis is an infectious disease that has spread from a non-human animal to humans. Zoonotic pathogens may be bacterial, viral or parasitic, or may involve unconventional agents and can spread to humans through direct contact or through food, water or the environment (WHO, 2020). Zoonotic diseases are a serious threat to public health and have a significant impact on both national and international economics (Morens *et al.*, 2004). Because developing nations are frequently found in the tropics, which are regions with a large diversity of pathogen species, they are disproportionately affected by zoonotic infections when compared to industrialized nations when it comes to veterinary and human health (Guernier *et al.*, 2004). Additionally, a variety of human populations may be exposed to zoonotic infections due to a community's dependence on livestock, the high incidence of bush meat consumption, inadequate food and water security, and frequent contacts with wildlife (Clifford *et al.*, 2013). According to recent study, the issue of pathogen detection in food is worse in low-income nations such as, Ethiopia (Asfaw *et al.*, 2022). Several pathogens were detected from contaminated food at every point in the food chain, including during agricultural production (soil and irrigation), when food is sourced from animals, and when it is being prepared by food handlers. Attempts were made to detect zoonotic pathogens from human, animal or environmental samples with conventional culture-based diagnostic methods in Ethiopia.

Different researchers have reported the prevalence of major foodborne pathogens from food of animal origin in Ethiopia; 4.8% to 28.4% of *L. monocytogenes* (Molla *et al.*, 2004, Garedew *et al.*, 2015, Yilma, 2004, Mengesha *et al.*, 2009, Derra *et al.*, 2013, Gebretsadik *et al.* 2011, Seyoum *et al.*, 2015); 2.1% to 39.3% of Bovine tuberculosis (Tulu *et al.*, 2021, Almaw *et al.*, 2021, Ameni and Erkihun, 2007); 8.0% to 88.0% of Campylobacter (Ewnetu and Mihret, 2010, Chala *et al.*, 2021, Wayou *et al.*, 2022, Terefe *et al.*, 2020); 5.5% to 11.5% of Salmonella (Ejo *et al.*, 2016, Alemu *et al.*, 2022, Mohammed & Dubie 2022, Asfaw *et al.*, 2021); 2.6% to 19.7% of bovine cysticercosis (Gutema *et al.*, 2020, Abera *et al.*, 2022, Tegegne *et al.*, 2018, Tolossa *et al.*, 2015).

1.1.2. Food safety

The World Health Organization (WHO, 2010) estimated that approximately 1.8 million children die each year from diarrhea, much of which is caused by consumption of contaminated food and water. WHO, (2014) also stated that global burden of foodborne diseases is estimated at 600 million people, almost 1 in 10 falls ill every year from eating contaminated food and 420, 000 die as a result. This is not only the problem of developing countries, but also in developed countries where every year one third of the total population are likely to be suffered from food borne diseases and from which 70% of the cases are linked with the consumption of contaminated food (Ifiadike *et al.*, 2012). It is also estimated that out of 10 people, 1 person fall ill and 420 000 die every year because of ingestion of food contaminated by microorganisms and most of these foodborne illnesses and deaths are accounted for poor food handling practice (WHO, 2015), in which substantial proportion goes to meat-related hazards (Mangen *et al.*, 2018).

Adugna *et al.*, (2018) revealed that occurrence of the contamination of beef while transporting beef from the abattoir to the butcher shops with the highest source of contamination which is attributed to because of abattoir workers. In recent years, foodborne illnesses are increasing with an enormous impact on the health and economy of developing countries than those of developed countries (Akabanda *et al.*, 2017), which is manifested by hampering the health-care system and harming national economies, tourism, and trade. Soriyi *et al.*, (2008) also stated that almost all fresh food, particularly those emanating from animals are highly vulnerable to microbial contamination and food poisoning. Food of animal origin accounts for the majority of foodborne diseases (Nyamakwere *et al.*, 2017). Bersisa *et al.*, (2019) and Akinyera *et al.*, (2018) also reported meat is

one of the foods that are easily perishable given its richness in nutrients composition that makes it an ideal medium for the growth of wide range of microorganisms.

The health status of food handlers and hygiene practices in the food industry are the major determinants of food contamination and poisoning (Tefera and Mebrie, 2014). The inadequate food safety knowledge and skills of food handlers can result in unsafe food handling practices and cross contamination in food service establishments (Rahman *et al.*, 2016). Causes of foodborne disease by food handlers include cross-contamination between raw and processed food items; storage and cooking of food in adverse conditions; and utilizing contaminated utensils and equipment (Al Suwaidi *et al.*, 2015). Food handlers can also be vehicles that carry organisms associated with foodborne-illnesses, such as salmonella, staphylococci, and *E. coli* (Rosmawati, 2015). Also, other causes include the probability that food handlers could carry pathogens (while not showing any symptoms) and transmit those pathogens in food (Al Suwaidi *et al.*, 2015; Egan *et al.*, 2007). In Denmark, asymptomatic handlers were associated with a quarter of food-borne outbreaks in the years between 2005 and 2011 (Franck, 2014). The urgency of the matter is stressed by the findings of the World Health Organization (WHO) that human actions are the leading cause of food contamination during food preparation in food service establishments as a result of non-adherence to good hygiene practices (WHO, 2013). Ensuring the safe handling of food in school feeding programmes remains a big challenge in many countries, considering that many of these school feeding schemes are often regarded as poverty and hunger alleviation initiatives (Jomaa *et al.*, 2011; WHO/FAO, 2010). Food safety assurance in food service establishments depends heavily on the availability of adequate infrastructure, appropriate management support and commitment, as well as knowledgeable and skilled food handlers (Rendall-Mkosi *et al.*, 2013). Outbreaks of many foodborne diseases are due to contamination that occurs during food preparation within food service establishments (Smigic *et al.*, 2016). Cases of food poisoning are prevalent in schools as a result of cross-contamination during food preparation (Sanlier & Konaklioglu, 2012). The outbreaks of foodborne diseases in school feeding programmes can result in life-threatening diseases, huge medical costs and the spread of infection to other children and staff, thereby leading to disruption of learning in schools (Scharff, 2012).

The issue of food handling and hygiene is a great concern across industries (Angelillo *et al.*, 2001), as foods can easily be mishandled during preparation, processing, or storage (Sani and Siow, 2014).

Improper food handling practices are found to be the front runner for most outbreaks related to food poisoning (Ansari-Lari *et al.*, 2010) that implicate the food handlers (Tegegne and Phyo, 2017). *Codex Alimentarius* Commission disclosed that improper food handling is a major cause of foodborne diseases and poor hand hygiene is a significant risk factor in the occurrence of food contamination (Al-Shabib *et al.*, 2016). Mishandling food supposed to be implicated in 97% of all food-borne illnesses associated with food service establishments (Egan *et al.*, 2007).

The food chain starts from farm to fork/plate while challenges include microbial, chemical, personal and environmental hygiene (Fung *et al.*, 2018). Food contamination that results in outbreaks of foodborne disease can be greatly influenced by improper food handling by food handlers during the food production processes, such as cooking, storing, and serving food (Zanin and Cunha, 2017). In order to avoid foodborne diseases, food handler engagement is crucial (Bou-Mitri *et al.*, 2018). Hence, in some cases, good education, a positive attitude, and appropriate food handling procedures can help prevent the emergence of foodborne diseases (Sharif and Al-Malki, 2010). Socio-demographic factors, such as the degree of education and food safety training that food handlers have received, play a significant influence in encouraging food handlers to adopt adequate food handling procedures in addition to having strong understanding of and a favorable attitude toward food safety (Al-Shabib and Mosi, 2016). Most of food handlers implicated outbreaks came from food service facilities, such as restaurants and cafeterias, followed by catered events, schools and day care centers, processing facilities, homes and community events. Improper food handling can be a major cause of food contamination. To prevent food contamination, the food handler should have good knowledge, display a positive attitude, and practice proper food handling practices (Putri and Susanna, 2021).

Many foodborne infections can be avoided with simple measures like appropriate washing facilities and careful hand washing. Food that is rotten and tainted accumulates as a result of inadequate recycling and waste disposal infrastructure. As a result, there are more insects and pests around, which raise the possibility of food spoiling and contamination. As a result, there are more insects and pests around, which raise the possibility of food spoiling and contamination. Poor food storage, transportation, and sales practices are all caused by unclean food being produced and processed in unsanitary environments (Fung *et al.*, 2018).

1.1.3. Status of E. coli O157:H7 in Ethiopia

The species *E. coli* consists of a diverse and large group of bacteria (Bintsis, 2017). The majority of *E. coli* strains are not harmful (Adzitey, 2015). Some strains, however, are dangerous and can cause serious human sickness (Stromberg *et al.*, 2018). *E. coli* O157:H7 is a frequent and virulent foodborne bacterial pathogen (Abdissa *et al.*, 2017), and it is a subtype of Shiga toxin-producing *E. coli* strains (Al-Ajmi *et al.*, 2020). In Ethiopia, like other developing countries, it is difficult to evaluate the situation and effect of *E. coli* O157:H7. This is mainly because of the limited scope of studies, lack of coordinated epidemiological surveillance system and inadequacy of laboratory facilities for culture. In addition, under reporting of cases and the presence of other diseases considered to be of high priority may have overshadowed the problem of this important foodborne pathogen. Several studies were conducted by some researchers to determine the occurrence and proportion of *E. coli* O157:H7 in faecal, skin swabs and carcasses of sheep, goat cattle in different areas of the country. The prevalence of *E. coli* O157:H7 (1.9% to 12.0%) was reported along supply food chain in Ethiopia (Gutema *et al.*, 2021; Ayenew *et al.*, 2021; Geresu and Regassa, 2021; Tadesse *et al.*, 2021; Hamid *et al.*, 2018; Atnafie *et al.*, 2017; Abdissa *et al.*, 2017; Haile *et al.*, 2022; Tassew, 2015; and Taye *et al.*, 2013).

1.1.4. Antimicrobial Usage and Antimicrobial Resistance

Globally, antimicrobial resistance (AMR) is a complex public, animal, and environmental health problem, primarily fueled by inappropriate use of antimicrobials. The trends and problems associated with AMR are observed naturally in pathogenic and commensal or non-pathogenic bacterial organisms (Prestinaci *et al.*, 2015; WHO, 2018). Currently, AMR is one of the greatest health threats that demand the most urgent attention in global health security. Antimicrobial abuse, misuse, and overuse in animals have devastating effects on humans, animals, and the environment, resulting in serious health and economic consequences (FMAEH, 2017; Manyi-Loh *et al.*, 2018). In Africa, these consequences are often underreported due to inadequate surveillance data (WHO, 2014; O'Neill, 2014; Tadesse *et al.*, 2017). AMR is projected to be attributed with about 4.15 million human deaths per annum in Africa by the year 2050 if left to continue uncontrolled (O'Neill *et al.*, 2014).

The association between antimicrobial use and increasing antimicrobial resistance (AMR) in animals has long been established (Leite-Martins *et al.*, 2014). There are direct and indirect association between antimicrobial use and increasing antimicrobial resistance in animals. The direct association is reported by previous research findings (Dohmen *et al.*, 2015; Liu *et al.*, 2015; Platell *et al.*, 2018; Worthing *et al.*, 2018) or indirect (Boost *et al.*, 2013; Grinberg *et al.*, 2005; Liu *et al.*, 2015) contact with animals can result in human–animal exchange of multidrug-resistant pathogens. Despite this established relationship, there is still widespread inappropriate prescribing of antimicrobials in all sectors of veterinary practice in Australia (Hardefeldt *et al.*, 2017), indicating a need for antimicrobial stewardship (AMS) programs. The research findings of Chokshi *et al.*, (2019) showed key factors for the development of antimicrobial resistance in poorer countries. Some of the risk factors are a lack of surveillance, inadequate quality control of the available antibiotics, clinical misuse, and ease of availability. Contrarily, in developed world, factors causing antibiotic resistance are inadequate hospital-level regulation and excessive antibiotic usage in animals used for food. Moreover, both developing and developed countries suffer from a serious dearth of research on novel antibiotics.

1.2. Objectives of the study

The main aim of this study was to assess the level of knowledge, attitudes, and practices about transmission of Zoonoses, food safety, and antimicrobial usage and antimicrobial resistance in Ethiopia.

Specific objectives were to:

1. Assess the level of knowledge, attitudes, and practices about transmission of Zoonoses among smallholder livestock keepers in Ada’a district of central Oromia, Ethiopia
2. Investigate abattoir and retail meat shop workers’ level of food safety knowledge, attitudes, and practices in Bishoftu city, Ethiopia
3. Detect *E. coli* O157:H7 from slaughterhouses and butcher shops and determine the antimicrobial resistance pattern of the isolates in Bishoftu city, Ethiopia
4. Assess knowledge, attitudes, and practices towards antimicrobial usage and antimicrobial resistance among public University students in Ethiopian

1.3. Overview of the study

This PhD research work was initiated with the conceptual framework of assessing perceptions regarding zoonosis transmission, and food safety along the meat value chain. Moreover, it is complemented with the assessment of KAP of University students regarding antimicrobial usage and antimicrobial resistance. Many Zoonoses are regarded as occupational health risks (Battelli, 2008). As a result, livestock farmers are vulnerable because different types and intensities of interaction with animals might result in zoonotic diseases (Klous *et al.*, 2016). On-farm *E. coli* O157:H7 control and reduction measures will minimize the risk of carcass contamination at slaughter and processing plants, but they will not eliminate *E. coli* O157:H7. Hence, KAP of zoonotic disease transmission among livestock keepers, food safety among meat handlers, and detection of *E.coli* O157:H7 in abattoirs and retail meat shops were carried out in Bishoftu city areas of Ada'a district, East Shewa zone (Figure, 1.3.1). Bishoftu city, which is found at 47 kilometers southeast of the capital, located at 38°51' to 39°04' East and latitudes 8°46' to 8°59' North, covering a land area of 1750 km². Moreover, KAP study on antimicrobial usage and antimicrobial resistance was conducted among students at 10 public University in Ethiopia (Figure, 1.3.2).

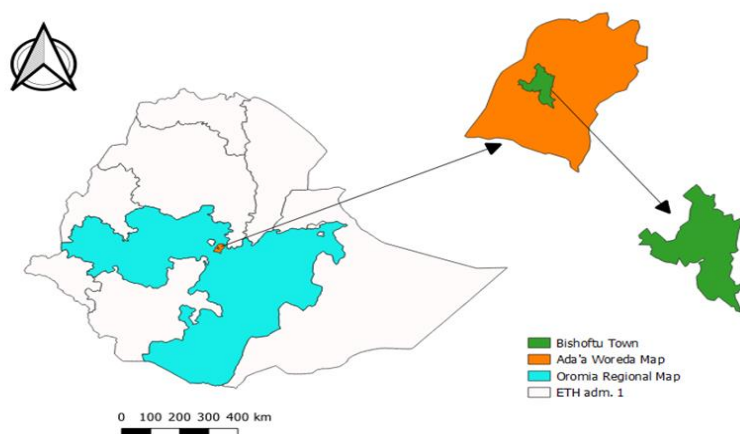


Figure 1.3.1. Map of the study area (Transmission of Zoonoses, KAP of food safety and detection of *E.coli* O157:H7)

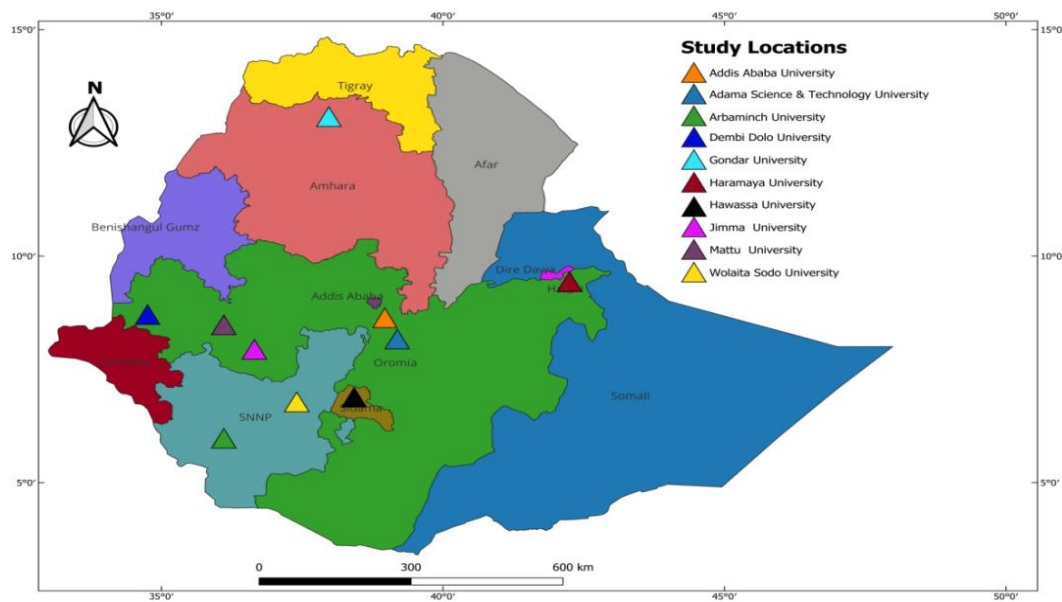


Figure 1.3.2. Map of the study area (KAP of AMR among University students)

A cross-sectional survey was conducted among meat handlers, farmers and University students about KAP towards food safety, transmission of Zoonoses and antimicrobial usage and antimicrobial resistance in Ethiopia. For the food safety study, 391 meat handlers working in retailers and abattoirs were recruited for this purpose and interviewed through face to face interview. The retail meat shops/abattoirs were selected using a purposive sampling technique. Following that, meat handlers were chosen using simple random sampling.

Similarly, KAP studies on Zoonoses among livestock keepers (n=388) in Ada'a district and antimicrobial uses and antimicrobial resistance among students (n=1252) in various Universities were also conducted. Moreover, for detection and isolation of *E.coli* O157:H7, a cross-sectional study was carried out at two slaughterhouses (Bishoftu Municipal and privately owned), and 92 butcher shops. A semi-structured, pre-tested questionnaire was used to collect information meat handlers', farmers' and University students' KAP about food safety, Zoonoses and AMU and AMR. Interviews were conducted in a private area within the respondents' workplace to ensure that the respondent was comfortable and there was no interruption. An interviewer read the questionnaire aloud and recorded the results on paper. For the AMU and AMR investigation, students were approached to participate in this study through trained data collectors (staff member of their universities) and verbal consent was obtained from the students and then they were invited to complete a self-administered questionnaire during their free time. The respondents were interviewed face-to-face during working hours with no advance notice. Individual interviews

typically took 10-15 minutes to complete. Because the study took place during the COVID-19 pandemic, preventive measures such as wearing facemasks, observing social distance (at least 2m), washing hands, using alcohol-based hand sanitizer, and conducting interviews in a well-ventilated space were implemented.

For detection of *E. coli* O157: H7, fecal contents of the animals, carcass swabs, and swab samples from the environment (knives, hook, hand swabs, water, and wastewater) were collected (IOS, 2001). All samples were labeled legibly with a permanent marker identifying the type/source of the sample, the date of sampling, and the code of the slaughterhouses/butcher shop. The samples were transported in an ice box containing ice packs to the Microbiology laboratory of the College of Veterinary Medicine and Agriculture of Addis Ababa University. The antimicrobial resistance test was also carried out by using the standard agar disc diffusion (Oxoid Ltd., Hampshire, UK) technique (CSLI, 2013) for 13 antimicrobial agents that are in regular use for ruminants, potential public health importance, and recommendations from the guideline of antimicrobial susceptibility test.

Data analyses of KAP studies on food safety, transmission of zoonosis and antimicrobial resistance were performed using Stata Statistical Software, version 14.2 (College Station, TX: StataCorp LP). For the detection of *E. coli*, the data were entered into Microsoft Excel spread sheet and checked for any error before analysis. Descriptive statistics were used to summarize the results. The significance of the association between *E. coli* O157 isolates and sample source, and type of sample was assessed using the univariate logistic regression. Odds ratio and 95% confidence intervals were used to measure the strength of associations. A p-value of less than 0.05 was considered significant.

1.4. Ethics approval and consent to participate

This research was reviewed and approved by the ethical committees of Addis Ababa University, College of Health Science, IRB (Protocol No.: 031/21), the University of Liverpool (Reference No.: 9935), Animal Research Ethical and Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University (Reference No.: VM/ERC/14/02/14/2022), and Oromia Health Bureau Research Ethics Committee (Reference No.: BEFO/HBTFH/H6/520). Moreover,

before the commencement of the interview, the purpose of the study was explained to the respondents, and requested their willingness to participate in this study.

1.5. Outline of the thesis

This thesis is organized into six chapters; the first and last chapters providing general introduction and general discussion, respectively. Chapter one gives general overview of the study, which include background, extended introduction, objectives, and overview and outline of the study. Chapters from 2, 3 and 5 elaborate the extent of knowledge, attitude and practices regarding transmission of Zoonosis, food safety and antimicrobial use and its resistance in Ethiopia. Chapter 4 addresses the occurrence and antibiotic susceptibility profile of *E coli* O 157 isolates in the meat value chain. Chapter 6 consolidates the findings of the four papers described from chapter 2 to 5. The strengths and limitation of the methodological approaches employed in this study were described and conclusive remarks are provided.

2. CHAPTER II: ASSESSMENT OF KNOWLEDGE, ATTITUDE AND PRACTICES (KAP) OF FARMERS ABOUT TRANSMISSION OF ZONOOSES IN ADA'A DISTRICT, OROMIA, ETHIOPIA

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This chapter is submitted to Heliyon Journal and it is under 2nd revision

ABSTRACT

Zoonotic diseases pose a significant health and economic burden, with high exposure of farmers to the infection given the prevailing traditional husbandry practices. A cross-sectional survey involving 388 farmers in the Ada'a district central Ethiopia was conducted to investigate the knowledge, attitudes, and practices (KAP) of livestock keepers regarding the transmission of zoonotic diseases. A semi-structured pretested questionnaire was used to collect information on farmers' knowledge, attitudes, and practices related to zoonotic diseases. The respondents' mean age was 41.0 ± 10.0 SD years. The majority of respondents (39.4%) were between the ages of 41 and 50, and 90.0% were married. Farmers claimed that conversations with family, colleagues, and friends were the primary sources of Zoonoses information. Farmers stated that the primary sources of information on zoonotic diseases as community. They had a relatively low level (66.8%) of knowledge on zoonotic diseases and half of them (54.9%) did not have good information on Zoonoses. Three-quarters (75.2%) of farmers reported that they neither consume meat and milk nor use offals and hide/skin from sick or dead animals. Similarly, 71.1% of them stated that they do not eat meat from regularly aborting sheep/goats, and never consume raw meat and milk. Most of the respondents correctly answered that raw meat (82.7%) and raw milk (79.9%) are means of disease transmission from animals to humans. However, considerable proportions of them were unaware of zoonotic transmission of bovine tuberculosis (61.3%) and brucellosis (74.7%) transmission through raw milk intakes, and risk of taeniasis (71%) due to raw meat consumption. But, considerable proportions had never dewormed (48.7%), or tested their animals for brucellosis (82.7%) and bovine tuberculosis (83.3%), and more than three quarters (77.3%) did not used personal protective equipment when handling sick animals. Farmers who had a secondary education or above (OR= 6.8, CI=2.4 - 18.0, p = 0.004), aged between 41 and 50 years (OR=3.0, 95% CI: 1.2–7.2, p=0.002), and those having good knowledge (OR=2.1, CI=1.3 - 3.5, p =0.002), and positive attitude related to zoonotic diseases (OR=7.8, CI=4.7 - 12.9, p <0.01) had better practices that reduce the risk of exposure to zoonotic infections than their counterparts. This study revealed a knowledge gaps, a low level of the desired attitude, and high-risk behavioral practices which call for awareness creation about zoonotic disease transmission.

Keywords: *Livestock keepers; Knowledge; Attitude; Practice; Zoonoses; Ethiopia*

2.1. Introduction

Zoonoses have a significant impact on both animal and human health, especially in developing countries. Zoonotic diseases place a double burden on people's well-being by jeopardizing the health and productivity of their livestock; however, they are frequently overlooked by health-care managers and policymakers in both the developed and developing world (WHO, 2006). Almost 60% of currently known infectious diseases are of zoonotic origin, and up to 75% of emerging infectious agents are as well (Woolhouse & Gowtage-Sequeria, 2005). Many Zoonoses are considered occupational health hazards (Battelli, 2008). Livestock farmers are at risk because various types and intensities of contact can result in zoonotic infections (Klous *et al.*, 2016). Ethiopia has Africa's second-largest human population and the continent's largest livestock population (Tilahun & Schmidt, 2012). Ethiopia is especially vulnerable to the effects of zoonotic diseases because its economy is heavily reliant on agriculture (McDermott & Grace, 2012), and roughly 80% of households have direct contact with domestic animals, creating opportunities for infection and disease spread (Lindahl *et al.*, 2015).

Ethiopia has the second largest human population in Africa and the largest livestock population on the continent. About 80% of Ethiopians are dependent on agriculture and have direct contact with livestock or other domestic animals. As a result, the country is vulnerable to the spread of zoonotic diseases (Pieracci *et al.*, 2016). Ethiopia had the second-highest zoonotic disease burden in Africa (Grace *et al.*, 2012). Human-animal interactions in the environment are exacerbating the ongoing transmission of Zoonoses from cattle to humans and vice versa (WHO, 2015). Rabies, brucellosis, bovine tuberculosis, food borne illnesses and poisoning, echinococcosis, and other zoonotic diseases continue to have an impact on human and animal health in many countries, particularly in developing countries (Meslin *et al.*, 2000). Previous research revealed the presence of several zoonotic diseases, including bovine tuberculosis, in Ethiopia (Endalew *et al.*, 2017; Sibhat *et al.*, 2017), brucellosis (Kebeta *et al.*, 2015), bovine cysticercosis and/or taeniosis (Abunna *et al.*, 2008; Terefe *et al.*, 2014; Emiru *et al.*, 2015) and common foodborne pathogens (Zelalem *et al.*, 2019). Surveys of Knowledge, Attitudes, and Practices (KAP) provide critical information for investigating risk factors and potential intervention strategies for disease management. As a result, farmers' behavior is heavily influenced by their knowledge and attitude (Dernburg *et al.*, 2007). Poor disease knowledge correlates with disease prevalence and can set off a vicious cycle of under-

diagnosis/underreporting and awareness deficit (Govindaraj *et al.*, 2016). Egypt (Holt *et al.*, 2011), Turkey (Çkmur *et al.*, 2015), India (John *et al.*, 2019), and other countries have highlighted the importance of zoonotic disease education and KAP surveys in understanding country-specific circumstances.

The development of an efficient plan for zoonotic disease control and prevention depends on consumers' awareness of the risk that zoonotic illnesses associated with the use of animal products represent as well as the protective measures available. Information on the public's beliefs of zoonotic disease risk and preventative strategies, however, is scarce in Ethiopia. As a result, the objective of this study was to assess the level of knowledge, attitudes, and practices about the transmission of zoonotic diseases among smallholder livestock keepers in central Ethiopia.

2.2. Methods

2.2.1. Description of study area

Ada'a district, where the research was carried out is located about 47 kilometers southeast of Addis Ababa. Geographically, it is found on the east side of Addis Ababa at 38°51' to 39°04' East and latitudes 8°46' to 8°59' North, covering a land area of 1750 km². The vast majority of the land (90%) is plain highland ranging from 1600 to 2000 meters above sea level. The district's receives average annual rainfall of 851 mm, and has minimum and maximum average temperatures of 11°C, and 29°C, respectively. In 2022, the district's total population is expected to be 182,162 (CSA, 2022).

2.2.2. Study design, sample size, and data collection

A cross-sectional survey was conducted among farmers in Ada'a district, central Ethiopia. A semi-structured pretested questionnaire was used to collect information on farmers' knowledge, attitudes, and practices related to zoonotic diseases. The questionnaire was adapted from previously published research articles (Kassahun & Mekonen, 2017; Abera *et al.*, 2016; Girma *et al.*, 2012) and translated into local language (Afan Oromo). A sample size of 384 was calculated using presumed proportion of farmers (50%) having knowledge about zoonotic diseases, a 95% confidence level, and a 5% marginal error. Finally, a total of 388 farmers were interviewed for this study. *Ganda* or

villages were selected using purposive sampling methods based on their accessibility by vehicle. Farmers were then chosen using simple random sampling.

The questionnaire was divided into four sections, the first part dealt with respondents' demographic characteristics such as gender, age, level of education, marital status, and toilet type. The second section of the questionnaire focused on Zoonoses knowledge, including questions about the routes of zoonosis transmission from animals to humans, the significance of uncooked meat and milk in transmitting diseases from animals to humans, diseases that humans can contract through raw milk and meat consumption, and so on. This part consisted of 23 questions. The third section of the questionnaire addressed farmers' attitudes toward Zoonoses. It included 13 questions such as whether farmers think consuming or using meat, milk, offal, hide/skin from sick or dead animals, whether they consume milk from regularly aborting sheep/goats, whether they consume raw or undercooked meat, and so on. The fourth section also focused on farmer Zoonoses practices. It included nine questions about raw milk and meat consumption practices, whether farmers wash their hands after having contact with animals, whether they have tested their animals for common zoonotic diseases in the area, and so on. The majority of the questions were closed-ended, requiring participants to respond with "Yes" or "No."

The aims of the research, willingness to communicate results after the end of the study and anonymity of the respondents and confidentiality of their information were explained to the livestock farmers prior to the start of the interviews, and an informed consent document was filled outlined. An interviewer read the questionnaire aloud and recorded the results on paper. The respondents were interviewed face-to-face in the early morning and late afternoon without interfering with their farming activities and without prior notice of the interview. Individual interviews typically took 10-15 minutes to complete. Because the study was conducted during the COVID-19 pandemic, preventive measures such as wearing facemasks, keeping social distance (at least 2m), washing hands, using alcohol-based hand sanitizer, and conducting interviews were used.

2.2.3. Statistical Analysis

The data were extracted using a standardized data extraction format created in Microsoft Excel, and the analysis was performed using STATA-14.2 statistical software. Because the interviews were

conducted face-to-face by a trained veterinarian, there were a few missing responses, which were identified as missing data in the analysis.

To total the farmer's knowledge on specific questions, a knowledge score (range 0-23) was created. If the participant chose the correct answer, he or she received a score of 1.0; otherwise, no score was given. The same scoring system was used for the attitude score (range 0-13) and the practice score (range 0-9). For the attitude part the median score is used instead of the mean score since the data is not normally distributed. The participants were divided into two groups; good and poor based on their mean scores. Respondents who scored above the mean on the knowledge, attitude, and practice items were deemed to have good knowledge, attitudes, and practices, while those who scored below the mean were deemed to have poor knowledge, attitudes, and practices (Kassahun & Mekonen, 2017). A series of univariable logistic regression analyses were used to investigate the unconditional relationship between each explanatory variable and knowledge score. Following the preliminary analysis with knowledge score as the outcome variable, the same series of univariable analyses with attitude score as the outcome variable was performed. Finally, the same univariable analyses were performed with the practice score as the outcome variable and knowledge and attitude scores as independent variables, along with the previously mentioned explanatory variables. Explanatory variables with p-values less than 0.25 were chosen for inclusion in the multivariable model construction. P-values were obtained from logistic regression models and are rounded to three decimal places.

For each of the three scores, a forward stepwise multivariable logistic regression analysis was performed, beginning with the variable with the lowest p-value in the univariable analyses. Explanatory variables with p-values less than 0.05 were kept in the final model. Model goodness of fit-test was assessed by Hosmer and Lemeshow test. The Cronbach's alpha coefficient was also used to determine the questionnaire's internal consistency.

2.3. Results

2.3.1. Demographic characteristics of participants

During the survey period, 388 farmers who were approached completed the questionnaires; with 80.4% of them were men and 68.0% having never attended formal education. The respondents' mean age was 41.0 ± 10.0 SD years. The majority of respondents were (90.0%) married and 54.4% used an open field toilet (Table 2.1).

Table 2.1. Socio-demographic characteristics of randomly selected farmers in Ada'aa district, Ethiopia (n= 388).

<i>Variable</i>	<i>Category</i>	<i>N (%)</i>
Age (years)	Mean (SD)	41.0 (10.0)
	Range	20 - 72
	20-30	59 (15.2)
	31-40	146 (37.6)
	41-50	153 (39.4)
Marital status	≥ 51	30 (7.7)
	Single	39 (10.1)
	Married	349 (89.9)
Gender	Female	76 (19.6)
	Male	312 (80.4)
Educational level	Informal education	264 (68.0)
	Primary education	95 (24.5)
	Secondary and above	29 (7.5)
Type of toilet used	Open field	211 (54.4)
	Ordinary with septic tank	177 (45.6)

Farmers claimed that conversations with community were the primary sources of Zoonoses information (Figure, 2.2).

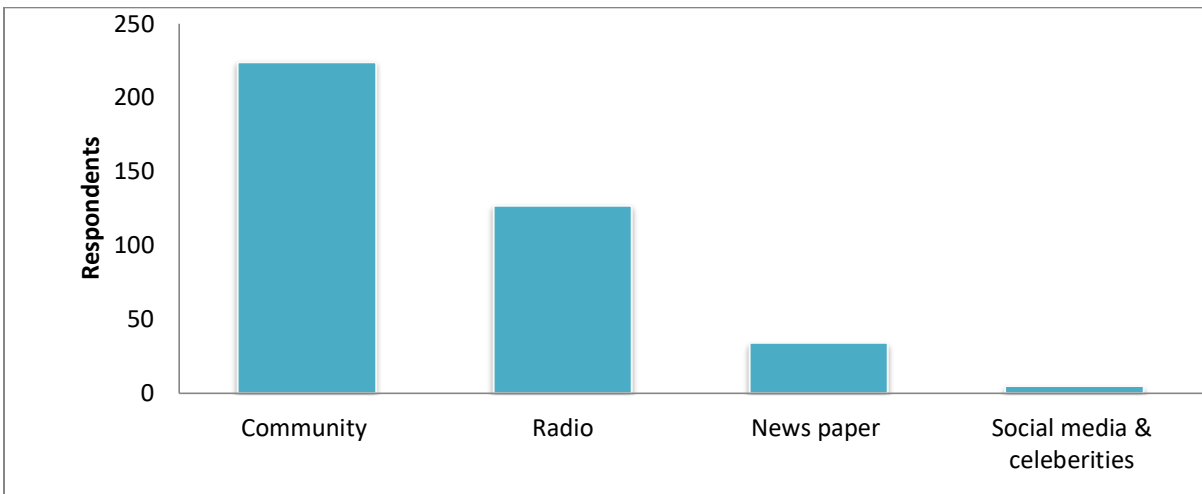


Figure 2.1. Farmers' sources of information about transmission of Zoonoses (n=388)

2.3.2. Knowledge about transmission of Zoonoses

Nearly 61.8% of farmers responded correctly to questions about knowledge about transmission of Zoonoses. As shown in Table 2.2, more than half of respondents (54.7%) were not aware of diseases transmitted between animals and humans (Zoonoses). The total mean score for correctly answered knowledge questions were (13.15 ±4.8).

Two hundred thirty nine farmers (61.6%) scored mean and above the mean were considered to be knowledgeable while 149 (38.4%) scored below the mean and considered as not knowledge-able. Respondents were asked if eating raw meat and milk could spread diseases from animals to humans. As a result, 82.7% and 79.9% of respondents correctly answered that raw meat and raw milk can transmit diseases from animals to humans, respectively. The majority of respondents (61.3%) and 74.7%) did not know that drinking raw milk can cause bovine tuberculosis and brucellosis. Furthermore, 71.1 percent of respondents had low awareness about taeniasis transmission or the risk of bovine cysticercosis due to raw meat consumption.

Table 2.2. Knowledge among farmers of Ada'a districts about transmission of zoonosis in Ethiopia (n= 388)

<i>Transmission of zoonosis knowledge questions</i>	<i>Response, n (%)</i>	
	No	Yes
Do you know what zoonosis is?	213 (54.9)	175 (45.1)
Many animal diseases can be transmitted from animals to humans	154 (39.7)	234 (60.3)
Animal disease can be transmitted via different routes to humans	95 (24.5)	293 (75.5)
Eating uncooked meat can transmit diseases from animals to human	67 (17.3)	321 (82.7)
Drinking raw milk can transmit diseases from animals to human	78 (20.1)	310 (79.9)
Close contact with sick/dead animal can transmit diseases to human	87 (22.4)	301 (77.6)
You can get infection from environment contaminated from secretions of sick animal	79 (20.4)	309 (79.6)
Insect bite can transmit animal diseases to human	123 (31.7)	265 (68.3)
Being bitten by animal can transmit diseases to human	56 (14.4)	332 (85.6)
Abortion in animals can cause a serious economic and public health problem	66 (17.0)	322 (83.0)
Infections can be transmitted to humans through:		
Raw milk consumption	146 (37.6)	242 (62.4)
Raw meat consumption	183 (47.2)	205 (52.8)
Unpasteurized yoghurt consumption	172 (44.3)	216 (55.7)
Unpasteurized cheese consumption	227 (58.5)	161 (41.5)
Being in close contact with sick animals	238 (61.3)	150 (38.7)
Being bitten by an animal	163 (42.0)	225 (60.0)
Humans can contract which of the following diseases via raw milk consumption		
Bovine tuberculosis	238 (61.3)	150 (38.7)
Anthrax	109 (28.1)	279 (71.9)

Brucellosis	290 (74.7)	98 (25.3)
Humans can contract which of the following diseases via raw meat consumption?		
Bovine tuberculosis	237 (61.1)	151 (38.9)
Anthrax	109 (28.1)	279 (71.9)
Cysticercosis/ taeniasis	276 (71.1)	112 (28.9)
Brucellosis	276 (71.1)	112 (28.9)

2.3.3. Attitude about the transmission of Zoonoses

The mean score of the farmers' attitude was 3.89 ± 3.7 . Farmers who scored below the mean score were 220 (56.7% which was considered negative attitude) and above the mean score 169 (43.3% which was considered positive attitude). Farmers generally have negative attitude toward the transmission of Zoonoses (43.3%). Almost three-quarters (75.2%) of respondents stated that they do not consume or use sick or dead animals' meat, milk, offal, hide/skin, or wool products. Additionally, a large proportion of farmers (71.1%) replied that they don't have the habit of consumption of meat from a regularly aborting sheep/goat, consumption of raw or undercooked meat, raw milk consumption, and touching stray dogs (Table, 2.3).

Table 2.3. Attitude of farmers of Ada’a district about the transmission of Zoonoses in Ethiopia (n= 388).

<i>Attitude questions on transmission of Zoonoses</i>	<i>Response, n (%)</i>	
	No	Yes
Do you consume or use the following products from sick or dead animals?		
Meat	282 (72.7)	106 (27.3)
Milk	280 (72.2)	108 (27.8)
Yoghurt products	108 (27.8)	280 (72.2)
Cheese products	124 (32.0)	264 (68.0)
Offal	323 (83.3)	65 (16.8)
Hide/skin	255 (65.7)	133 (34.3)
Wool	319 (82.2)	69 (17.8)
Do you mind consumption of the following activities?		
Consumption of meat from a regularly aborting sheep/goat	313 (80.7)	75.9 (19.3)
Consumption of raw or undercooked meat	276 (71.1)	112 (28.9)
Raw milk consumption	230 (59.3)	158 (40.7)
Raw yoghurt	162 (41.8)	226 (58.3)
Raw cheese	182 (46.9)	206 (53.1)
Touching stray dogs	285 (73.5)	103 (26.6)

2.3.4. Practices about the transmission of Zoonoses

The mean score of the farmers’ practice towards Zoonoses was 2.8 ± 2.0 . Farmers who scored below the mean score were 195 (50.3% which was considered poor practice) and above the mean score 193 (49.3% which was considered good practice). Table 2.4 summarizes farmers’ practices concerning the transmission of zoonosis. About 265 (68.3%) and 287 (74.0%) of the respondents claimed they consume raw milk and raw meat, respectively. A large proportion of farmers walk at home in bare feet (66.2%) and in the farm or garden (68.3%). Furthermore, (48.7%), (82.7%), and (83.3%) have never been dewormed their animals or tested for brucellosis or bovine tuberculosis. More than three quarters (77.3%) respondents stated they had never used personal protective equipment when dealing with sick animals.

Table 2.4. Practices among farmers of Ada’a districts about the transmission of zoonosis in Ethiopia (n= 388).

<i>Transmission of zoonosis practice questions</i>	<i>Response, n (%)</i>	
	No	Yes
Do you drink raw milk?	123 (31.7)	265 (68.3)
Do you eat raw meat?	101(26.0)	287(74.0)
Do you wash your hands after having contact with animals?	267 (68.8)	121 (31.2)
Do you walk bare feet at home?	257 (66.2)	131 (33.8)
Do you walk bare feet at farm or garden?	265 (68.3)	123 (31.7)
Have you ever done deworming of your animals	189 (48.7)	199 (51.3)
Have you ever tested your animals for Brucellosis?	321 (82.7)	67 4(17.3)
Have you ever tested your animals for B.T.B*?	323 (83.3)	65 (16.8)
Do you use personal protective equipment when dealing with sick animals	300 (77.3)	22.7)

**B.T.B=Bovine tuberculosis*

2.3.5. Logistic regression analysis

The association between the demographic variables and the knowledge, attitude, and practices of farmers were determined using logistic regression analysis (Table 2.5). Individuals with good knowledge of transmission of zoonotic diseases were found to have good practices that minimize risk of exposure to zoonotic infections (OR= 2.1, 95% CI= 1.3 - 3.5, $p < 0.01$). Male individuals and the use of toilet with septic tank were 2.2 and 1.7 times more likely to have positive attitude towards transmission zoonotic diseases compared to their counterparts. Good knowledge on transmission Zoonoses was significantly associated with good practices towards zoonotic diseases transmission. Farmers who attained a secondary education or above (OR= 6.8, CI=2.4 - 18.0, $p = 0.004$), aged between 41 and 50 years (OR=3.0, 95% CI: 1.2– 7.2, $p=0.002$), had toilet with septic tank (OR= 0.5, CI= 0.3 - 0.8, $p =0.008$), and good knowledge (OR=2.1, CI=1.3 - 3.5, $p =0.002$), and a positive attitude (OR=7.8, CI=4.7 - 12.9, $p <0.01$) had better practices of transmission of zoonosis that reduce the risk of exposure to zoonotic infections than their counterparts.

Table 2.5. Multivariable logistic regression of factors associated with good knowledge, positive attitude and good practices about the transmission of zoonotic diseases among farmers in Ada’a district in Ethiopia (n= 388).

Variable	Good Knowledge		Positive attitude		Good Practices	
	AOR (95% CI)	p -value	AOR (95% CI)	p -value	AOR (95% CI)	p - value
Gender						
Female	Ref.	-	Ref.	-	Ref.	-
Male	1.1 (0.6- 1.9)	0.87	2.22 (1.2- 4.3)	<0.05	0.7 (0.4-1.3)	0.27
Age						
20-30	Ref.	-	Ref.	-	Ref.	-
31-40	0.70 (0.31-1.55)	0.38	1.38 (0.6 -3.2)	0.45	1.2 (0.5-2.9)	0.63
41- 50	0.75 (0.33- 1.72)	0.50	0.85 (0.4-2.0)	0.72	3.0 (1.2-7.2)	0.002
≥ 51	2.00 (0.6-7.0)	0.28	0.34 (0.1-1.2)	0.09	2.43 (0.8-7.7)	0.13
Marital status						
Single	Ref.	-	Ref.	-	Ref.	-
Married	1.82 (0.8- 4.0)	0.13	1.0 (0.4- 2.3)	0.98	1.1 (0.4-2.6)	0.88
Education						
Informal	Ref.	-	Ref.	-	Ref.	-
Elementary	1.1 (0.6- 2.0)	0.77	0.7 (0.4-1.4)	0.31	1.63 (0.9-3.1)	0.13
Secondary & above	1.7 (0.6- 4.6)	0.29	1.92 (0.7-5.0)	0.18	6.8 (2.4-18.0)	0.004
Type of toilet used						
Open field	Ref.	-	Ref.	-	Ref.	-
Toilet with septic tank	1.0 (0.7-1.7)	0.907	1.7 (1.0 - 2.8)	0.03	0.51 (0.3-0.84)	0.008
Good knowledge	-	-	1.4 (0.9- 2.1)	0.14	2.09 (1.3- 3.5)	0.002
Positive Attitude	1.4 (0.9- 2.1)	0.137	-	-	7.8 (4.7- 12.9)	p<0.01
Good Practice	2.1 (1.3- 3.5)	p<0.01	7.8 (4.7- 12.9)	p<0.01	-	-
Goodness of fit-test	(Chi2(73) = 83.9, p=0179)		(Chi2(73) = 84.0, p=177)		(Chi2(106) =113.6, p=0.290)	

2.4. Discussion

This study provided vital information on knowledge and risky behaviours or practices related to transmission of Zoonoses among smallholder farmers in the Ada'a district of Ethiopia. It revealed that smallholder farmers had a good level of knowledge, a negative attitude and poor practices about transmission of zoonotic disease. In this study, over half of the respondents (54.9%) didn't know about transmission zoonosis. The observed proportion of farmers with poor knowledge on transmission zoonosis is lower than another Ethiopian study finding (Emiru *et al.*, 2015) in which the majority of their study participants (91.2%) had heard of transmission zoonosis. Farmers' levels of knowledge may vary with diseases prevalence, time and place due to differences in access to information and educational level.

In this study, most of the respondents were not knowledgeable about the transmission of bovine tuberculosis (61.3%) and brucellosis (74.7%) through consumption of raw dairy products. This is an alarming situation that an immediate action is needed to create awareness among farmers about the danger of raw milk consumption. Abera *et al.* (2016) reported that 83.4% of respondents had heard of rabies, tuberculosis (61.6%), taeniasis (33.2%), anthrax (22.2%), and brucellosis (21.6%), while very few individuals (8.8%) had not heard of zoonosis. Tesfaye *et al.* (2013) also reported that rabies was the most common disease in Southwestern Ethiopia, followed by taeniasis (83.4%), anthrax (55.4%), bovine tuberculosis (29.1%), and hydatidosis (4%). Another study from Ethiopia indicated that 70% of the participants were aware that rabies is one of the pet contact-associated diseases (Zelalem *et al.*, 2019). This finding is consistent with previous reports from Mekelle (Kebeta *et al.*, 2015) and Gonder (Teferi, 2020) where rabies is considered a serious problem. This clearly demonstrates that rabies is the oldest and a well-known zoonotic disease in Ethiopia. In general, zoonotic disease awareness varied low to moderate among farming communities in Ethiopia. In Addis Ababa, however, it was reported that a higher level of awareness (100%) among respondents naming rabies as a zoonotic disease, followed by anthrax (94.3%), taeniasis (89.1%), bovine tuberculosis (88.5%), and brucellosis (49.5%), suggesting urban residents have higher awareness on zoonotic diseases compared to those from rural settings. The overall disparity awareness among studies for common zoonotic diseases could be attributed to differences in educational status, accessibility, proximity to information sources and frequencies, lifestyle, and disease prevalence.

In this study, more than half of the farmers (57.7%) said they get information about transmission of Zoonoses primarily from conversations with family, colleagues, and friends, followed by radio (32.7%). This finding indicated that there is a knowledge gap among farmers. According to Abera *et al.* (2016),

31.8% of respondents get information from family and friends, while only 4.6% get information from the media. In another study conducted in and around Addis Ababa (Girma *et al.*, 2014), 85.4% of respondents received information in the form of advice from their family. However, electronic media such as radio and television were the major source of information among high school students in Addis Ababa, Ethiopia. According to a study done in Nairobi, evidence-based communication through the media to the general population could be helpful in reducing the dangers of zoonotic diseases (Kang'ethe *et al.*, 2012). The differences in the level of knowledge among farmers from place to place could be attributed to the respondents' age group, proximity to towns, and access to electronic media (Kidane *et al.*, 2015).

In this study, nearly three-quarters (75.2%) of the respondents claimed that animal diseases can be transmitted to humans through various routes. Furthermore, respondents were asked if consumption of raw meat and milk can transmit diseases from animals to humans. As a result, 82.3% and 79.9% of respondents correctly answered that consumption of raw meat and raw milk can transmit diseases from animals to humans, respectively. The majority of respondents (61.4%) and 74.4%) did not know that drinking raw milk can cause bovine tuberculosis and brucellosis. Furthermore, 71.1% of respondents were unaware that raw meat consumption causes bovine cysticercosis. Even though eating raw meat might expose to health concerns including tapeworms, salmonella, and *E. coli*, Ethiopians continue to do so during family gatherings and festive occasions. Nearly, 93.2%, 81.6%, and 15.6% of respondents in and around Asella, Arsi Negele, and South West Ethiopia, respectively, diseases could transmit from animal to human (Abera *et al.*, 2016; Amenu *et al.*, 2010, Kumna *et al.*, 2013). A large proportion of farmers responded that uncooked meat (82.73%), raw milk (79.0%), close contact with sick/dead animals (77.58%), sick animal secretions from contaminated environments (79.4%), insect bite (68.0%), and animal bites (85.6%) can transmit infection from animals to humans. Another report from Ghana indicated that 88% of respondents knew about tuberculosis and 76% knew about brucellosis, which contradicts the findings of this study (Addo *et al.*, 2011), which might be attributed to the fact that the diseases reporting practice and the level of awareness towards Zoonoses among farmers might be better in Ghana than Ethiopia. Despite the fact that 63.5% of respondents were aware of diseases that could be transmitted through cow milk, 61.3% did not know the specific names of milk-borne zoonotic diseases (Negash *et al.*, 2012).

According to the findings of this study, respondents were aware of general milk-borne zoonosis but did not know the specific names of the diseases. The possible reasons for variations in the level of knowledge from place to place could be attributed to the access to information, educational background, life

experience and the prevalence of the respective diseases. Only 3.5% of respondents were aware that pasteurization could be used to prevent milk-borne zoonosis (Delelegn & Girma, 2018). Similarly, 61.3% of respondents were aware that boiling could be used to prevent milk-borne zoonosis. Unpasteurized or raw milk has been linked to milk-borne zoonotic diseases like brucellosis and bovine tuberculosis (Fetene *et al.*, 2011). The majority of respondents were aware that diseases can be transmitted through the consumption of raw cow milk (Mandefero & Yeshibelay, 2018). This result was due to the fact that 100% of students in high school and higher were aware that diseases can be acquired through the consumption of raw cow milk. A study from Punjab indicated that 69.6% of respondents drink raw milk and 55.6% are aware that diseases can be transmitted through the consumption of contaminated milk (Hundal *et al.*, 2016). Only 61.3% of the respondents are aware that boiling milk can prevent milk-borne zoonosis (Mandefero & Yeshibelay, 2018). The disparity in milk-borne zoonosis awareness is due to the study area's unique circumstances. Remoteness, a lack of health facilities, poor extension services, a lack of training in animal rearing and handling, and a low literacy rate as major contributors to a lack of awareness among smallholder dairy farmers as major contributors to a lack of awareness (Ameni *et al.*, 2007). Furthermore, many African communities' associate diseases shared by livestock and humans with misbehavior or witchcraft, and all of these practices are the result of a lack of information or knowledge about milk quality at the farm level and about various aspects of dairy husbandry issues (Marcotty *et al.*, 2009). A higher proportion of respondents (94.3%) believe dog bites are the cause of rabies, and consumption of raw milk and meat is a transmission route of bovine tuberculosis from cattle to humans (Addo *et al.*, 2011). Amenu *et al.*, (2010) reported 15.0% of respondents identified contact with sick animals as a mode of zoonotic disease transmission. Kidane *et al.*, 2015 also claimed contact and ingestion of animal products serve as modes of zoonotic disease transmission. The variations in these findings indicate the presence of a knowledge gap between cities and rural residents. This variation could be attributed to differences in participants' educational levels, access to media and other public health information services, and the prevalence of diseases in the specific area.

Raw milk consumption is an age-old tradition and widespread practices throughout Ethiopia. As a result, in this study, 59.3% of respondents consumed raw milk and half (50.0%) of milk produced by smallholder farmers in the Ethiopian Rift Valley areas has been reported to be consumed fresh at home, without being boiled or pasteurized (Negash *et al.*, 2012). Approximately, 35.0% of dairy farmers drank raw milk, and only 13.0% of these farmers were aware of foodborne diseases that can be transmitted through drinking raw milk (Ayele *et al.*, 2017). In most parts of Ethiopia, the consumption of pasteurized milk is low and limited to urban areas given the underdeveloped milk processing plants in the country.

The vast majority (95%) of all milk produced in the country being sold through informal marketing systems rather than pasteurization plants (Getabalew *et al.*, 2020). A large proportion (67.0%) of farmers in North Western Ethiopia claimed to drink raw milk (Nuru *et al.*, 2017). In the Jimma zone of western Ethiopia, 57.0% of adults drank fermented milk on occasion and 14.0% of interviewees did not boil milk for their children (Tolosa *et al.*, 2016). As a result, these findings imply that a sizable portion of Ethiopian society consumes raw milk, either fresh or fermented, on a regular or sporadic basis. This clearly indicates that farmers lack knowledge about the zoonotic risks associated with drinking raw milk. In this study, nearly three-quarters (75.1%) of farmers stated that they didn't consume or used animal products such as meat, milk, offal, hide/skin, and wool products from sick or dead animals. Cooking animal products such as meat (33.7%) and meat products (64.3%) is also practiced by respondents in fear of disease transmission (Amenu *et al.*, 2010). Contrary to this study finding, reports from Africa and Southeast Asian countries suggested socio-cultural practices such as slaughtering sick animals, eating or handling meat from infected animals, and dumping carcasses in the open have been linked to anthrax transmission (Islam *et al.*, 2014).

Furthermore, a large proportion of farmers (77.3%) responded that they had never used personal protective equipment when dealing with sick animals. It is stated that due to the scarcity of personal protective equipment in their areas; farmers and pastoralists do not use it when dealing with animal abortion (Alemayehu *et al.*, 2021). Approximately, 60.0% of respondents were exposed to the risk of zoonotic diseases due to their frequent consumption of raw meat (Deneke *et al.*, 2022). This was despite the fact that over 90% of the respondents were aware of possible zoonotic risks of raw meat consumption. Location, gender and age of the household head, household size, meat type preference, per-capita meat consumption, knowledge about disease transmission risks, and training on Zoonoses were all associated with raw meat consumption behaviors. It was also reported , 58.2% and 57.1% of respondents from the Arsi-Negele district of Ethiopia consumed raw meat and milk, respectively (Amenu *et al.*, 2010). Moreover, it was reported that 56.8% of respondents in Mana and Limmu-Kosa districts of Jimma Zone, South West Ethiopia, consumed raw food of animal origin (Kuma *et al.*, 2013). A large proportion of respondents (69.1%) in Jimma, Southwestern Ethiopia also consume raw meat (Tesfaye *et al.*, 2013). In Ethiopia, meat consumption is a deep-rooted cultural behavior. Meat is often consumed as part of the staple diet of the people and also during special occasions of festivity. Its cultural symbolism is more significant than that of any other cuisine. Eating raw meat or half cooked meat is very common and although Ethiopians from various cultures enjoy eating meat, they are generally very selective, in that only poultry, beef, mutton, goat and fish (not including shell-fish) are culturally and religiously

acceptable. Eating other kinds of meat, such as pork, is a cultural taboo among most Ethiopians (Seleshe *et al.*, 2014). Consumption of raw meat is practiced in some parts of the world as a cultural heritage passed down through many generations. Raw and/or undercooked meat is known to be consumed in countries such as Russia, Cuba, and many social groups on the African continent (Suarez *et al.*, 2005). To safeguard the public health, it needs an intensive awareness programs about the dangers of raw meat consumption. Because raw meat consumption may not only predisposes to taeniasis but also to other extremely dangerous foodborne pathogens such as anthrax and bovine tuberculosis. In one study conducted in Ethiopia's Arsi-Negele district, respondents revealed that butchery (15.3%), slaughtering at home (59.2%), and backyard slaughtering (94.9%) were the sources of meat for home consumption (Amenu *et al.*, 2010). There is no discernible trend in the sources of meat and meat products purchased. It may be determined by consumer needs, festivals, or everyday use, as well as the availability of markets in urban and rural areas. This is due to the fact that there is a weak regulatory body to implement the zero tolerance for backyard slaughtering.

This study also showed that nearly 68.8% of respondents' didn't wash their hands after coming into contact with animals. A similar study from Turkey shows over 90.0% of cattle farmers had both positive attitudes and good practices regarding hand-washing after contact with an animal, the burial of dead animal bodies, and the separation of sick animals from the herd (Özlu *et al.*, 2020). Incorrect perceptions and attitudes toward zoonotic disease prevention underscore the importance of culturally appropriate health education in rural communities. As a result, it is critical to change the community's attitude in order to improve their behavioral practices regarding zoonotic disease transmission prevention practices (Nijland *et al.*, 2013). It was also reported that awareness of zoonotic disease transmission by consumption of meat (96.3%) and milk (51.3%) among the respondents in the Arsi-Negele district of Ethiopia (Amenu *et al.*, 2010). Similar study from Ethiopia indicated that in the southwestern part of Ethiopia, 82.3% of respondents were aware of raw/undercooked meat consumption as a vehicle for the transmission of taeniasis to humans (Tesfaye *et al.*, 2013). Such variation could be explained by differences in educational attainment, media access, and disease prevalence.

The majority of farmers in this study had never dewormed their animals (48.7%), had never tested for brucellosis (82.7%), and had never tested their animals for bovine tuberculosis (83.3%). One study from India indicated that 23% of farmers consumed raw milk, while only 10% and 8% of livestock farmers had their animals tested for brucellosis and tuberculosis, respectively. A low level of education and being a cattle farmer were negatively associated with the farmer's knowledge of zoonotic diseases. The

participants' attitude score was positively related to their practice score. The findings highlight the importance of educating livestock farmers, especially those with a low level of education, in order to reduce the health and economic impact of zoonotic diseases (Singh *et al.*, 2019).

In this study, there was no significant difference in farmers' knowledge of zoonotic diseases based on their gender, marital status, or educational level. Farmers' good practices, on the other hand, were found to be positively associated with Zoonoses knowledge. Farmers who have good zoonotic practices were found to have better knowledge than their counterparts. It was also reported that respondents' knowledge was positively associated with desired attitudes and practices (Alemayehu *et al.*, 2021). Similarly, it was reported that gender and age are important demographic factors that can influence farmer knowledge, attitude, and practices (Sadati *et al.*, 2010). The educational background of farmers has been linked to improved zoonotic disease knowledge and practices (Moutos *et al.*, 2022). Different studies have reported different outcomes from different countries regarding the role of demographic profiles of the respondents; which might be due the fact that different countries have different level of Zoonoses awareness, some countries have strict regulations regarding Zoonoses, and countries have different cultural backgrounds.

Regarding attitude, those farmers who use a toilet with an ordinary septic tank, and those who practice good hygiene had a more positive attitude toward zoonosis than their counterparts. This might be explained by the fact that in Ethiopia, individuals who use toilets are those who are better informed about the transmission of zoonotic diseases; however large proportion of individuals are still using open defecation. An encouraging efforts made by the government of Ethiopia is that it has set ambitious targets for water, sanitation, and hygiene (WASH). Total Sanitation to End Open Defecation and Urination (TSEDU) campaign also aims to make Ethiopia open defecation free (ODF) by 2024. Finally, farmers with a secondary or higher level of education had better Zoonoses practices than their counterparts. People will use hygienic practices if they have good knowledge (Adhikari & Bagale, 2019). Higher education, on the other hand, only significantly improves their knowledge while having no effect on their attitude or practices. It was also reported that higher education levels were associated with better knowledge and practices (Moutos *et al.*, 2022). This might be because farmers with better levels of education are more progressive or informed than others.

Furthermore, toilet usage among farmers is found to be significantly associated ($p = 0.008$) with good Zoonoses practice. This suggests that raising awareness among farmers about the use and importance of toilets in disease prevention and control is critical. Furthermore, farmers who had good knowledge and a

positive attitude practiced zoonosis better than their counterparts. There is a positive relationship between correctly answering knowledge questions and a positive attitude and self-reported good practice (Alemayehu *et al.*, 2021).

2.5. Conclusions

The results of this study indicated that most of the farmers are not aware of the transmission, prevention and control of zoonosis in the study area. Numerous possible problems stem from farmers' attitudes and practice towards transmission of Zoonoses. For instance, the consumption of raw milk is a potential risk for the transmission of brucellosis and tuberculosis. Similar to this, barefoot walking at home and field or garden may increase the risk of contracting cutaneous larva migrans. Hence, awareness creation among farmers about zoonotic disease transmission, prevention and control-through mass media, and education and training programs could enhance the knowledge of farmers.

Strength of this study

An important strength of this study was that it used an appropriate sample size of farmers with random sampling methods; therefore, the study findings can be generalizable to the district population. Farmers were randomly sampled from their peasant associations (PA) not more than once to avoid bias. The sample size was estimated before the start of the study and considered to provide adequate predictive power. The questionnaire was adapted from published instruments and revised by experts to ensure content validity. It was carefully translated into local languages Amharic/Afan Oromo and tested to ensure clarity of questions and respondents' ability to provide accurate answers.

Limitations of this study

As an observational and cross-sectional study, our study has several limitations. We cannot document causal relationships because of the current survey's cross-sectional nature. Because our study was questionnaire-based, there is a possibility of recall bias. Because of the use of multiple-choice answers, response bias is unavoidable, though it may have been mitigated in the construction of the knowledge score due to some stringent requirements. The attitude responses clearly do not correspond to practices, as many respondents admitted that despite knowing the proper action, they do not implement it due to cost or the amount of handwork required. This is even more understandable given the highly risky behaviors

revealed by self-reported practices. However, the Cronbach's alpha results indicate that our questionnaire had good internal consistency.

Declarations

Ethical Approval

This research was reviewed and approved by the ethical committees of Addis Ababa University (Protocol number 031/21), the University of Liverpool (Reference No.: 9935).

Authors' Contributions

FA participated in conception, design of the study, field work, and manuscript drafting. GG participated in manuscript revision. BM participated in supervision of the research, revising and approving the manuscript for submission.

Funding statement

This work was supported by Addis Ababa University

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper

2.6. References

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3. CHAPTER III: ASSESSMENT OF FOOD SAFETY KNOWLEDGE, ATTITUDES, AND PRACTICES AMONG MEAT HANDLERS IN BISHOFTU CITY, ETHIOPIA

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This chapter is published on American Journal of Tropical medicine and Hygiene 2023, 108(1):200-205: doi: 10.4269/ajtmh.22-0268

ABSTRACT

Meat handlers play a critical role in food safety by preventing contamination of food for human consumption. A cross-sectional survey was undertaken with 391 meat handlers working in abattoirs and retail meat stores in Bishoftu, Ethiopia, to investigate their food safety knowledge, attitudes, and practices (KAP). Data were collected in interviews using a semi-structured questionnaire adapted from previous research. An overall score for each topic area was calculated based on the responses to individual questions. Almost all meat handlers were males (97.2%) and more than half (51.9%) had primary-level education. Most (72.4%) meat handlers had a good knowledge level with a median score of 16 out of 21 (interquartile range [IQR] = 6). Similarly, most (94.6%) meat handlers had a positive attitude toward food safety with a median score 18 out of 20 (IQR = 1). However, most (83.7%) meat handlers had poor food safety practices with median score of 11 out of 20 (IQR = 3). In multivariable models, good knowledge was significantly ($p < 0.05$) associated with male gender and older age; positive attitudes were associated with lower educational attainment and good knowledge; and good practices were associated with working in an abattoir and having received training on food safety. Regular hands-on training and enforcement of general and personal hygiene is recommended.

Keywords: Meat-handlers, Food safety, KAP, Ethiopia

3.1. Introduction

Globally, about 600 million people fall ill after consuming contaminated food each year, equivalent to 27 million healthy life years lost) (WHO, 2015). Foodborne illness is one of the most important causes of human disease, with consumption of contaminated meat playing a major role in illness (Jacob *et al.*, 2010). Meat is a common source of foodborne illness as it provides a good medium for multiplication of microorganisms, including pathogens and spoilage organisms (Scharff *et al.*, 2009). Because of the high potential for meat to support growth of pathogens, meat has to be handled with care from the point of slaughter as well as post-harvest to avoid contamination (Abdul-Mutalib *et al.*, 2012). Contamination and cross-contamination of raw meat is a major cause of foodborne diseases, particularly in developing countries (Adesokan & Raji, 2014). In many developing countries, strict control of meat hygiene and safety is not easy and meat for human consumption is approved mainly based on visual inspection (Bakhtiary *et al.*, 2016).

Like many developing countries, the majority of foodborne illness in Ethiopia can be attributed to organisms that are shed intermittently in feces of infected animals and which contaminate carcasses during the slaughter process as a result of unhygienic handling and cross-contamination (Eguale *et al.*, 2018, Atnafie *et al.*, 2017). Studies of children with diarrhea in Ethiopia, for example, reveal a high prevalence of pathogenic *E. coli* (28.8%) Zelelie *et al.*, (2019), *Salmonella* (3.1%), Zelelie *et al.*, (2019) and (1.3%) Tosisa *et al.*, (2020). Other studies have revealed a *Salmonella* prevalence of 7.2%, 4.7%, and 4.4% in human patients, poultry, and swine, respectively (Eguale *et al.*, 2018, Eguale *et al.*, 2015). Microbial contamination of animal source foods along the value chain has also been reported in Ethiopia. A recent meta-analysis of studies on the prevalence of *E. coli* in milk and meat found an overall random pooled prevalence of 15% (95% CI = 13-17%) (Assefa & Bihon, 2018) while another review highlighted the occurrence *E. coli*, *Salmonella*, *Listeria*, *Campylobacter* in beef sold in retail stores (Edget *et al.*, 2014).

Given the critical role that slaughterhouses and meat retailers play in food safety, it is useful to have insight into the level of food safety knowledge, attitudes, and practices (KAP) of meat handlers, so that interventions can be developed accordingly. Studies conducted in different parts of Ethiopia have revealed that meat handlers' behavior is an important risk factor for food contamination and can reduce the quality of food served for human consumption. For example, in one study conducted in eastern Ethiopia, meat handlers had poor knowledge regarding foodborne pathogens, time-temperature control to prevent bacteria growth, cross-contamination, and the difference between cleaning and sanitation (Tegegne *et al.*, 2017). Another study in northern Ethiopia found that about 15.4% of abattoir personnel had no health certificate and there was no hot water, sterilizer, or cooling facility in the abattoir while 11.3% of butchers did not use protective clothes when handling meat (Haileselassie *et al.*, 2013).

In Ethiopia, "downstream" food safety measures such as investigating food establishments and recalling food products are not well established. For this reason, special attention must be given to "upstream" factors including personal hygiene of meat handlers in slaughterhouses and retail shops to ensure safe foods are available for consumers. Inadequately trained employees practicing unhygienic handling of meat can expose the public to meat-borne illness, which could be ameliorated through training and implementation of quality control systems (Gutema *et al.*, 2010). While there have been some studies on food safety KAP in food handlers in food establishments in Ethiopia (Adane *et al.*, 2018, Admasu & Kelbessa, 2018, Tesfaye & Tegene, 2019) only one study (Tegegne *et al.*, 2017) has undertaken an in-depth investigation of food safety KAP of meat handlers in abattoirs and retail meat shops. Other studies

in this setting have focused mainly on food safety practices (Haileselassie *et al.*, 2013, Gutema *et al.*, 2021, Yenealem *et al.*, 2020). Therefore, this study aimed to assess the food safety KAP and the influence of knowledge and attitudes on food safety practices of abattoir and retail meat shop workers' in central Ethiopia.

3.2. Methods

3.2.1. Study setting

This study was undertaken in Bishoftu city, in the Oromia region of Ethiopia, located 47 km southeast of the capital, Addis Ababa. Geographically, the city is located at 8°45'8.1" N latitude and 38°58.708' E longitude. The city is located in mid-highland at an altitude of about 1920 meters above sea level. It is characterized by moderate weather conditions with an average annual temperature ranging between 16°C and 24°C. Most precipitation occurs in August with an average precipitation of 220 mm. The annual amount of precipitation is 968 mm and humidity is close to 75%. The total population of the city was projected to be 197,557 in 2021 (Central Statistics Agency, 2021). Retail meat shops purchase cattle from different open markets or fattening farms located in neighboring towns and bring the animals to abattoirs for slaughter. There are five abattoirs with different slaughtering capacities and about 137 retail meat shops operating in the study area.

3.2.2. Study design, sample size, and data collection

A cross-sectional survey was undertaken with meat handlers working in abattoirs (involved in slaughtering and handling of carcasses) and retail meat shops in Bishoftu city. Assuming a 50% level of knowledge/attitudes/practices, with 95% confidence level and $\pm 5\%$ precision, a minimum sample size of 384 was calculated. This was increased to 391 meat handlers assuming 10% non-response. A purposive sampling technique was employed to select the retail meat shops/abattoirs. Subsequently, meat handlers were selected using simple random sampling.

A semi-structured, pre-tested questionnaire was used to obtain data on meat handlers' knowledge, attitudes, and practices towards food safety. The questionnaire was adapted from previously published research articles (Tegegne *et al.*, 2017, Al-Shabib *et al.*, 2016, Soares *et al.*, 2012, and Akabanda *et al.*, 2017) and translated into the local languages (Afan Oromo and Amharic). The questionnaire was

organized into four distinct parts. The first section included questions on demographic characteristics of respondents such as sex, age, years of experience, and level of education. The second section of the questionnaire focused on food safety knowledge, and questions on personal hygiene, cross-contamination, and causes of foodborne diseases. The third part of the questionnaire dealt with the food safety attitude of meat handlers. It comprised 20 questions such as hand washing, cross-contamination, food handling, and storage. The fourth section of the questionnaire addressed food safety practices amongst meat handlers. It comprised 20 questions on topics related to basic food safety and hygiene, protective clothing, and prohibited and equipment handling practices. Questions were mostly closed-ended and required participants to respond with “Yes” or “No” (“Agree” or “Disagree”).

Interviews were conducted in a private area inside the respondents’ work establishment in order to make the interview comfortable for the respondent and without disturbances. The questionnaire was read out loud by an interviewer who recorded the results on a paper form. The respondents were interviewed face-to-face during working hours without prior notice of the interview. On average individual interviews took 10-15 minutes to complete. As the research took place during the COVID-19 pandemic, preventive measures were practiced during face-to-face data collection by wearing facemasks, observing social distance (at least 2m), washing hands, using alcohol-based hand sanitizer, and conducting interviews in a well-ventilated space.

3.2.3. Statistical analyses

Data analysis was performed in Stata Statistical Software, release 14.2 (College Station, TX: StataCorp LP). For knowledge questions, a score of one was given for each correct answer. The sum of scores (ranging between 0 and 21) was then calculated giving an overall score on food safety knowledge. Meat handlers that had an overall score of <15 were considered to have “poor knowledge” while those that scored ≥ 15 (i.e. $\geq 70\%$ of questions answered correctly) were deemed to have “good knowledge” of food safety. Similarly, for attitudes and practices questions, a score of one was given to each answer that was consistent with “good attitudes/practices” and an overall score out of 20 was calculated. Consistent with other studies (Tegegne *et al.*, 2017; Akabanda *et al.*, 2017), meat handlers with a score of ≥ 14 (i.e. $\geq 70\%$ of questions answered in the affirmative) were deemed to have “good attitudes/practices” whereas respondents with scores ≤ 13 were deemed to have “poor attitudes/practices”. This cut-off point was used to generate a binary dataset (i.e. good =1, poor=0) for each indicator. Descriptive statistics (median, interquartile range [IQR]) were used to summarize the overall scores of knowledge, attitude, and practice

questions. Mann Whitney U test was used to compare scores by socio-demographic characteristics. Finally, logistic regression was used to assess the independent associations between socio-demographic characteristics of the study participants (independent variables) and good knowledge, attitudes and practices (dependent variables; separate models each variable). Consistent with the KAP model of health behavior, knowledge was included as a predictor in the model exploring factors associated with good attitudes, while knowledge and attitudes were included as predictors in the model exploring factors associated with good practices. Predictor variables with p-value <0.25 in univariable analysis were retained in the final multivariable logistic model.

3.2.4. *Ethics approval and consent to participate*

This research was reviewed and approved by the ethical committees of Addis Ababa University (Protocol number 031/21) and the University of Liverpool (Reference No. 9935). Before the commencement of the interview, the purpose of the study was explained to the respondents and requested their willingness to participate in this study.

3.3. Results

3.3.1. *Demographic characteristics*

The socio-demographic profile of respondents is summarized in Table 3.1. The majority (61.6%) of the respondents were aged 16-25 years. The mean age of the meat handlers was 25.2 ± 6.6 years (range 16-68). The majority of the meat handlers were males (97.2%) and around half (51.9%) had primary-level education. Participants had worked for 3.6 ± 3 years of service on average (range 0.1-30 years). Around one third (34.5%) of respondents indicated they had received training in food safety, all of whom worked in abattoirs; no formal training had been given to any meat handler working in retail meat shops.

Table 3.1. Socio-demographic characteristics of randomly selected meat handlers working in abattoirs and retail meat shops in Bishoftu, Ethiopia (n= 391). RMS = retail meat shop.

Variable	Category	Frequency, n (%)
Age (years)	16-25	241 (61.6)
	26-68	150 (38.4)
Gender	Female	11 (2.8)
	Male	380 (97.2)
Education	Primary	203 (51.9)
	Above primary	158 (40.4)
Year of service	≤3 years	230 (58.8)
	>3 years	161 (41.2)
Training	No	256 (56.5)
	Yes	135 (34.5)
Place of work	RMS	223 (57.0)
	Abattoir	168 (43.0)

3.3.2. Food safety knowledge of meat handlers

Food safety knowledge of meat handlers is presented in Table 3.2. Nearly three quarters (72.4 %) of the respondents had a good knowledge level with a median score of 16 out of 21 (IQR = 6). Most participants correctly identified that improper meat handling posed a risk to consumers and that good hand hygiene practices and proper cleaning, sanitization, and disinfection could reduce the risk of contamination. Knowledge of specific food-borne pathogens such as bacteria and viruses was, however, poor with only around half of the participants able to identify that meat contaminated with fecal material could cause food-borne illnesses. The importance of meat workers' health status was also not well appreciated by a sizable proportion of the respondents.

Table 3.2. Food safety knowledge of meat handlers working in abattoirs and retail meat shops of Bishoftu city, Ethiopia (n= 391).

Food safety knowledge questions	Response, n (%)		χ^2	p-value
	Good knowledge)	Poor knowledge		
When meat is handled improperly, it could pose health hazards to consumers	381 (97.4)	10 (2.6)	147.6	0.000
Risk of contamination can be reduced by regular washing of hands before and during meat processing	386 (98.7)	5 (1.3)	182.6	0.000
The use of gloves during handling meat reduces the risk of contamination	288 (73.7)	103 (26.3)	151.3	0.000
Knives and hooks should be properly cleaned and sanitized to reduce the risk of meat contamination	372 (95.1)	19 (4.9)	153.5	0.000
In the workplace, eating and drinking increase the risk of meat contamination	331 (84.7)	60 (15.3)	145.7	0.000
Washing and disinfection of working surfaces and tools are important for safety of meat	387 (99)	4 (1)	78.8	0.000
Regular application of disinfectants for cleaning can reduce the risk of meat contamination from working surfaces and cutting tools	358 (91.6)	33 (8.4)	113.9	0.000
Insects and pests could be a source of contamination to raw meat	379 (96.9)	12 (3.1)	221.6	0.000
The cause for diarrhea could be food contamination	347 (88.7)	44 (11.3)	106.8	0.000
Meat contaminated with fecal materials can cause food-borne illness	211 (54)	180 (46)	271.1	0.000
A virus can cause food-borne illness	187 (47.8)	204 (52.2)	284.0	0.000
Bacteria can cause food-borne illness	206 (52.7)	185 (47.3)	283.5	0.000
Microbes are on the skin, nose, and mouth	243 (62.1)	148 (37.9)	211.7	0.000

of healthy meat handler				
Cleaning is the same as sanitizing	143 (36.6)	248 (63.4)	98.9	0.000
Cross-contamination occurs when microorganisms from contaminated meat are transferred by the meat handler's hands or utensils to another	316 (80.8)	75 (19.2)	119.6	0.000
The ideal place to store raw meat is in the refrigerator	335 (85.7)	56 (14.3)	176.9	0.000
Freezing preserves food for extended periods because it prevents the growth of microorganisms (bacteria, yeasts, and molds) that cause both food spoilage and foodborne illness	348 (89)	43 (11)	175.4	0.000
High temperature or freezing is a safe method to destroy bacteria	306 (78.3)	85 (21.7)	141.4	0.000
Contaminated meat always have some change in color, odor, or taste	383 (98)	8 (2)	107.5	0.000
Employees with open injury on skin, gastroenteritis, and ear or throat infections should not be allowed to handle meat	251 (64.2)	140 (35.8)	198.8	0.000
It is recommendable to evaluate the health status of workers before employment	196 (50.1)	195 (49.9)	219.1	0.000

3.3.3. Food safety attitude of meat handlers

Table 3.3 shows the frequency distribution of meat handlers' attitudes towards food safety. Most respondents (94.6%) had a positive attitude toward food safety with a median score of 18 out of 20 (IQR = 1). In general, meat handlers appreciated their role in reducing contamination through measures such as washing hands regularly, keeping surfaces clean, and inspecting meat for freshness. Nonetheless, views on cleaning methods were not always consistent with good food hygiene practices with a majority (90.3%) expressing that the same towel could be used to clean many places. Further, attitudes towards raw meat were not consistent with good food safety, with the majority of meat handlers believing that raw

meat is healthier and more nutritious than cooked meat. A considerable proportion (33.5%) also expressed that it was not unsafe to leave meat out of the refrigerator for 2 hours.

Table 3.3. Food safety attitudes of meat handlers' working in abattoirs and retail meat shops of Bishoftu city, Ethiopia (n= 391).

Food safety attitude questions	Response, n (%)			
	Positive attitude	Negative attitude	χ^2	p-value
It is not recommended for meat handlers with wounds, bruises, or injuries on their hands to touch or handle meat	365 (93.4)	26 (6.6)	128.8	0.000
The use of personal belonging such as watches, earrings, and rings in the work establishments will increase the risk of meat contamination	321 (82.1)	70 (17.9)	35.9	0.000
Mishandling and improper meat storage is dangerous to health	379 (96.9)	12 (3.1)	181.4	0.000
The risk of food contamination could be reduced by regular hand washing before handling meat and equipment	380 (97.2)	11 (2.8)	101.0	0.000
Regular training could improve meat safety and hygiene practices	382 (97.7)	9 (2.3)	126.4	0.000
Safe meat handling is part of the responsibilities of meat handlers to avoid contamination and diseases	361 (92.3)	30 (7.7)	50.0	0.000
If we keep working surfaces and utensils clean, it reduces the risk of illness	380 (97.2)	11 (2.8)	101.0	0.000
If we use different knives and cutting boards for meat and offal processing, it reduces contamination	312 (79.8)	79 (20.2)	36.0	0.000
It is unsafe to leave meat out of the refrigerator for more than 2 hours	260 (66.5)	131 (33.5)	11.0	0.001
It is valuable to inspect meat for freshness	377 (96.4)	14 (3.6)	124.7	0.000

and wholesomeness

Working environments such as surfaces and equipment should be clean before re-using for meat processing	374 (95.7)	17 (4.3)	148.7	0.000
After meat processing, any leftovers should be kept in a cool place to minimize food contamination	368 (94.1)	23 (5.9)	86.7	0.000
Raw meat is preferred to cooked meat because it is healthier and more nutritious	87 (22.3)	304 (77.7)	11.6	0.001
Mishandling of knives, hooks and cutting boards could be the source of food contamination	368 (94.1)	23 (5.9)	105.3	0.000
Properly sanitized knives and cutting boards could help to minimize and prevent cross-contamination	382 (97.7)	9 (2.3)	162.3	0.000
It is possible to use the same towel to clean many places	38 (9.7)	353 (90.3)	56.9	0.000
Failure to cover our noses or mouth while sneezing or coughing could potentially contaminate the meat	368 (94.1)	23 (5.9)	172.2	0.000
Food safety could be improved by wearing protective clothing and shoes	384 (98.2)	7 (1.8)	37.6	0.000
It is good practice to put on hair cover on the head in the food industry	375 (95.9)	16 (4.1)	107.1	0.000
The use of potable water is important to wash working surfaces and cutting tools after disinfection	383 (98)	8 (2.1)	108.4	0.000

3.3.4. Food safety practices by meat handlers

Table 3.4 summarizes the meat handlers' practices regarding food safety. Most of the meat handlers (83.7%) had below the acceptable level of food safety practices with median score of 11 out of 20 (IQR =

3). While a majority of meat handlers practiced good hand washing before and after handling meat, after handling waste/garbage, and after using the toilet, lapses in practices were reported around the time of donning/doffing gloves and after smoking, sneezing, or coughing. Moreover, while hairnets or caps were worn by a majority of meat handlers, gloves, masks, aprons, or other protective clothing were not commonly used. Close to half of the respondents stated they handled/processed meat when they were ill (45%) or when they had cuts or other injuries on their hands (47.1%).

Table 3.4. Food safety practices of meat handlers' working in an abattoir and retail meat shops of Bishoftu city, Ethiopia (n= 391).

Food safety practice questions	Response, n (%)	
	Yes	No
Basic food hygiene practices		
Do you wash your hands after handling waste/garbage?	339 (86.7)	52 (13.3)
Do you have the habit of washing your hands before & after handling meat, meat products, and equipment?	334 (85.4)	57 (14.6)
Do you wash your hands before and after using the toilet?	381 (97.4)	10 (2.56)
Do you wash your hands after smoking, sneezing, or coughing?	149 (38.1)	242 (62)
Do you wash and sterilize your protective clothes or aprons after each day's work?	61 (15.6)	330 (84.4)
Do you wash your hands properly before or after using gloves?	22 (5.6)	369 (94)
Do you wear a hairnet or a cap in the working establishment?	297 (76)	94 (24)
Do you wear protective clothes or aprons in the working establishment?	62 (15.9)	329 (84.1)
Do you wear a mask while working?	138 (35.3)	253 (65)
Do you eat or drink at your workplace?	287 (73.4)	104 (26.6)
Do you handle food with long or painted fingernails?	29 (7.4)	362 (93)
Do you smoke inside meat processing areas?	14 (3.58)	377 (96.4)
Do you handle/process meat when you are ill?	215 (55)	176 (45)
Do you handle/process meat when you have cuts, wounds, bruises, or injuries on your hands?	207 (52.9)	184 (47.1)
Do you use sanitizer when washing service utensils (knives, hooks, and cutting boards)?	270 (69.1)	121 (30.9)
Do you change knives or sterilize them after each meat processing?	279 (71.4)	112 (29)
Do you remove your personal items such as rings, necklaces, watches, etc. while processing meat?	254 (65)	137 (35)
Do you properly clean hooks, cutting boards, and meat storage area before storing new products?	379 (96.9)	12 (3.1)
Do you use gloves while handling meat?	19 (4.9)	372 (95)
Do you remove your work equipment when going to the toilet?	360 (92.1)	31 (7.9)

Median KAP scores by socio-demographic characteristics of the respondents are presented in Table 3.5. Male individuals had a higher median score for food safety knowledge, attitudes and practices than

females. Older age and longer service was associated with higher median knowledge. KAP scores showed disparity with the age of the respondents. Meat handlers in the age range of 25 to 45 years scored better in terms of knowledge and attitudes towards food safety compared to younger and older individuals (Fig. 3.1a). In contrast, older individuals scored higher in terms of food safety practices. Good knowledge was associated with higher median attitude score, while positive attitude was associated with higher median practice score. Both food safety attitudes and practices scores increased with knowledge score, albeit to a lesser degree for food safety practices scores (Fig. 4.1b).

Table 3.5. Median (IQR, interquartile range) knowledge, attitudes and practices scores of meat handlers' working in abattoir and retail meat shops of Bishoftu city, Ethiopia (n= 391), by socio-demographic characteristics*.

Variable	Level	Knowledge score		Attitudes score		Practices score
		Median (IQR)	p-value	Median (IQR)	p-value	Median (IQR)
Gender	Female	13 (5)	0.00	15 (8)	0.00	10 (4)
	Male	16 (6)		18 (1)		11 (3)
Age (years)	16-25	16 (6)	0.02	18 (1)	0.18	11 (3)
	>25	18 (5)		18 (2)		11 (2)
Education	Primary	17 (6)	0.16	18 (1)	0.03	11 (3)
	Above primary	16 (6)		18 (2)		11 (3)
Service	≤ 3 years	16 (6)	0.05	18 (1)	0.71	11 (3)
	> 3 years	18 (5)		18 (1)		11 (3)
Training	No	16 (6)	0.33	18 (1)	0.20	11 (2)
	Yes	16 (6)		18 (1)		11 (4)
Place of work	RMS	16 (6)	0.66	18 (2)	0.14	11 (2)
	Abattoir	17 (6)		18 (1)		11 (4)
Knowledge	Poor	na	na	17 (3)	0.00	11 (4)
	Good	na	na	18 (1)		11 (3)
Attitude	Negative	na	na	na	na	10 (6)
	Positive	na	na	na	na	11 (3)

*P-values were obtained from Mann Whitney U tests and are rounded to two decimal places. na = not applicable, RMS = retail meat shop.

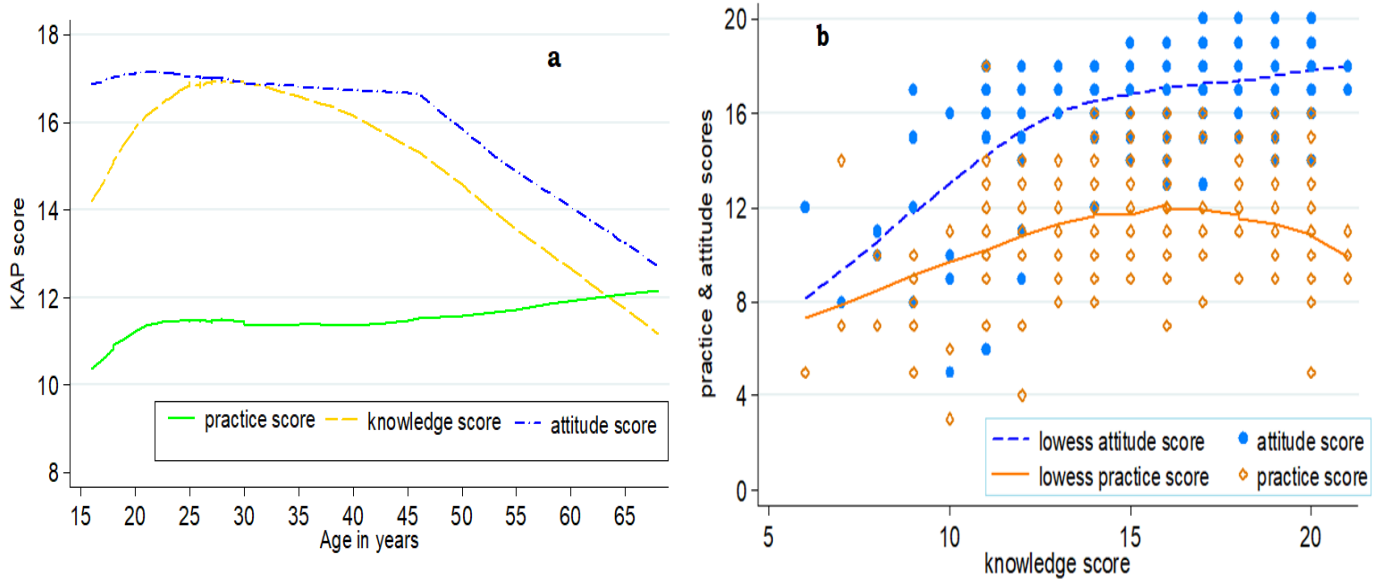


Figure 3.1. Loess curve showing the relationship between age and KAP scores (a), and knowledge score versus attitude and practice scores (b).

Table 3.6 shows the results of regression analysis exploring the association between respondents' socio-demographic characteristics and food safety knowledge, attitudes, and practices. In multivariable models, good knowledge was significantly ($p < 0.05$) associated with male gender and older age; positive attitudes were associated with lower educational attainment and good knowledge; and good practices were associated with working in an abattoir and having received training on food safety. An interaction term between training and place of work was explored in the latter model but it was found to be non-significant ($p = 0.28$).

Table 3.6. Multivariable logistic regression of factors associated with food safety knowledge, attitude and practices of meat handlers' working in an abattoir and retail meat shops of Bishoftu city, Ethiopia (n= 391)*.

Variable	Level	Good knowledge (n=283)					Positive attitude (n=370)					Good practices (n=103)				
		n (%)	Univariable OR(95% CI)	p-value	Multivariable aOR(95% I)	p-lue	n (%)	Univariable OR(95% CI)	p-alue	Multivariable aOR(95% CI)	p-alue	n (%)	Univariable OR(95% CI)	p-alue	Multivariable aOR(95% I)	p-value
Gender	Female	3(27.3)	Ref	na	Ref	na	6(54.5)	Ref	na	Ref	na	1(9.1)	Ref	na	na	na
	Male	280 (73.7)	7.5(1.9, 28.7)	0.00	9.3 (2.3, 37.4)	0.00	364 (95.8)	19.0 (5.3, 68.7)	0.00	4.5 (0.9, 21)	0.06	63 16.6)	2.0 (0.3, 15.8)	0.52	na	na
Age (years)	16-25	161(66.8)	Ref	na	Ref	na	233 (96.7)	Ref	na	na	na	33(13.7)	Ref	na	na	na
	>25	122 (81.3)	2.2(1.3, 3.5)	0.00	1.9 (1.1, 3.2)	0.03	137 (91.3)	0.4 (0.1, 0.9)	0.28	na	na	31 20.7)	1.6 (1.0, 2.8)	0.72	na	na
Education	Primary	146(71.9)	Ref	na	na	na	198 (97.5)	Ref	na	Ref	na	34 16.7)	Ref	na	na	na
	Above primary	137 (72.9)	1.0 (0.7, 1.6)	0.83	na	na	172 (91.5)	0.3 (0.1, 0.8)	0.01	0.3 (0.1, 0.9)	0.03	30 16.0)	0.9 (0.6, 1.6)	0.83	na	na
Service (years)	≤ 3	154 (67.0)	Ref	na	Ref	na	219 (95.2)	Ref	na	na	na	34(14.8)	Ref	na	na	na
	>3	129 (80.1)	2.0(1.2, 3.2)	0.00	1.7 (1.0, 2.9)	0.05	151 (93.8)	0.8 (0.3, 1.8)	0.54	na	na	30 18.6)	1.3 (0.8, 2.3)	0.31	na	na
Training	No	185 (72.3)	Ref	na	na	na	239 (93.4)	Ref	na	Ref	na	8 (3.2)	Ref	na	Ref	na
	Yes	98 (72.6)	1.0(0.6, 1.6)	0.95	na	na	131 (97.0)	2.3 (0.7, 7.0)	0.14	1.6 (0.5, 5.7)	0.43	56 (41.5)	22.0 (10.0, 48.1)	0.00	21.2 (9.5, 47.1)	0.00
Place of work	RMS	163 (73.1)	Ref	na	na	na	207(92.8)	Ref	na	Ref	na	17 (7.6)	Ref	na	Ref	na
	Abattoir	120(71.4)	0.92 (0.6, 1.4)	0.72	na	na	163 97.0)	2.5 (0.9, 7.0)	0.08	1.8 (0.6, 5.7)	0.32	47 (28.0)	4.7 (2.6, 8.6)	0.00	4.4 (2.2, 8.6)	0.00
Knowledge	Poor	na	na	na	na	na	90 (83.3)	Ref	na	Ref	na	19 (17.6)	Ref	na	na	na
	Good	na	na	na	na	na	280 (98.9)	18.7 (5.4, 64.8)	0.00	17.3 4.8, 62.2)	0.00	45 15.9)	0.9 (0.5, 1.6)	0.69	na	na
Attitude	Negative	na	na	na	na	na	na	na	na	na	na	3 (14.3)	Ref	na	na	na
	Positive	na	na	na	na	na	na	na	na	na	na	61 16.5)	1.2 (0.3, 4.1)	0.79	na	na

* p-values were obtained from regression models and are rounded to two decimal places. OR: odds ratio, CI: confidence interval, aOR: adjusted odds ratio, RMS: retail meat shop, na: not applicable.

3.4. Discussion

This study investigated the food safety knowledge, attitudes and practices of meat handlers working in abattoirs and retail meat shops in Bishoftu city, Ethiopia. In this study, meat handlers were found to have satisfactory knowledge of food safety, including of the importance of general sanitary measures such as frequent hand washing, proper cleaning and sanitization of equipment, avoidance of eating and drinking in the workplace, and cross-contamination. Likewise, most of the meat handlers had positive attitudes toward food safety and their role in reducing contamination through washing hands regularly, keeping surfaces clean and inspecting meat for freshness. However, this study showed that despite good knowledge and positive attitudes towards food safety, this did not always translate into adequate practices during meat handling.

The level of food safety knowledge reported in this study was higher than those reported amongst meat handlers in western Ethiopia (Tegegne *et al.*, 2017), but similar to those reported amongst food handlers working in hotels in northwestern Ethiopia (Admasu & Kelbessa, 2018). Despite the good food safety knowledge in this study, there were still several misunderstandings which could be improved through training. Specifically, gaps in knowledge related to the exact causes of food-borne illness were identified along with lack of understanding of the difference between cleaning/washing and sanitizing. The importance of the health status of the worker also seemed to be a knowledge gap and may again reflect a poor understanding of the causes of food-borne illness and the fact that humans are the source of some pathogens. Indeed, another study with meat handlers in Ethiopia (Tegegne *et al.*, 2017) revealed that most of the respondents did not know that hepatitis A and *Staphylococcus aureus* were the cause of foodborne diseases.

Positive attitudes towards food safety were also documented in this study with the main deficiencies related to beliefs about raw meat and towel use. Eating raw meat is a common tradition in Ethiopia and so the finding that raw meat is perceived to be healthier and more nutritious is not surprising. Use of the same towel to clean many places can contribute to cross-contamination. Most (90.3%) participants in this study believed it was possible to use the same towel to clean many surfaces. This is considerably higher than another study of meat handlers in Ethiopia (53%) (Tegegne *et al.*, 2017) and may reflect a specific gap in training or equipment available in the workplaces in this study.

Codex Alimentarius Commission recommends food handlers wash their hands, particularly before handling food, after using the toilet and after handling raw food or contaminated material. Further it recommends personal effects such as jewelry not be worn and that cuts and abrasions on hands be covered whilst working (Codex Alimentarius Commission, 2020). While the majority of meat handlers in this study did practice good hand hygiene, lapses in hand washing practices were reported around the time of donning/doffing gloves and after smoking (both rare in this setting) as well as after sneezing/coughing. Notably, only a third of meat handlers reported wearing a mask in this study. The latter findings are particularly important given this study was undertaken during the COVID-19 pandemic. Indeed, slaughterhouses around the world were implicated in outbreaks (Waltenburg *et al.*, 2020, Ijaz *et al.*, 2021). Again, this would seem to suggest that meat handlers may not appreciate the role they may play in being a source of infection to others.

Discordance between knowledge/attitudes and practices was observed across several areas in this study. For instance, while nearly all (93.4%) respondents agreed that meat handlers with injuries on their hands should not touch or handle meat; more than half stated that they did, in fact, do this. Similarly, a majority (98.2%) believed that wearing protective clothing improves food safety, yet a minority reported wearing protective clothes, aprons or gloves. These findings are not unlike other studies in Ethiopia where a sizeable proportion of meat handlers reported handling meat when they have injuries on their hands and/or not using gloves (Tegegne *et al.*, 2017, Haileselassie *et al.*, 2013, Yenealem *et al.*, 2020).

Of the several factors identified in multivariable analysis as being associated with good food safety KAP, training was the only modifiable factor. That is to say, food hygiene training which specifically targets gaps identified in this study could potentially improve food safety in slaughterhouses and retail meat shops in Ethiopia. This may be particularly important in retail meat shops where food safety practices were lower and training stated to be non-existent at least amongst respondents in this study. Personal communication with the abattoir managers and retail meat shop owners revealed that there are attempts to train employees regarding meat hygiene but it is not on regular basis and not sufficient. There was also a positive association between knowledge and attitudes of meat handlers toward food safety, which suggests that training which leads to improved knowledge, will lead to improved attitudes of meat handlers towards food safety. Similar positive association between food safety knowledge and attitudes was observed in slaughterhouse workers in Cameroon (Matchawe *et al.*, 2019).

However, it is notable that good food safety knowledge and positive attitudes were not in themselves associated with good food safety practices in this study. Similar findings have been made in other studies. For example, a study of food handlers in the UK revealed that 63% of respondents with good knowledge of food safety practices acknowledged they did not always practice the corresponding positive behavior (Clayton *et al.*, 2002). Barriers to good food safety practices in that study included time constraints and lack of staff. In Ethiopia, further reasons could be poor regulatory systems and enforcement, inhibitory attitudes of supervisors and colleagues, as well as structural factors, such as lack of facilities and availability of supplies. Indeed, informal observation during the interviews identified several factors that likely contributed to poor practices such as lack of hot water baths for hand washing and dipping of knives/equipment, and lack of cooling facilities. Similar observations were also reported in another study which used direct observation and checklist of beef handling practices in slaughterhouses and beef retail shops in the same area (Gutema *et al.*, 2021). Thus, while quality and reach of food safety training clearly needs to be improved in Ethiopia, these other factors must be simultaneously addressed to reduce the risk to consumers.

There are several limitations in this study which need to be acknowledged. In particular, the number of female respondents is too small to make this comparison meaningful; this may have inflated the odds ratio and contributed to spurious findings for gender in logistic regression models. Secondly, assessment of practices was largely done using self-report. Given participants typically had good knowledge and attitudes towards food safety it is likely that they over-reported good practices. Personal observation was made informally during the study and some discrepancies between what people said they did and what they were observed to do were noted. However, for the majority of the questions, the respondents' responses did match our observations at the meat establishments. In any case, our conclusion based on the self-reported data in this study and confirmed by direct observation in another study in the same area (Gutema *et al.*, 2021) is that food safety practices were poor.

3.5. Conclusions

This study contributes to an understanding of food safety in abattoirs and retail meat shops in Bishoftu city, Ethiopia. The study showed that there was a good level of food safety knowledge, which was reflected in a positive attitude toward food safety; however this did not adequately translate into practice. In particular, gaps related to the use of personal protective equipment and other hygienic practices necessary to reduce the risks emanating from the contamination of food during handling were documented. It is recommended that hands-on induction and continuing/refreshment training be provided

to meat handlers working in abattoirs and retail meat stores. In addition, this training needs to be accompanied by improvements to work conditions and supportive infrastructure that remove barriers to enacting good food safety practices in these environments.

Acknowledgments

The authors would like to acknowledge Dr. Zerihun Asefa and Dr. Sara Amanuel for the technical support provided. The authors also thank all the meat handlers who took part in this study.

Financial support:

This project was funded by the Global Challenges Research Fund (GCRF) One Health Regional Network for the Horn of Africa (HORN) Project, from UK Research and Innovation (UKRI) and Biotechnology and Biological Sciences Research Council (BBSRC) (project number BB/P027954/1) and Addis Ababa University Thematic Research Project (Ref. No. RF/LT-407/2021).

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4. CHAPTER IV: DETECTION AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *E. COLI* O157:H7 FROM SLAUGHTERHOUSES AND BUTCHER SHOPS IN BISHOFTU CITY, CENTRAL OROMIA, ETHIOPIA

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This chapter has been submitted to Journal of Consumer Protection and Food Safety (under revision).

ABSTRACT

Raw beef consumption is an old tradition and much favored dish by majority of Ethiopians. However, prevailing unhygienic processing and distribution practices likely contribute to beef contamination leading to foodborne infections. A cross-sectional study was carried out to investigate the occurrence of *E. coli* O157:H7 and evaluate its antimicrobial resistance profile in slaughterhouses and butcher shops in Bishoftu town, Ethiopia. A total of 352 samples (120 fecal, 92 beef cut, and 140 environmental swabs) were collected. The isolation and identification process were carried out using selective enrichment media, followed by latex agglutination test. The isolates were tested for their resistance against 13 antimicrobials using the standard disk diffusion method. Out of 352 samples, 14 (4.0%) were found to be positive for *E. coli* O157:H7 serotype; of which, 28.6% (4/14), 21.4% (3/14), and 50% (7/14) were from fecal, beef, and environmental swab samples respectively. A significant difference in the occurrences of the pathogen was observed among the sources of samples ($p < 0.05$). Results of antimicrobial susceptibility test revealed high resistance to three commonly used drugs: tetracycline (100%), erythromycin (92.8%), and ampicillin (64.3%). All of the *E. coli* O157 isolates were found to be susceptible to azithromycin, cefotaxime, and chloramphenicol. Of the 14 isolates, 12 (85.8%) of them were found to be resistant to three or more classes of antimicrobial agents. *E. coli* O157:H7 was detected in samples collected from meat and environmental samples implying the health risk of raw and undercooked meat consumption. Results also showed occurrences of multiple antimicrobial resistant *E. coli* O157:H7. Therefore, the current study warrants the need of implementing appropriate hygienic measures in the slaughterhouses and butcher shops to safeguard the public health. .

Keywords: *Beef, Contamination, E. coli* O157:H7; Drug resistance, Ethiopia

4.1. Introduction

Despite of the improved technology and hygienic practices at all stages of food production, foodborne diseases are continued to be great public health and well-being concerns of individuals and countries across world (Moawad *et al.*, 2017). Especially, developing countries are largely vulnerable to foodborne infections and costs billions of dollars in medical care and social costs (Havelaar *et al.*, 2015). Studies indicated that, each year, 1 out of 10 people get ill from microbial food contamination, resulting in 600 million illnesses, 420 000 deaths and the loss of 33 million healthy years of life globally (Pires *et al.*, 2021). It often follows the consumption of contaminated foodstuffs especially from animal products

such as meat from infected animals or carcasses contaminated with pathogenic bacteria including *Escherichia coli*.

E. coli O157: H7, an entero-hemorrhagic *E. coli* (EHEC), is one of the most common causes of foodborne infections in humans. It infects all age groups and the pathogen is noted for its severe consequences following infection, low infective dose and acid resistance (Ferens & Hovde, 2011). Depending on the immune status and the general health of the infected individual, and the dose and virulence of the bacteria, infection with *E. coli* O157: H7 can result in mild diarrhea, severe bloody diarrhea, hemorrhagic colitis, or hemolytic uremic syndrome (HUS) leading to kidney failure (Smith *et al.*, 2014). Globally, EHEC O157:H7 causes 2, 801, 000 acute illnesses annually, with an incidence rate of 43.1 cases per 100,000 persons per year. Among those, a total of 10,200 cases of STEC infections occur in Africa with an incidence rate of 1.4 cases per 100,000 people per year (Majowicz *et al.*, 2014).

Cattle are the primary reservoirs of *E. coli* O157:H7 and consumption of beef and beef products are identified as major sources of foodborne transmission (Money *et al.*, 2010). Carcass contamination occurs through skin-to-carcass or fecal-to-carcass transfer of the pathogen during slaughter process at processing plants (Abdissa *et al.*, 2017). Furthermore, microbial cross-contamination can occur during processing and manipulation, such as dehidung, evisceration, storage and distribution at slaughter houses and butcher shops (Zweifel *et al.*, 2014).

To increase the production output, the animal production sector in developing countries have been regularly using antimicrobials for therapy, diseases prevention and growth purpose (Boeckel *et al.*, 2015). Antimicrobials are widely used in cattle for disease prevention and as growth promotion (Herago & Agonafir, 2017). In Ethiopia, different studies have shown that multidrug resistance among *E. coli* O157:H7 of animal food origin (Shecho *et al.*, 2017). *E. coli* O157:H7 can be transmitted to humans through contaminated food and water, directly between persons, and through contact with animals or their environment. The use of antimicrobials in food cattle leads to the development of resistance pathogenic *E. coli* O157:H7 that can reach humans through the beef food chain (Ma *et al.*, 2021).

E. coli O157:H7 detection can be done by various methods that include culture based, immunological based, nucleic acid based and biosensors (Valderrama *et al.*, 2016). The value of a latex agglutination test for rapid presumptive detection of *E. coli* serotype O157:H7 was determined by laboratory trials and

during an outbreak of hemorrhagic colitis. The latex test was found to be a simple, highly efficient and reliable test in detecting *E. coli* O157:H7 with 100% sensitivity and specificity.

In Ethiopia, animals are commonly slaughtered and dressed under unhygienic conditions in the open air or sub-standard slaughterhouses which compromises the microbiological quality and safety of the meat obtained from the animals (Atnafie *et al.*, 2017). Lack of surveillance of food-borne pathogens, lack of education and training among slaughterhouse and butcher shop workers, and poor hygienic practice of food handlers are major factor contributing to the high risk of exposure of Ethiopians to food-borne pathogens such as EHEC (Assefa, 2019). Furthermore, raw meat is widely consumed in the country and if hygiene and adequate temperature control is not maintained, these may pose a potential risk for the occurrence of foodborne disease because of a widespread tradition of raw meat consumption in the country. There is a need to investigate the possible sources of *E. coli* O157:H7 in the beef supply chain, quantify risk factors, and evaluate the hygienic performance of slaughterhouses and butcher shops to ensure that prevention and control strategies are appropriate. However, in Ethiopia, it has not yet been well handled to evaluate the standards of slaughterhouses and their environment which could serve as sources of *E. coli* O157: H7. Therefore, the objective of this study was to investigate the occurrences and antimicrobial resistance profiles of *E. coli* O157:H7 from slaughterhouses and butcher shops.

4.2. Materials and methods

4.2.1. Study area

The study was conducted in Bishoftu city, located at 9°N latitude and 40°E longitudes at an altitude of 1850 m above sea level in central high lands of Ethiopia. According to the CSA, (2021), the total population of the town is 197,557. Bishoftu is being a resourt city that attracts local and international visitors and guests; as a result the booming of hotels and reaturants, and business is believed to increasing number of butcher shops. In Bishoftu town, there are three export abattoirs, two medium scale slaughterhouses (one municipal and one private) and 131 officially registered butcher shops.

4.2.2. Study design and sampling methods

A cross-sectional study type was carried out from November 2021 to May 2022 at two slaughterhouses (Bishoftu municipal and privately owned), and 92 butcher shops. Sampling was carried out once a week, where 10 cattle were selected by using a simple random sampling method. Fecal contents of the animals, carcass swabs, and swab samples from the environment (knives, hook, hand swabs, water, and wastewater) were collected. Fecal samples were collected from each selected animal directly from the rectum using rectal gloves in the lairage. Carcass swab samples were collected after skinning and evisceration. A simple random sampling technique was employed to select 92 butcher shops out of 131, from which beef cuts (at least 25 g) and parallel pooled swab samples of butcher's knife, hand, and cutting board were collected.

4.2.3. Sample size determination and sample collection and transportation

The sample size was determined by the formula (Thrusfield, 2005); and the prevalence of *E. coli* O157:H7 in cattle and carcass samples was 7.1% and 6.3%, respectively (Gutema *et al.*, 2021). Therefore, using the 7.1% and 6.3% expected prevalence, 95% confidence interval, and 5% marginal error, the number of cattle and butcher shops was estimated to be 102 and 91, respectively. However, the sample size was increased to to compensate for any sample losses as well as to minimize margin of error. Hence, a total of 120 fecal samples were collected using a sterile arm-length glove from the abattoirs in sterile and leak-proof universal tubes. Rectal fecal sample collections were made. Moreover, a total of 92 beef samples from 92 butcher shops were collected (International Organization for Standardization, 2001). At least 25g of beef was taken from the exterior of the carcass (fat tissue) and the surface of lean beef using sterile scalpels and forceps and put into sterile, separately labeled plastic bags. Scalpel and forceps were cleaned with pieces of gauze dipped in 70% ethanol after each sampling to minimize cross-contaminations.

Pooled carcass swabs (n =12) were collected from four different sites of the carcass (thorax, brisket, flank, and crutch by using a method described by McEvoy *et al.*, (2003), one site covering 100 cm² by placing a sterile template (10 x 10 cm) on a carcass. For each sampling area, a sterile cotton-tipped swab (2 X 3 cm) fitted with a shaft was moistened in approximately 10 ml of buffered peptone water (Oxoid, Hampshire, England), and was rubbed first horizontally and then vertically several times across the carcass surface. On the completion of the rubbing process, the shaft was broken by pressing it against the

inner wall of the test tube and disposed of leaving the cotton swab in the test tube. The four swabs were put into one screw-capped test tube containing 10 ml of sterile buffered peptone water and transported to the Microbiology laboratory of the College of Veterinary Medicine and Agriculture of Addis Ababa University.

A total of 128 swab samples (36 from the two slaughterhouses and 92 from butcher shops) were collected. Swab samples collected from the slaughterhouses were the knife, rasp, axe, and hook (n= 12) and hand swabs (n= 12), and tap water (n=12). Similarly, butcher's hand swabs, knives, and chopping boards were collected during the operation at the butcher shops. Environmental swab samples were taken from meat contact surfaces of 15-20 cm² using a sterile cotton swab moistened in buffered peptone water. Swab samples were immersed in a test tube containing sterile buffer peptone water. In addition, slaughterhouse wastewater samples (50 ml) in the drainage line were collected at every single visit to the slaughterhouses. All samples were labeled legibly with a permanent marker identifying the type/source of the sample, the date of sampling, and the code of the slaughterhouses/butcher shop. The samples were transported in an ice box containing ice packs to the Microbiology laboratory of the College of Veterinary Medicine and Agriculture of Addis Ababa University.

4.2.4. Isolation of *E. coli* O157:H7

Twenty-five grams of each fecal and beef cut sample was aseptically transferred into a stomacher bag containing 225mL of modified tryptone soya broth (Oxoid, Basingstoke, United Kingdom) supplemented with 20mg/L Novobiocin (NTSB; Sigma Aldrich, MO), homogenized using a stomacher blender (Stomacher 400, Seward Medical, England) for 1 min at normal speed (200 rpm); and incubated at 41.5 °C for 6 hours. Similarly, all swab samples from the slaughterhouses and butcher shops were homogenized in 9 mL of mTSBn and incubated at 37°C for 24 hrs.

The enriched samples were streaked onto MacConkey agar (Oxoid, England) plates for primary isolation and incubated at 37°C for 24 hrs. Following incubation, the plates were observed for the growth of pink colonies (lactose fermenter). A single, isolated colony was then picked and sub-cultured on Eosin Methylene Blue (EMB) agar (Oxoid, England) for 24hrs at 37°C for the formation of metallic sheen. Suspected colonies of *E. coli* (pinkish color appearance on MacConkey agar and metallic sheen on EMB) were then sub-cultured onto nutrient agar (Oxoid, England) at 37°C for 24 hrs. From nutrient agar,

relevant biochemical tests that included indole, methyl red, Voges-Proskauer reaction, citrate utilization (IMViC) tests, and H₂S production tests were performed. A citrate utilization test was also done using Simon's citrate agar (Difco, Detroit, USA). The test reagents used were Kovac's reagent for the indole test, methyl red for the methyl red test, and alpha-naphthol and 40% KOH chemicals for Voges-Proskauer reaction tests. The H₂S production and citrate utilization test results were observed and interpreted (Quinn *et al.*, 2002).

The bacterium that was confirmed as *E. coli* was sub-cultured onto Sorbitol MacConkey agar (SMAC) (Oxoid, England) supplemented with 0.05 mg/l Cefixime- 2.5 mg/l potassium tellurite, and plates were incubated at 35 °C for 20 to 22 hrs. *E. coli* O157:H7 does not ferment sorbitol and, thus, produces slightly transparent colorless colonies. Then, up to six colorless colonies (non- Sorbitol fermenters) on SMAC agar were picked and sub-cultured onto nutrient agar slants and incubated at 37°C for 24 hrs for a further confirmatory test.

4.2.5. Identification of *E. coli* O157:H7

Identification and confirmation of non-sorbitol fermenting *E. coli* O157:H7 were done by latex agglutination test using a latex kit (Oxoid, DR0620). The latex kit consists of four components: latex test reagent, latex control reagent, positive controls, and negative controls. The test reagent contains blue latex particles sensitized with a specific antibody against the *E. coli* O157:H7 antigen and the control reagent consist of latex particles sensitized with rabbit globulin. The positive controls are suspensions of inactivated *E. coli* O157:H7 cells, whereas the negative controls are suspensions of inactivated non-specific *E. coli* cells. The test was performed according to the manufacturer's instructions (Oxoid, Hampshire, England). The latex kit was first checked for its performance by using the control suspensions in the kit, the test was continued after the positive control reacts with the test latex showing a positive result. Briefly, one drop of 0.85% saline water and latex test were dispensed into the reaction card separately. Using a sterile wire loop, a few presumptive colonies of *E. coli* O157 were taken and emulsified into the saline water on the latex card, then slowly mixed with the test latex and checked for agglutination within 1 minute. A result was positive if agglutination of the latex particles occurred within 1 minute. The negative result was obtained if no agglutination occurred and a smooth blue suspension remained after 60 seconds in the test area. Test positive isolates were stored in glycerol using cryovials for further antimicrobial resistance determination.

4.2.6. Antimicrobial susceptibility testing

The antimicrobial resistance test was carried out by using the standard agar disc diffusion (Oxoid Ltd., Hampshire, UK) for 13 antimicrobial agents which are in regular use for ruminants, potential public health importance, and recommendations from the guideline of antimicrobial susceptibility test in; ampicillin (AMP, 10 µg), azithromycin (AZM, 15µg), cefotaxime (CTX, 30 µg), ceftazidime (CTZ, 30µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 5 µg), colistin sulfate (CT, 10 µg), erythromycin (ERY, 15 µg), gentamicin (GEN,10 µg), Kanamycin (KAN, 30 µg), nalidixic acid (NA, 30 µg), sulfamethoxazole (SXT, 25 µg), and tetracycline (TET, 30 µg).

Pure colonies of *Escherichia coli* O157:H7 isolates were transferred into a test tube of 5 ml tryptone soya broth (Oxoid, England) and incubated at 37°C for 6 hours. The turbidity of suspension broth was adjusted using sterile saline solution, or more colonies were added to obtain turbidity that is usually comparable with that of 0.5 McFarland standards. A sterile cotton swab was immersed into the suspension and rotated against the side of the tube to remove the excess fluid and then swabbed uniformly on the surface of already prepared Mueller-Hinton agar (Oxoid,) plates. As soon as the plates dried, antimicrobial discs were placed on the inoculated plates using sterile forceps and incubated at 37°C for 24 hrs. The results were interpreted as resistance, intermediate, or susceptible after the zone of inhibition of the strain was appreciated (CLSI, 2014).

4.2.7. Data management and analysis

The collected data were entered into Microsoft Excel and checked before analysis. The data were analyzed using R version 4.1.2. Descriptive statistics were used to summarize the results. The significance of the association between *E. coli* O157 isolates and sample source, and type of sample was assessed using the univariate logistic regression. Odds ratio and 95% confidence intervals were used to measure the strength of associations. A p-value of less than 0.05 was considered significant.

4.3. Results

4.3.1. Prevalence of *E. coli* O157:H7

Table 4.1 shows summary of *E. coli* O157: H7 detections by sample source and type. Accordingly, *E. coli* were detected in 69 out 352 (19.6%, 95% CI: 15.6 – 24.1), of which *E. coli* O157: H7 strains were detected in 14 (4.0 %, 95 % CI: 2.2 – 6.6) samples. *E. coli* O157: H7 was more prevalent in slaughterhouse samples (5.95%, 95% CI: 2.9 – 10.7) than butcher shop (2.2%, 95% CI: 0.6 – 5.5). Accordingly, *E. coli* O157: H7 was prevalent in 12.5 % of the slaughterhouse samples namely carcasses, carcass contact surfaces, and wastewater samples. *E. coli* O157: H7 was also detected in 3.33% of fecal samples. Similarly, 3 (3.3%) beef cut samples and 1(1.1%) carcass contact surfaces sample was positive for *E. coli* O157:H7 from butcher shops. Higher occurrence of *E. coli* O157: H7 was observed in the municipal slaughterhouse (8.3 %, 95% CI: 3.4 – 16.4) compared to the private slaughterhouse (3.6%, 95% CI: 0.7 – 10.1).

Table 4.1. Occurrence of *E. coli* O157: H7 by sample sources and type

Sample source	Sample type (p* =pooled sample)	No. of samples tested	No.(%)
Municipal slaughterhouse	Fecal	60	3 (5)
	Carcass swab (p*)	6	2 (33.3)
	Knife swabs (p*)	6	0
	Hand swabs (p*)	6	1 (16.7)
	Water/wastewater	6	1 (16.7)
	Sub-total	84	7 (8.3)
Private slaughterhouse	Fecal	60	1 (1.7)
	Carcass swab (p*)	6	1 (16.7)
	Knife swabs (p*)	6	1 (16.7)
	Hand swabs (p*)	6	0
	Water/wastewater	6	0
	Sub-total of slaughterhouse	168	10 (6.0)
Butcher shops	Beef /meat	92	3 (3.3)
	Butcher swab sample (p*)	92	1 (1.1)
	Sub-total	184	4 (2.2)
Total		352	14 (4.0)

A univariable logistic regression test was used to assess the associations of *E. coli* O157: H7 occurrence with sample source and type. Hence, samples from municipal slaughterhouse had a significantly higher prevalence of *E. coli* O157: H7 compared to butcher shops. The likelihood of *E. coli* O157:H7 detections from municipal and private slaughterhouses were 4.1 and 1.67 times more than butcher shops. However, no significant association was observed between *E. coli* O157:H7 detection and sample types (Table 4.2). Although there was no significant association with sample type, higher odds of *E. coli* O157:H7 detection was observed in water (OR=2.6) and swab (OR=1.4) samples than in fecal samples.

Table 4.2. Association of *E. coli* O157:H7 with sample source and sample type

Categories		No. of positive	Odd Ratio [95% CI]	P =value
Sample source	Butcher house	4	Ref	Ref
	Municipal abattoir	7	4.09 [1.20-16.0]	0.028
	Private abattoir	3	1.67 [0.32-7.73]	0.510
Sample type	Fecal	4	Ref	Ref
	Beef /meat	3	0.98 [0.19-4.54]	0.977
	Swab samples	6	1.43 [0.40-5.70]	0.590
	Water/wastewater	1	2.64 [0.13-19.89]	0.404

The observational survey revealed that besides the lack of training on food safety, the shortage of facilities hindered workers to maintain the minimum acceptable hygienic practices. None of the slaughterhouse and butcher shop workers had proper hand washing using soap and disinfection, and washing and disinfection of their operational tools and floor after each working interval. There were no specifically separate equipments such as cutting boards, knives, or rasps used for both cutting meat and abdominal organs. Moreover, the substandard slaughtering practices were made without necessary precautions in place to avoid cross contaminations.

4.3.2. Antimicrobial resistance profile

Antimicrobial susceptibility testing was performed for all confirmed positive isolates against 13 different antimicrobial agents. Accordingly, *E. coli* O157: H7 was found to be resistant to tetracycline (100%), erythromycin (89.9%), and ampicillin (64.3%). Furthermore, the resistance of 14.3% was observed to

ceftazidime, colistin sulfate, kanamycin, nalidixic acid, and sulfamethoxazole. However, none of the isolates was resistant to azithromycin, cefotaxime, and chloramphenicol (Table 4.3 and Figure 4.1).

Table 4.3. Antimicrobial resistance profile of *E. coli* O157: H7 isolates

Antimicrobial disc	Diameter of zone of inhibition (mm)				Resistance profile (n=14)		
	Con.(µg)	R (≤)	I	S (≥)	S No. (%)	I No. (%)	R No.(%)
Ampicillin (AMP)	10	13	14-16	17	3 (21.4)	2 (14.3)	9 (64.3)
Azithromycin (AZM)	15	12		13	14 (100)	0	0
Cefotaxime (CTX)	30	22	23-25	26	14 (100)	0	0
Ceftazidime (CTZ)	30	14	15-17	18	11 (78.6)	1 (7.1)	2 (14.3)
Chloramphenicol (C)	30	12	13-17	18	14 (100)	0	0
Ciprofloxacin (CIP)	5	15	16-20	21	12 (85.8)	1 (7.1)	1 (7.1)
Colistin sulphate (CT)	10	16	17-21	21	12 (85.8)	0	2 (14.3)
Erythromycin (ERY)	15	13	14-22	23	0	1 (7.1)	13 (89.9)
Gentamicin (GEN)	10	12	13-14	15	12 (85.8)	1 (7.1)	1 (7.1)
Kanamycin (KAN)	30	13	14-17	18	11 (78.6)	1 (7.1)	2 (14.3)
Nalidixic acid (NA)	30	13	14-18	19	12 (85.7)	0	2 (14.3)
Sulfamethoxazole (SXT)	25	10	11-15	16	12 (85.7)	0	2 (14.3)
Tetracycline (TTC)	30	11	12-14	15	0	0	14 (100)

*S=Susceptible, I=Intermediate, R=Resistant

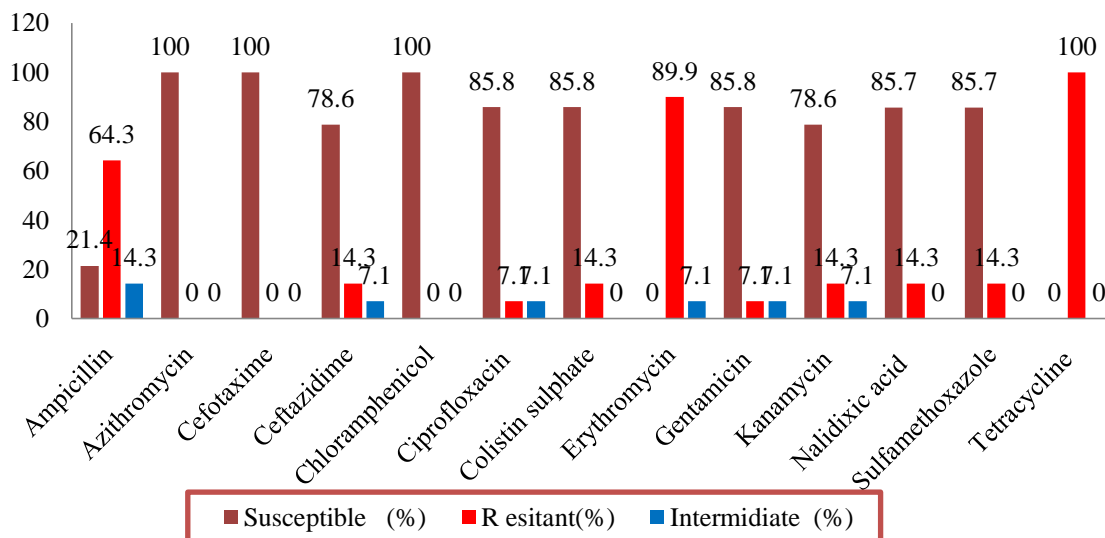


Figure 4.1. Antimicrobial susceptibility profile of *E. coli* O157: H7 isolates

4.3.3. Multi-drug resistance profiles

This study showed that 12 (85.8%) *E. coli* O157: H7 isolates were resistant to three or more classes of antimicrobial agents. Multi-drug resistance (MDR) profiles of the isolates 5/14 (35.7%), 5/14 (35.7%), and 1 (7.1%), against three, four, and five antimicrobials were registered respectively. Most of the multi-drug resistant isolates were originated from swab and fecal samples. A multi-drug resistance profile consisting of six drugs was also documented in 1/14 (7.1%) of the fecal isolate (Table 4.4).

Table 4.4. Multi-drug resistance (MDR) profile of *E. coli* O157:H7

Source of Isolates	Resistance profile	Sources of MDR				Total MDR
		Beef n=3	Fecal n=4	Swab n=6	Water n=1	
Municipal slaughter house	CT, ERY, TTC		1			1
	AMP, ERY, TTC			1	1	2
	AMP, ERY, NA, TTC		1			1
	AMP, ERY, GEN, TTC			1		1
	AMP, CTX, CTZ, ERYNA, TTC		1			1
Private slaughter house	ERY, KAN, TTC			1		1
	AMP, ERY, TTC			1		1
	AMP, CIP, ERY, TTC		1			1
Butcher house	ERY, KAN, TTC	1				1
	AMP, ERY, SXT, TTC			1		1
	AMP, CTZ, ERY, SXT, TTC	1				1
Overall MDR (%)		2 (14.3)	4(28.6)	5 (35.7)	1 (7.1)	12 (85.7)

Key: Ampicillin (AMP), Azithromycin (AZM), Cefotaxime (CTX), Ceftazidime (CTZ), Chloramphenicol (C), Ciprofloxacin (CIP), Colistin sulphate (CT), Erythromycin (ERY), Gentamicin (GEN), Kanamycin (KAN), Nalidixic acid (NA), Sulfamethoxazole (SXT) and Tetracycline (TTC), R3-R6, resistance to three, four, five, and six classes of antibiotics

4.4. Discussion

The overall prevalence of *E. coli* O157:H7 was 3.6% which is in agreement with the national prevalence estimate (4%) in Ethiopia (Assefa, 2019). This finding was slightly higher than 2.4% (Atnafie *et al.*, 2017), and lower than 5.4% (Sebsibe & Asfaw, 2020). Regarding to sample source, the prevalence of *E. coli* O157:H7 at the slaughterhouse level was 5.95%; which is in line with the findings (Bekele *et al.*, 2014) 5.7%. A lower prevalence than the present finding was reported from Ethiopia (Atnafie *et al.*, 2017), United Kingdom (De Boer & Heuvelink, 2000) and Ireland (Carney *et al.*, 2006) which reported 2.7%, 3.2% and 3.0%, respectively. The variation in the prevalence could be attributed to the fact that there is a difference in slaughterhouse standards, hygienic practice of workers, sampling and isolation methodology, season, geographical origins and number of cattle (Varela-Hernández *et al.*, 2007).

In previous study by Bekele et al. (2014) the occurrence of *E. coli* O157:H7 in butcher shops was observed to be higher, which was 13.3% (17/128) collected from butcher shops in central Ethiopia. Likewise, the prevalence was recorded in Ambo (Tadese *et al.*, 2021) (19.1%) and Bishoftu Gutema *et al.*, (2021) (6.3%) butcher shops. The variation in the prevalence of *E. coli* O157:H7 in butcher shops could be due to the status of hygiene and sanitation practices of the butcher shops, sample size and sampling technique. For example, positive isolates were recovered from knife, (1/30), cutting board (3/30), and protective clothes (1/30) (Sebsibe & Asfaw, 2020). However, Pooled sampling method, which was used in this study, recovers only one (1/92); showing sampling methods can be big factor.

The occurrence of *E. coli* O157:H7 was significantly higher at the municipal slaughterhouse (8.3%) than at the private slaughterhouse (3.5%). The prevalence of *E. coli* O157 in the beef carcass was 3.3%, which is comparable to a report from Haramaya University slaughterhouse (2.65%, 3/113) (Taye *et al.*, 2013). The occurrence of *E. coli* O157 was significantly higher at the municipal slaughterhouse (8.3%) than at the private slaughterhouse (3.5%). The slaughterhouse facility, origin of the cattle, worker's hygiene, and transportation of cattle from origin to the slaughterhouse might have contributed to the differences in the occurrence of the pathogen. The slaughterhouse facility, origin of the cattle, worker's hygiene, and transportation of cattle from origin to the slaughterhouse might have contributed to this difference. A survey in Ethiopia discussed that public slaughterhouses have poor management and facilities than private abattoirs (Yusuf & Cottingham, (2015).

Regarding to sample type, carcass and carcass contact surface swabs (12.5 %,) had higher proportion of *E. coli* O157:H7 followed by fecal, content (3.3 %) and beef samples (3.3 %). The occurrence of *E. coli* O157:H7 in fecal, swab and beef samples were also reported in different studies (Gutema *et al.*, 2021; Sebsibe & Asfaw, 2020; Atnafie *et al.*, 2017). In this even though the prevalence of *E. coli* O157: H7 was low, it was observed that there was no declining profile of prevalence from fecal (3.3%), carcass and carcass in contact surface with wastewater (12.5%), and beef sample (3.3%), demonstrating that cross-contaminations of carcass occur during slaughtering process which in overall reflect the general unhygienic conditions in employees, utensils and environmental sanitation of the slaughter house under study. It also indicates that the sanitary and hygienic measures at the slaughterhouses were ineffective against *E. coli* O157:H7.

Similarly to what is reported by Beyi *et al.*, (2017), which detected (3.6%, 4/110) *E. coli* O157:H7 on the surface of wooden cutting boards, this survey also isolated (1.1%, 1/92) *E. coli* O157:H7 from a pooled

swab sample of butcher's hand, knife and cutting board. However, in this study *E. coli* O157: H7 was not detected at the same time from the beef cut and beef contact surface samples swabbed from the same butcher shop.

In the present study, *E. coli* O157:H7 was isolated from slaughterhouse wastewater (1/12, 8.3%), which is alarming. This finding was lower than a previous study conducted in other countries. Barel et al. (2022) reported a relatively higher 11% prevalence of from slaughterhouses in Kayseri, Turkey. Similar higher prevalence 16%, in Nigeria (Oluwawemimo *et al.*, 2016) and 20.8% in Turkey (Ayaz *et al.*, 2014) was also reported. This finding notes the importance of ensuring establishment of water treatment facilities to slaughterhouse wastewater efflux.

In general, the result of the present study was lower compared to previous studies conducted in Ethiopia and other countries. The recto-anal junction (RAJ) of cattle is the principal site of colonization for *E. coli* O157: H7 (Cobbold *et al.*, 200) and it was argued that *E. coli* O157: H7 detected in higher proportion from the intestinal mucosa proximal to RAJ than in the feces (Greenquist *et al.*, 2005). Therefore, in the present study, the low prevalence of *E. coli* O157: H7 in the fecal sample observed may be associated with low shedding profile of the pathogen by beef cattle included in this study. The present study was also supported by other studies for the absence of the association between occurrences of *E. coli* O157: H7 and sample type (Abdissa *et al.*, 2017 ; Bekele *et al.*, 2014). In contrast, other researchers have reported that sample type and *E. coli* O157: H7 have a significant association.^{24,42} This study has also considered observational survey so as to assess the hygiene and sanitary practices of slaughterhouses and butcher shops and the overall slaughtering operations. Hygienic practices during beef production, processing and distribution is essential to formulate a preventive measures to mitigate the contribution of meat to foodborne diseases (Sebsibe & Asfaw, 2020; Abreham *et al.*, 2019).

Unfortunately, this study was not able to collect samples from the truck, lairage and hide. However, upon visual observation, the lairage was dirty and there were no compartments which increase fecal contamination of hide due to close contact of animals. Previous works has revealed that the major source of beef carcass contamination was skin/hide of cattle entering to the processing facilities (Fegan *et al.*, 2009). In a study done by Mersha *et al.* (2010), a significant association was found between carcass swabs and skin swabs. Arthur *et al.* (2007) support these findings by demonstrating that antimicrobial

interventions targeting cattle hides lead to drastic reductions in the rates of carcass contamination with *E. coli* O157:H7.

Stressful stunning and dragging of cattle on the floor, lack of clear demarcation between dirty and clean area (municipal slaughterhouse), careless fisting and evisceration, sharing of knife, axe, and rasps, uncovered drainage line, lack of sequential decontamination at various stages, infrequent postmortem examination (municipal slaughterhouse), lack of hot water baths for hand washing and dipping of knives, dirty protective cloths (except in few butcher shops), infrequent hand washing were bad practices observed at the slaughterhouses and butcher shops. At slaughterhouses, workers transported the carcass from the hook bar to the vehicle on their shoulders. They used plastic gown which covered their back. However, the hygienic status of these protective clothes was not up to the standard required for abattoir workers. Personnel working at the slaughterhouses did not wear clean aprons, boots, and hair caps during meat processing. This might be the reason for the occurrence of *E. coli* O157:H7 in the beef sold at butcher shops. Hence, slaughterhouse workers should wear clean protective cloths, and also ensure their hands are always clean so as to produce high quality beef. Microbial contamination of carcasses is most likely occurred during evisceration (Wambui *et al.*, 2018). However, in the slaughterhouses under study, evisceration was conducted without considering the spillage of fecal material into the carcass from the gut.

With regard to hygiene and sanitation, it is a documented fact that lack of education and training on food safety can contribute to unhygienic handling, processing and display of meat at the slaughtering places and at butcher shops (Jeffer *et al.*, 2021; Tegegne & Phyto, 2017). The slaughterhouses and butcher shops were not equipped with the necessary equipment which might enable them to maintain the general hygienic practice. For instance, there were no hot water for hand washing and knife dipping, clean towels, foot bath, separate rooms to process rumen and intestine (municipal slaughter house). Above all, inadequate supply of tap water was one of the greatest challenges to maintain hygiene.

Thus, the relative higher prevalence of *E. coli* O157:H7 on carcass swab and beef in the current study might be due to the contamination of carcass with fecal material during the slaughter operation or from different contaminated materials and hands of meat handlers. Hence, because of an increasing trend of raw meat consumption throughout the country, this is an alarming situation. Studies clearly documented that, raw beef can harbor shiga toxin producing *E. coli* (STEC) that causes diarrhea, hemorrhagic colitis,

hemolytic uremic syndrome (HUS) in human (Sethulekshmi *et al.*, 2016). Antimicrobial resistance has come recently a challenge for 'One Health' due to the rapid emergence and spread of resistant bacteria among animals, humans, and the environment (McEwen & Collignon, 2018). Antimicrobial resistance may developed spontaneously either by selective pressure or due to misuse by humans or overuse in feeding or treatment of beef cattle by owners ((Schroeder *et al.*, 2002). Antimicrobial resistance development may also associated with exchange of resistance factors between related bacteria (Tenover, 2006).

The present study indicated that all *E. coli* O157:H7 isolates were susceptible to three antimicrobials namely azithromycin, cefotaxime, and chloramphenicol which was in agreement with a local Ethiopian study isolates from goat (Dulo *et al.*, 2015). Likewise, ceftazidime, ciprofloxacin, gentamycin, kanamycin, nalidixic acid, and sulfamethoxazole showed greater activities against the isolates. In contrast, all *E. coli* O157:H7 isolates were resistant to tetracycline which again comes in parallel with the previous result (Chinwe *et al.*, 2017). This is not surprising since tetracycline is often used as a first-choice antimicrobial for disease prevention and treatment in food animals and its widespread use has likely contributed to high rates of resistance (Mayrhofer *et al.*, 2007). Closely related tetracycline resistance to this finding was also reported by Disassa *et al.* (2017) and Ababu and Fesseha (2020), from raw milk, in Ethiopia. Higher resistance to erythromycin (92.8%) and Ampicillin (64.3%) was also observed among *E. coli* O157:H7 isolates. The current finding for tetracycline resistance disagree with other study on *E. coli* O157:H7 isolated from feces and carcass of cattle slaughtered in the abattoir (Osaili *et al.*, 2013).

Multiple antimicrobial resistance is becoming a common phenomenon among *E. coli* O157:H7 isolates (Ahmed & Shimamoto, 2015). It may arise from the spread of genetic materials such as plasmids, integrons, and transposons drawn from different sources (Zhao *et al.*, 2001). In this study, multidrug resistance to three or more classes of antimicrobial classes was detected in 12 (85.7%) of the isolates. The most frequently observed resistance profile in the isolates was resistance to tetracycline in combination with erythromycin, and ampicillin. Among the *E. coli* O157:H7 isolates, 42.8%, 28.6%, 7.1%, and 7.1% developed resistance to three, four, five and six antimicrobials classes respectively.

Similar finding of multiple antimicrobial resistance on STEC strains has been documented from Ethiopia (Haile *et al.*, 2022; Shecho *et al.*, 2017), and other part of the world (Amézquita-López *et al.*, 2016 ;

Maal-bared *et al.*, 2013 ; Govaris *et al.*, 2011). Statistically significant association was observed between the sample source (municipal slaughterhouse), and multiple antimicrobial resistance. Accordingly, isolates from municipal slaughterhouse were 5.84 time more likely to get multiple antimicrobial resistance than isolates from butcher shops. However, no statistical significance was established between sample type and multiple antimicrobial resistance which was in contrast with a previous report (Sebsibe & Asfaw, 2020). Based on sample type, from fecal 28.6% (4/14), carcass and carcass contact swabs 35.7% (5/14), beef 14.3% (2/14), and from water and wastewater 7.1% (1/14) multiple antimicrobial resistance profile was observed. The higher multiple antimicrobial resistance seen in this study might be due to presence of only few isolates tested for susceptibility compared to overall study sample, difference in sample source, variability of resistant gene within isolates, and type of antimicrobials used in the study. This result, multi drug resistant wastewater isolates particularly, suggests slaughterhouse efflux would become source of resistance pathogen to the environment.

4.5. Conclusion

E. coli O157: H7 was detected from the feces, carcass swab, and environmental samples with a slightly higher occurrence in carcass swab, possibly suggesting the role of hide and gastro-intestinal content as key sources of microbial contamination of the beef. The organism was also isolated from the butcher's hand, knife, and cutting board (pooled sample) indicating high potential of cross-contamination of carcass during the slaughter operations, carcass transportation, loading and unloading. Thus, control measures to reduce carcass contaminations were not absolute even if the prevalence is lower in this study. Isolation of *E. coli* O157:H7 from raw beef, which is resistant to multiple clinically important antimicrobials, highlights the potential threat to public health. This study has also attempted to assess the hygienic and sanitary practices in the slaughterhouses and butcher shops, and their respective workers. The results reflect that there were poor personal and general hygiene measures in place and that the workers did not focus on hygienic practices. A significant higher occurrence of *E. coli* O157:H7 in municipal than private slaughterhouse indicates less effective interventions measures of carcass contaminations in the former than the latter. The occurrence of multidrug-resistant *E. coli* O157:H7 isolate in slaughterhouse wastewater is a serious matter of concern as resistance genes are easily transferable into the environment as well as food chain and human populations. Therefore, the consumption of raw beef may be an important source of *E. coli* O157 infections in the country and standard hygienic practice should be implemented in the meat establishments.

Declarations

Conflict of interest: None.

Ethical consideration: Ethical clearance was obtained from the Animal Research Ethical and Review committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University (Ref. No: VM/ERC/14/02/14/2022). Moreover, verbal consent was obtained from the abattoir and retail meat shop owners before the commencement of sample collection. All authors approved to participate in this research work and in the manuscript.

Consent for publication: All authors approved this manuscript to be published.

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5. CHAPTER V. KNOWLEDGE, ATTITUDE, AND PRACTICES (KAP) OF UNIVERSITY STUDENTS TOWARDS ANTIMICROBIAL USAGE (AMU) AND ANTIMICROBIAL RESISTANCE (AMR) IN ETHIOPIA

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This chapter has been accepted by Ethiopian Veterinary Journal, Vol. 27(2): (2023)

ABSTRACT

A cross-sectional questionnaire-based study on 1252 University students from VM (490), NHS (349) and HS (413) were conducted in Ethiopia to assess their knowledge, attitude, and practices (KAP) towards antimicrobial resistance (AMR) and antimicrobial usage (AMU). A verbal consent was obtained from randomly selected students to participate in this study. The Kruskal-Wallis and chi-square tests were used to examine how the median scores in each of the knowledge, attitude, and practice categories varied across study participants. A p-value of less than 0.05 was considered significant. The overall median AMR and AMU knowledge score was 13 (IQR = 11, 14). The median AMR and AMU attitudes score was 15 (IQR: 13, 15). The median AMR and AMU practice score was 10 (IQR: 10, 11). Logistic regression analysis revealed that the students' birthplace was found to be significant factor ($p < 0.01$) regarding the knowledge of students. The analysis further revealed that students' birthplace, field of studies, and good knowledge were significant factors ($p < 0.01$) affecting their attitudes. Students with good knowledge had 3.9 times more positive attitudes than those with poor knowledge (OR = 3.9, CI = 3.0 -5.2, $p < 0.01$). Students from VM had 1.6 times better attitude than students from HS and NHS (OR = 1.6, CI=1.2- 2.1, $p < 0.05$). Finally, students in the field of veterinary medicine and those having good knowledge had 1.4 and 0.5 times better practice than their counterparts (OR=1.4; CI=1.2, 2.1, $p < 0.01$ and OR= 0.5, CI=0.4, 0.6, $p < 0.01$ respectively). In conclusion, this study demonstrated that there were critical gaps regarding knowledge, attitudes and practices among University students towards antimicrobial resistance and antimicrobial usage. Hence, it is advisable to encourage students to exhaustively utilize the digital era to advance their knowledge. Intervention to raise awareness should also target students majoring in fields other than health sciences.

Keywords: AMR and AMU, KAP, students, Ethiopia

5.1. Introduction

Antimicrobial resistance (AMR) is a multifaceted global public health issue affecting both human and animal populations. Antibiotic resistance must be carefully monitored, yet in the vast majority of developing nations; it is minimal to nonexistent (WHO, 2014). Additionally, most developing countries lack the necessary quality control practices to ensure that the antibiotics given are of the highest quality (Obodozie *et al.*, 2006). Antibiotic overuse, underuse, and misuse will result in the emergence of antimicrobial resistance (Ventola, 2015) and manifested in an attempt to treat infectious diseases in humans and animals as well as when used as a growth promoter in animals (Robinson *et al.*, 2016). In addition to other equally significant social and cultural factors, self-medication, erroneous prescription, inappropriate intake, and excessive use of these antimicrobial medications are the main causes of the development and spread of AMR. Antibiotic self-medication has emerged as a significant issue and a major contributor to antibiotic resistance. It can be caused by a variety of issues, including a lack of awareness regarding appropriate antibiotic usage policies, a lack of public understanding and attitude toward antibiotics, and easy access to antibiotics in many locations (Jairoun *et al.*, 2019).

Sunusi *et al.* (2019) reported inappropriate antibiotic usage among college students is documented as a result of self-medication and inadequate antibacterial agent understanding; particularly, their indications, their pathogen specificity, and the adherence to dosing schedules. Taking antibiotics for a short period can lead to drug resistance development in pathogenic bacteria. Stopping a course of antibiotics early may lead to a relapse of illness for the patient and increase the risk of infected individual passing drug-resistant pathogens to others. Some people stop taking prescribed antibiotics early due to unpleasant side effects, because they are feeling better, or because they want to save the remaining antibiotics for later use or to share with others (Mallhi *et al.*, 2019).

Future prescribers, such as human and animal health students, must be well prepared throughout their training to contribute to the prevention of AMR. As demonstrated previously, the information learned during training will aid in forming future prescribers' proper attitudes and views regarding the usage of antimicrobials and antimicrobial resistance (Heaton *et al.*, 2008). Therefore, it is undeniable that educational interventions, particularly those at the undergraduate level are still essential and should receive special emphasis in the effort to combat antimicrobial resistance. If educational interventions are not properly managed, AMR will continue to be a significant public health issue; with microbes gaining resistance to almost all antimicrobials (Ross and Maxwell, 2012). Antibiotic misuse and the pace of

resistance development are closely related, therefore, it is crucial to use antibiotics responsibly in both animal and human health systems to stop the emergence of resistance (Hockenhull *et al.*, 2017).

Knowledge, attitude, and practice (KAP) surveys are representative of a specific population to collect information on what is known, believed and done in relation to a particular topic, and are the most frequently used study tool in health-seeking behavior research (WHO, 2008). Knowledge is usually assessed in order to see how far students' knowledge corresponds to biomedical concepts (Perring, 1994). Attitude has been defined as “a learned predisposition to think, feel and act in a particular way towards a given object or class of objects” (Ribeaux *et al.*, 1978). As such, attitude is a product of a complex interaction of beliefs, feelings, and values. Practices in KAP surveys usually enquire about the use of antimicrobials. Finding out what the public knows, think, and do about using antimicrobials help to plan effective AMR prevention initiatives. As far as we are aware, no research has been conducted in Ethiopia to highlight an in-depth KAP among wider University students. Hence, this study aims to ascertain KAPs toward antimicrobial usage and antimicrobial resistance among Ethiopian public University students.

5.2. Methods

5.2.1. Study area

The study was carried out at purposely selected 10 public Universities located at different regional states of Ethiopia: University of Gondar (Northern), Metu, and Dambi Dolo Universities (Western), Jimma University (Southwest), Adama Science and Technology and Addis Ababa Universities (central Ethiopia), Arbaminch, Wolaita Sodo, and Hawassa Universities (Southern), and Haramaya University (Eastern). The Universities were selected purposively based on their geographical location, accessibility and willingness to participate in this study.

5.2.2. Study design

A University-based cross-sectional survey was conducted among randomly selected senior students of human health-related sciences (medicine, pharmacy, nursing, public health, and midwifery), non-human health-related sciences (engineering, computational, social, law and business and agriculture), and veterinary medicine students to assess their KAP towards antimicrobial usage and antimicrobial resistance. In order to overcome the multiplicity of sources of data collection that might confuse the

assessment of study results, the directory of admission and registration department of each University was used as the sampling frame. This directory was considered as a pre-existing frame with officially recognized and listed information on students' names, university ID numbers, and field of study, and gender.

5.2.3. Sample size determination and distribution

The calculation for the single population proportion was used to determine the sample size (Thrusfield, 2005) by using the following assumptions: a 95% level of confidence, a 5% margin of error, a 50% proportion of low knowledge, and a 10% non-response rate. A 10% non-response rate was included in the minimum estimated sample size of $423 \times 3 = 1269$ for each field of study (Bolarinwa, 2020). Of 1269 students, only 1,252 students filed the questionnaire for participation (the response rate was 98.7%).

5.2.4. Data collection

Based on a review of the literature, a structured questionnaire was developed and modified to address all of the major themes of the research issue of antimicrobial usage and resistance (Jairoun *et al.*, 2019; Sylvia, 2019; WHO, 2017 and Huang *et al.*, 2014). The questionnaire's content, design, relevance, readability, and comprehension were then examined and evaluated by subject-matter experts. Ten students and 10 subject-matter specialists participated in a pilot research to evaluate the validity and reliability of the questions. Data from the pilot study were not included in the findings but were used to make minor changes to the questions based on the review of the comments that were gathered.

Based on their fields of study, students were divided into three groups: 33.0% ($n = 413$) from human health-related sciences (HS) (medicine, pharmacy, nursing, public health, midwifery, and biomedical sciences); 27.9% ($n = 349$) from non-health related sciences (NHS) (engineering, computational, social, business administration, agriculture, arts, and education); and the remaining 39.1% (490) were majoring in veterinary medicine (VM). Overall, more than half of the participants 760 (60.7%) were males, and the mean age of the respondents was 22.31 ± 1.35 years.

5.2.5. Data management and analysis

Data were entered into MS Excel spread sheet and the analysis was made using SPSS version 26.0. To summarize the data, descriptive statistics including frequency, percentages, and median (IQR) were generated. Each respondent's responses to questions on their knowledge, attitude, and practices received a score. Participants received a score of "0" for each question with 'No' response and a score of "1" for each question with the 'Yes' response for all knowledge, attitude, and practices questions. Following the addition of all the scores, those with scores equal to and above the median (IQR) (13(3), (15(2) and (10 (3), were classified as "good" knowledge, 'positive' attitude, and 'good' practice, respectively (Kassahun

and Mekonen, 2017). The Kruskal-Wallis test and chi-square tests were used to examine how the median scores in each of the knowledge, attitude, and practice categories varied across study participants. To identify the factors that are associated with good knowledge, positive attitudes, and good practices of antimicrobial usage and antimicrobial resistance, multivariate logistic regression was used in conjunction with the independent variables of socio-demographic profiles (Fetensa *et al.*, 2020). In the final model, variables with a p-value of 0.05 or lower were deemed significant.

5.2.6. Ethics approval and consent to participate

This research was reviewed and approved by the ethical committees of Addis Ababa University, College of Health Science, IRB (Protocol No.: 031/21) and the University of Liverpool (Reference No.: 9935). A verbal consent was obtained from the students and thereafter they were requested to finish a self-administered survey during lectures (at the end of their classes) or during their free time.

5.3. Results

5.3.1. Knowledge of antimicrobial usage and antimicrobial resistance

The overall median antimicrobial usage and antimicrobial resistance knowledge score was 13 (IQR = 11, 14). Table, 5.1 shows Ethiopian public University students' knowledge of antimicrobial resistance and antimicrobial usage. The percentage of students in the VM, HS and NHS field of studies who correctly answered antimicrobial resistance and antimicrobial usage knowledge questions was 72.0%, 70.0%, and 15.8%, respectively. When asked if they had heard of antimicrobial resistance, 83.3%, 82.9%, and 72.2% of HS, VM, and NHS students responded that they had heard and are aware of antimicrobial resistance ($p < 0.01$). A large proportion of students from HS (79.9%), NHS (55.6%), and VM (62.0%) stated that treatment of sick animals should be performed by both human and animal health professionals ($p < 0.01$). The interesting point is that 89.2%, 70.0%, and 66.2% of VM, HS, and NHS students correctly answered that antibiotics should be purchased from drug stores or pharmacies on the basis of a prescription ($p < 0.01$).

Table 5.1. Knowledge about antimicrobial usage and antimicrobial resistance among University students in Ethiopia (n= 1252)*

Questions regarding AMU, AMR and Knowledge	Study discipline	No n (%)	Yes n (%)	χ^2	P value
AMU					
Can antibiotics be used to cure infections caused by viruses?	Human health sciences	132 (32.0)	281(68.0)	157.8	<0.001
	Non health science	213 (61.0)	136 (39.0)		
	Veterinary medicine	95 (19.4)	395 (80.6)		
Antibiotics should be purchased from drug stores or pharmacy based on prescription	Human health sciences	121 (22.3)	292 (70.0)	72.6	<0.001
	Non health science	118 (33.8)	231 (66.2)		
	Veterinary medicine	53 (10.8)	437 (89.2)		
It is good to use antimicrobials as additives with animal feeds	Human health sciences	103 (24.9)	310 (75.1)	85.7	<0.001
	Non health science	197 (56.4)	152 (43.6)		
	Veterinary medicine	235 (48.0)	255 (52.0)		
Antimicrobials used to treat animals can remain within their tissues	Human health sciences	265 (64.2)	148 (35.8)	114.6	<0.001
	Non health science	213 (61.0)	136 (39.0)		
	Veterinary medicine	156 (31.8)	334 (68.2)		
Antibiotics shouldn't be obtainable without a prescription at pharmacies?	Human health sciences	245 (59.3)	168 (40.7)	59.1	0.001
	Non health science	206 (59.0)	143 (41.0)		
	Veterinary medicine	181 (36.9)	309 (63.1)		
Frequent use of antimicrobials will lead to decrease in number of treatment options	Human health sciences	88 (21.3)	325 (78.7)	24.1	0.001
	Non health science	94 (26.9)	255 (73.1)		
	Veterinary medicine	66 (13.5)	424 (86.5)		
Is efficacy better if the antibiotics are newer and the price is higher?	Human health sciences	171 (41.4)	242 (58.6)	5.0	0.082
	Non health science	157 (45.0)	192 (55.0)		
	Veterinary medicine	183 (37.3)	307 (62.7)		
Treatment of sick animals should be carried out by both human and animal health professionals	Human health sciences	83 (20.1)	330 (79.9)	56.0	<0.001
	Non health science	155 (44.4)	194 (55.6)		
	Veterinary medicine	186 (38.0)	304 (62.0)		
AMR					
Microbes can become resistant to antimicrobials	Human health sciences	28 (6.8)	385 (93.2)	345.8	<0.001
	Non health science	199 (57.0)	150 (43.0)		
	Veterinary medicine	49 (10.0)	441 (90.0)		
Have you heard of antimicrobial resistance?	Human health sciences	69 (16.7)	344 (83.3)	18.6	0.001
	Non health science	97(27.8)	252 (72.2)		
	Non health science	84 (17.1)	406 (82.9)		

Bacteria that developed resistance in animals can become resistant in human through the consumption of food of animal origin	Veterinary medicine				
	Human health sciences	216 (52.3)	197 (47.7)		
	Non health science	174 (49.9)	175 (50.1)	119.3	<0.001
	Veterinary medicine	100 (20.4)	390 (79.6)		
Knowledge					
Are there bacteria in the human body that are good for our health?	Human health sciences	16 (3.9)	397 (96.1)		
	Non health science	152 (43.6)	197 (56.4)	169.6	<0.001
	Veterinary medicine	154 (31.4)	336 (68.6)		
Amoxicillin is an antibiotic?	Human health sciences	38 (9.2)	375 (90.8)		
	Non health science	119 (34.1)	230 (65.9)	123.2	<0.001
	Veterinary medicine	40 (8.2)	450 (91.8)		
Penicillin is an antibiotic	Human health sciences	19 (4.6)	394 (95.4)		
	Non health science	67 (19.2)	282 (80.8)	54.0	<0.001
	Veterinary medicine	33 (6.7)	457 (93.3)		
Tetracycline is an antibiotic	Human health sciences	33 (80.0)	380 (92.0)		
	Non health science	85 (24.4)	264 (75.6)	44.1	<0.001
	Veterinary medicine	60 (12.2)	430 (87.8)		

*'yes or no' is exclusively the response of the respondents

5.3.2. Attitudes on the antimicrobial resistance and antimicrobial usage

Students were given various statements about their attitudes toward the use of antimicrobials and antimicrobial resistance and asked how many of them they thought were correct or incorrect (Table, 5.2). The overall median antimicrobial usage and antimicrobial resistance attitudes score was 15 (IQR: 13, 15). A higher proportion of students from veterinary medicine (95.9%), human health-related sciences (89.0%), and non-human health-related sciences (NHS) (83.1%) agreed that antimicrobial resistance affects animal health and production ($\chi^2=38.2$, $p = 0.01$). When students were asked about inappropriate use of antibiotic, human health-related science students have a positive feeling that inappropriate use of antimicrobials is the one of the reasons for the occurrence of antimicrobial resistance (93.5%), followed by veterinary medicine (92.0%), and non-health-sciences (84.5%) ($\chi^2=31.6$, $p< 0.01$).

Table 5.2. Summary of attitude of University students towards AMU and AMR in Ethiopia (n= 1252)*.

Questions regarding attitude towards AMU and AMR	Study discipline	No n (%)	Yes n (%)	χ^2	<i>p value</i>
Antimicrobial resistance will affect you and your family's health	Human health sciences	92 (22.3)	321 (77.7)	42.3	<0.001
	Non health science	60 (17.2)	289 (82.8)		
	Veterinary medicine	35 (7.1)	455 (92.9)		
Antimicrobial resistance will affect animal health and production	Human health sciences	45 (10.9)	368 (89.1)	38.2	< 0.001
	Non health science	59 (16.9)	290 (83.1)		
	Veterinary medicine	20 (4.1)	470 (95.9)		
I have sufficient knowledge on antibiotics use	Human health sciences	300 (76.3)	102(24.7)	22.7	< 0.001
	Non health science	211 (60.5)	138 (39.5)		
	Veterinary medicine	307 (62.7)	183 (37.3)		
It is necessary to give more education to clinical level students about antimicrobial resistance	Human health sciences	39 (9.4)	374 (90.6)	13.3	<0.001
	Non health science	54 (15.5)	295 (84.5)		
	Veterinary medicine	39 (8.0)	451 (92.0)		
Inappropriate use of antimicrobials causes antimicrobial resistance	Human health sciences	27 (6.5)	386 (93.5)	31.6	< 0.001
	Non health science	59 (16.9)	290 (83.1)		
	Veterinary medicine	32 (6.5)	458 (93.5)		
Poor infection control practices by health care professionals will cause spread of antimicrobial resistance	Human health sciences	33 (8.0)	380 (92.0)	3.7	0.156
	Non health science	34 (9.7)	315 (90.3)		
	Veterinary medicine	58 (11.8)	432 (88.2)		
Clinical level students should get special training on the appropriate ways of prescribing antimicrobials before	Human health sciences	99 (24.0)	314 (76.0)	19.0	< 0.001
	Non health science	63 (18.1)	286 (81.9)		
	Veterinary medicine	63 (12.9)	427 (87.1)		

graduation					
Currently, antimicrobial resistance is a major problem in the world as well as in Ethiopia	Human health sciences	46 (11.1)	367 (88.9)	18.1	< 0.001
	Non health science	46 (13.2)	303 (86.8)		
	Veterinary medicine	25 (5.1)	465 (84.9)		
Prescribing antibiotics should be more closely controlled	Human health sciences	29 (7.0)	384 (93.0)	39.0	< 0.001
	Non health science	68 (19.5)	281(80.5)		
	Veterinary medicine	37 (7.6)	453 (92.4)		
Dispensing antibiotics without prescription or over-the-counter should be more closely controlled	Human health sciences	49 (11.9)	364 (88.1)	25.8	< 0.001
	Non health science	92 (26.4)	257 (73.6)		
	Veterinary medicine	98 (20.0)	392 (80.0)		

*'yes or no' is exclusively the response of the respondents

6.4.4. Practice on AMU and AMR

Students were given various statement practices about AMU and AMR and asked how many of them were correct or incorrect (Table, 5.3). The overall median AMU and AMR practice score was 10 (IQR: 10, 11). A higher proportion of NHS (81.1%), HS (72.2%), and VM (62.0%) students stated that they discontinue antibiotics as soon as their complaints subside ($\chi^2=32.3$, $p<0.01$). A higher proportion of HS students (71.9%) testified that they use antibiotics without a doctor's prescription, as do (66.8%) of NHS and 48.6% of VM ($\chi^2=57.5$, $p<0.01$). Overall, a large proportion of students reported using antibiotics to treat a common cold (69.4%), sore throat (76.1%), when coughing up yellow-green (71.7%), and flu (76.4%).

Table 5.3. Summary of practice towards AMU and AMR among University students in Ethiopia (n= 1252)*.

Questions regarding practice towards AMU and AMR	Study discipline	No, n (%)	Yes, n (%)	χ^2	P value
I stop antibiotics as soon as complaints lessen	Human health sciences	115 (27.8)	298 (72.2)	36.3	<0.001
	Non-health science	66 (18.9)	283 (81.1)		
	Veterinary medicine	186 (38.0)	304 (62.0)		
I use antibiotics prescribed by doctors	Human health sciences	15 (3.6)	394 (95.4)	3.2	0.203
	Non-health science	14 (4.0)	335 (96.0)		
	Veterinary medicine	10 (2.0)	479 (98.0)		
I follow prescription after choosing antibiotic	Human health sciences	32 (7.7)	381(92.3)	0.6	0.737
	Non-health science	23 (6.6)	326(93.4)		
	Veterinary medicine	32 (6.5)	458(93.5)		
I use antibiotics without doctor's instructions	Human health sciences	116 (28.1)	297 (71.9)	57.5	<0.001
	Non-health science	116 (33.2)	233 (66.8)		
	Veterinary medicine	252 (51.4)	238 (48.6)		
I ask the doctor to prescribe antibiotics for common cold	Human health sciences	214 (51.8)	214 (51.8)	68.1	<0.001
	Non-health science	267 (76.5)	267 (76.5)		
	Veterinary medicine	362 (73.9)	362 (73.9)		
I use antibiotics for common cold	Human health sciences	173 (41.9)	240 (58.1)	37.1	<0.001
	Non-health science	89 (25.5)	260 (74.5)		
	Veterinary medicine	121 (24.7)	369 (75.3)		
I use antibiotics for acute bronchitis	Human health sciences	130 (31.5)	283 (68.5)	22.6	<0.001
	Non-health science	114 (32.7)	235 (67.3)		
	Veterinary medicine	97 (19.8)	393 (81.0)		
I use antibiotics when coughing up yellow/green phlegm	Human health sciences	158 (38.3)	255 (61.7)	31.1	<0.001
	Non-health science	92 (26.4)	257 (73.6)		
	Veterinary medicine	104 (21.2)	386 (78.8)		
I use antibiotics for sore throat caused by any microbe	Human health sciences	105 (25.4)	308 (74.6)	29.0	<0.001
	Non-health science	113 (32.4)	236 (67.6)		
	Veterinary medicine	81 (16.5)	409 (83.5)		
I use antibiotics for flu with fever	Human health sciences	259 (62.7)	154 (37.3)	64.1	<0.001
	Non-health science	288 (82.5)	61 (17.5)		
	Veterinary medicine	409 (83.5)	81 (16.5)		

*'yes or no' is exclusively the response of the respondents

6.4.5. Factors associated with good knowledge, positive attitude, and practice on AMR and AMU

Multivariable logistic regression analysis revealed that students' fields of study were closely related to their level of good knowledge. Students from the field of HS had 18.4 times (OR = 18.4, CI =12.1 - 28.0; p<0.01) and those from VM had 12.7 (OR = 16.8, CI = 11.2 – 25.2, p<0.01) times better knowledge of AMU and AMR compared to students from NHS field of studies. Most of the demographic variables were significantly related to attitudes toward AMU and AMR. Moreover, students having good

knowledge had 3.9 times more positive attitudes than students with poor knowledge (OR = 3.9, CI = 3.0 - 5.2, $p < 0.01$) (Table, 5.4).

Table 5.4. Multivariable logistic regression analysis of factors associated with AMR and AMU among University students in Ethiopia (n= 1252)*.

Variables	Good knowledge			Positive attitude			Good practices		
	n (%)	aOR (95% CI)	p-value	n (%)	aOR (95% CI)	p-value	n (%)	aOR (95% CI)	p-value
Birthplace									
Town	265 (67.9)	Ref		282 (72.3)	Ref		237 (60.8)	Ref	
Rural	593 (91.0)	0.5 (0.4,0.7)	<0.01	541 (62.8)	0.5 (0.4,0.7)	<0.01	587 (68.1)	0.5 (0.4,0.7)	<0.01
Gender									
Male	511 (67.2)	Ref		516 (67.9)	Ref		532 (70.0)	Ref	
Female	347 (70.5)	1.0 (0.8,1.3)	0.20	307 (62.4)	1.8 (1.4,2.3)	<0.01	292 (59.3)	1.8 (1.4,2.3)	<0.01
Year of study									
3 rd	281 (73.6)	Ref		244 (63.9)	Ref		242 (63.4)	Ref	
4 th	317 (65.9)	1.4 (1.0, 2.0)	0.05	334 (69.4)	1.0 (0.7,1.3)	0.98	313 (65.1)	1.0 (0.7,1.3)	0.98
5 th	260 (90.0)	1.2 (0.9,1.6)	0.43	245 (63.0)	1.3 (1.0, 1.3)	0.60	269 (69.2)	1.3 (1.0,1.7)	0.10
Field of study									
NHS	101 (28.9)	Ref		186 (53.3)	Ref		230 (66.0)	Ref	
HS	417 (85.1)	18.4 (12.1,28.0)	<0.01	384 (78.4)	0.9 (0.6, 1.2)	0.34	364 (74.3)	0.9 (0.6, 1.2)	0.4
VM	340 (82.3)	16.8 (11.2,25.2)	<0.01	253 (61.3)	1.6 (1.2,2.1)	<0.001	230 (55.7)	1.4 (1.2, 2.1)	<0.01
Knowledge									
Poor	0(0)			176 (44.7)	Ref		286 (72.6)	Ref	
Good	858(100)			647 (75.4)	3.9 (3.0, 5.2)	<0.001	538 (62.7)	0.5 (0.4, 0.6)	<0.01
Attitude									
Negative	211 (49.2)	Ref			Ref		315 (73.4)		
Positive	647 (78.6)						509 (61.8)	0.6 (0.5,0.8)	<0.01

*P-values were obtained from regression models. AMR: antimicrobial resistance, OR: odds ratio, CI: confidence interval, aOR: adjusted odds ratio, na: not applicable, NHS: non-health science, HS: human health science, VM: veterinary medicine

5.4. Discussion

Overall, this study showed critical gaps in knowledge, and practices among University students. Accordingly, it is only 15.8% of non-health science students who correctly answered knowledge questions about AMU and AMR. Students in the health-related fields (both human and animal health) had better knowledge (higher percentages of correct answers) in almost all knowledge-related questions, and they also had positive attitudes toward AMU and AMR (higher percentages of correct answers in all attitude-related questions). This study revealed that field of studies has an impact on the level of knowledge among students. Our finding showed that 90.7% of students irrespective of their field of studies were aware that antibiotics are used to treat bacterial infections. This result is consistent with Jairoun *et al.* (2019). However, Chang *et al.* (2021) and Pogurschi *et al.* (2022) have reported that only 49.0% and 22.2% of the respondents claimed that antibiotics kill bacteria, respectively. Hence, our result is very encouraging that almost all students are aware that antibiotics kill bacteria. Strikingly, 80.6% of VM students reported that viral infections can be treated with antibiotics. Pogurschi *et al.* (2022) reported that antibiotics are ineffective against viruses, however, 37.5% of respondents believing that antibiotics treat viral infections. Similar confusions have been reported by previous researchers (Alqarni and Abdulbari, 2019; Yusef *et al.*, 2018). Seid & Hussen (2018) reported that approximately 28.0% of the participants believed that antibiotics could kill both viruses and bacteria. Our findings were consistent with a study conducted in Portugal, in which more than 60.0% of participants agreed that antibiotics should be prescribed for viral illnesses (Azevedo *et al.*, 2009). Encouraging results were also recorded in this study that a large proportion of students had heard about AMR, and frequent use of antibiotics reduces treatment efficacy antibiotics. Jairoun *et al.* (2019) reported that large proportion of respondents has similar agreements. This implies that a rigorous assessment of University students' knowledge would aid in the development of an educational program.

In terms of attitude, approximately 63.4% of students have correctly responded to questions regarding antimicrobial resistance and antimicrobial usage. Similar results (66.0%) were reported from India (Sadasivam *et al.*, 2016). This result, however, was lower than the report of Seid & Hussen (2018) in which 96.0% of students had a positive attitude toward antimicrobial resistance. The majority of students (85.1%) also stated that improper antibiotic usage can have a negative impact on their families. Similarly, Seid & Hussen (2018) found that 90.7% of the students agreed that their health and the health of their family will be impacted by antibiotic resistance. The vast majority of students (80.8%) irrespective of their field of studies agreed that dispensing antibiotics without a prescription should be strictly regulated.

However, only a few students (26.0%) had not received instructions on how to take antibiotics or the importance of completing the entire duration of the treatment. It is worth noting that those who did not seek advice are more likely to discontinue antibiotics when they feel better, resulting in the spread of resistance in the community. Similar studies in Ethiopia and India found that 82.4% (Seid & Hussien, 2018) and 65.0% (Sadasivam *et al.*, 2016) of participants believed that antibiotics should never be purchased as over-the-counter drugs.

In terms of practice, nearly 54.6% students have correctly answered AMR and AMU practice questions; however, the appropriate use of antibiotics was not demonstrated in their daily lives. This is demonstrated by the proportion of students (70.7%) who reportedly stopped using antibiotics after symptom improvement; of which 72.2% (HS), 81.1% (NHS), and 62.0% (VM) students feeling similarly. Previous studies in the UAE (Jairoun *et al.*, 2019) (44.5%), Pakistan (Limaye *et al.*, 2019) (28%), and Lebanon (Mouhieddine *et al.*, 2015) (51.5%) found similar results. The tendency of stopping antibiotics after symptom improvement might be related to self-medication. Self-medication could be a potential factor in the medication's discontinuance. Medical students appear to disregard proper antibiotic use, which sets a poor example for the general public (Sylvia, 2019). Antibiotics were among the top three medications used for self-medication among undergraduate healthcare students in Nigeria, who believed they had acquired the necessary medical knowledge of what to use for a specific condition treated (Akande-Sholabi & Ajamu, 2021). However, Akande-Sholabi & Ajamu (2021) reported that the majority of participants (91.0%) always completed the entire course of treatment. Saving money on expensive consultation fees and the inconvenience of making a doctor appointment for a mild illness are two possible justifications for storing antibiotics for potential respiratory illnesses and using leftover antibiotics.

This study also showed critical gaps towards AMR and AMU practice among students; a large proportion of students 69.4%, 76.1%, 71.7%, and 76.4%, respectively, claimed using antibiotics to treat a common cold, sore throat, when coughing up yellow-green, and flu. Similar studies in Nigeria and Rwanda found that 50.0% and 20.0% of study participants had used antibiotics to treat the common cold and sore throat, respectively (Akande-Sholabi & Ajamu, 2021; Nisabwe *et al.*, 2020). This inappropriate use of antibiotics could be attributed to a lack of knowledge about antimicrobial use. A systematic review of antibiotic use for the common cold and acute purulent rhinitis, on the other hand, found that antibiotics have no benefit for the common cold, sore throat, and cough (Kenealy & Arroll, 2013). Colds, sore throats, and coughs

are mostly caused by viruses, and antibiotics only fight bacteria, not viruses. Moreover, a majority of students (61.3%) reported using antibiotics without a doctor's prescription. In a similar study from Nigeria, however, 86% of participants always consulted a doctor before beginning an antibiotic regimen. Nonetheless, only 65.0% agreed that antibiotics should never be purchased as over-the-counter drugs, and doctors take the time to explain the do's and don'ts of antibiotics in detail. According to Kalungia *et al.* (2019) and Fadare *et al.* (2019), reinforcing antimicrobial stewardship in all health facilities would raise antimicrobial resistance awareness among healthcare professionals.

This study also revealed that 61.3% of the students claimed to have obtained antibiotics without a prescription from vendors or drug stores. Reynolds & Rodin (2009) supplemented to our finding that due to relatively lax antibiotic regulations, it is not difficult to obtain antibiotics without a proper prescription (over-the-counter acquisition) in China. Personal observation also showed that purchasing antibiotics without a prescription is a common phenomenon in Ethiopia. This indicates a lack of stringent and strict enforcement of laws and regulations governing how antibiotics are prescribed and dispensed in retail pharmacies. Lukovic *et al.* (2014) revealed that the prevalence of non-prescription drug use is quite high and common among medical students. Personal observation also revealed that there is an indiscriminate abuse of prescription drugs for non-prescription drugs among several pharmaceutical shops in different areas of the country. As far as someone can afford the prices, several pharmaceutical shops can sell them to their customers. Alnasser *et al.*, (2021) stressed that the public should be educated on the effectiveness of these medications and how they should be used in order to reduce AMR. Age, educational level, family attitudes, drug manufacturer advertising, laws governing the dispensing and sale of pharmaceuticals, prior experiences with the symptoms or condition, and significance attached to the disease are factors that have been found to influence how often people self-medicate (Zhu *et al.*, 2016), prescription medicines kept at home (Klemenc-Ketis and Kersnik, 2010) and financial circumstance of responders (James *et al.*, 2006). It has to be noted that there is a risk of overprescribing antibiotics in retail pharmacies and among vendors if antimicrobial susceptibility tests are not performed. Hence, the findings this study supports strict enforcement of laws and regulations governing how antibiotics are prescribed and dispensed in retail pharmacies. In conclusion, this study has demonstrated there were critical gaps regarding antimicrobial resistance and antimicrobial use among University students. Hence, students are encouraged to use additional online training platforms to advance their knowledge. Moreover, interventions to raise awareness should also target students majoring in fields other than health sciences is recommended.

Limitations of the study

As the study is a cross-sectional survey; we cannot document causal relationships. Moreover, the survey was a self-administered questionnaire; thus, the use of antimicrobials was basically self-reported, and the frequency of antimicrobial use may be overestimated or underestimated due to recall bias. The results obtained in this study were dependent on the recall ability and honesty of the respondents and hence, underreporting or over reporting could not be ruled out.

Acknowledgments

The authors would like to thank all the students who took part in this study. This work was supported in various ways by Addis Ababa University Research Directorate.

5.5. References

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6. CHAPTER VI: GENERAL DISCUSSION

This chapter provides general discussion on the four research findings described earlier and give interpretations and implication of the major findings. The purpose of this study was to look into the level of knowledge, attitudes, and practices towards transmission of Zoonoses, food safety, antimicrobial usage and antimicrobial resistance and occurrence *E. coli* O157 H: 7 in beef in Ethiopia. The study attempted to address the knowledge, attitude and practices of different community members regarding transmission of Zoonoses, food safety along the beef value chain starting from production (smallholder livestock keepers) to consumption (slaughterhouses and butcher shops), in addition to detecting a major food pathogen (*E.coli* O157:H7). This may provide baseline information on KAP among the beef values chain actors and helps to address the food safety. The study further dealt with one of the growing public health challenges globally namely the KAP on antimicrobial uses and development of drug resistance among the University students.

The study on farmers KAP regarding transmission of Zoonoses in the Ada'a district of Oromia region revealed a good/medium level of knowledge on zoonotic disease, but more of negative attitude and poor practices that may expose them to zoonotic infection. In this study, over half of the respondents (54.90%) didn't know about zoonosis. However, in another study carried out in Ethiopia, the majority of participants had heard of zoonosis (Emiru *et al.*, 2015). Farmers' levels of knowledge may vary with time and place due to differences in access to information and educational level. Their source of knowledge was mostly community members and few of them mentioned radio. This finding indicated that there is a knowledge gap among farmers. The research findings of Abera *et al.*, (2016) and Sisay, (2012) indicated that respondents get information from community and media as well. A study carried out in Nairobi indicated that evidence-based communication through the media to the general public could be helpful in reducing the dangers of zoonotic diseases (Kang'ethe *et al.*, 2012). The differences in the level of knowledge among farmers from place to place could be attributed to the respondents' age group, proximity to towns, and access to electronic media and other information generation platforms (Kidane *et al.*, 2015).

A vast majority of farmers correctly answered that consumption of raw meat and raw milk can transmit diseases from animals to humans. Strikingly, the majority of them did not know that drinking raw milk can cause bovine tuberculosis and brucellosis. Moreover, most of the respondents were unaware that raw

meat consumption causes bovine cysticercosis. Meat is often consumed as part of the staple diet of the people and also during special occasions of festivity in Ethiopia. Eating raw or half cooked meat is very common. However, eating other kinds of meat, such as pork, is a cultural taboo among most Ethiopians (Seleshe *et al.*, 2014).

In this study, more than half of farmers claimed to consume raw milk. It is often consumed in its natural state or as a fermented form in Ethiopia; some even believe that boiling or pasteurizing processes destroy the quality of the milk (Seyoum *et al.*, 2016). Negash *et al.*, (2012) disclosed that about 50.0% of milk produced by smallholder farmers in the Ethiopian Rift Valley areas was consumed fresh at home, without being boiled or pasteurized. In many societies around the world, raw (unpasteurized or un-boiled) milk consumption is a deeply rooted cultural habit. Hence, the vast majority of all milk produced in the country being sold through informal marketing systems rather than pasteurization plants (Mebrate *et al.*, 2020).

Almost all farmers stated that they had never used personal protective equipment when dealing with sick animals. Alemayehu *et al.*, (2021) reported that due to the scarcity of personal protective equipment, farmers and pastoralists do not use personal protective equipment when dealing with animal abortion. It is worth mentioning that a vast majority of farmers claimed that they didn't wash their hands after coming into contact with animals. Hence, it should be an assignment for all stakeholders and regulatory bodies to use this finding as a point of discussion in diseases prevention and control strategies. Moreover, animal health professionals can play their pivotal role in the awareness creation and dissemination of information among farmers about the importance of hand washing and the use of personal protective equipment in diseases prevention and control. On the other hand, Özlü *et al.*, (2020) for instance in their research findings reported that almost all cattle farmers had both positive attitudes and good practices regarding handwashing after contact with animals, after the burial of dead animal bodies, and the separation of sick animals from the herd. Variation in geographical location, education background, media access, and variation in animal husbandry practices might be attributed to the probable reason for variation in basic hygienic practices. Incorrect perceptions and attitudes toward zoonotic disease prevention underscore the importance of culturally appropriate health education in rural communities. As a result, it is critical to change the community's attitude in order to improve their behavioral practices regarding zoonotic disease transmission and prevention practices (Nijland *et al.*, 2013).

The second study dealt with food safety knowledge and practices and showed meat handlers were found to have adequate knowledge of food safety, including the importance of general sanitary measures such as frequent hand washing, proper cleaning and sanitization of equipment, avoidance of eating and drinking in the workplace, and cross-contamination. Similarly, most meat handlers had favorable attitudes toward food safety and their role in reducing contamination by regularly washing hands, keeping surfaces clean, and inspecting meat for freshness. However, this study found that, despite good knowledge and positive attitudes toward food safety, this did not always translate into proper meat handling practices. The level of food safety knowledge reported in this study was higher than that reported among meat handlers in eastern Ethiopia (Tegegne *et al.*, 2017), but similar to that reported among hotel food handlers in northwestern Ethiopia (Admasu & Kelbessa, 2018). Despite the high level of food safety knowledge in this study, there were several knowledge gaps on specific issues that could be minimized with adequate training of the meat handlers. Gaps in knowledge about the causes of food-borne illness were identified, as well as a lack of understanding the difference between cleaning/washing and sanitizing. The importance of the worker's health status also appeared to be a knowledge gap, possibly reflecting a lack of understanding of the causes of foodborne illness and the fact that humans are the source of foodborne illness and the fact that humans are the source of some pathogens that contaminate meat.

This study also showed positive attitudes toward food safety, with the main deficiencies being beliefs about raw meat consumption and towel use. Eating raw meat is a common tradition in Ethiopia, so it is not surprising that raw meat is perceived to be healthier and more nutritious. The use of the same towel to clean multiple locations can lead to cross-contamination. The majority of study participants (90.3%) believed it was possible to clean multiple surfaces with the same towel. This is significantly higher than another study of meat handlers in Ethiopia (53%) Tegegne and Phyto, (2017), and may reflect a specific gap in training or equipment available in the workplaces in this study.

The *Codex Alimentarius* Commission (CAC) advises food handlers to wash their hands before handling foods, after using the restroom, and after handling raw food or contaminated materials. Furthermore, it is advised that personal effects such as jewelry be avoided and that cuts and abrasions on hands be covered while working (CAC, 2020). While the majority of meat handlers in this study practiced good hand hygiene, lapses in hand washing practices were reported when donning/doffing gloves, smoking (both uncommon in this setting), and sneezing/coughing. Notably, only one-third of meat handlers in this study reported wearing a mask. The latter findings are especially significant given that the study was conducted

during the COVID-19 pandemic. Indeed, slaughterhouses all over the world have been linked to outbreaks (Waltenburg *et al.*, 2020; Ijaz *et al.*, 2021). Again, this appears to imply that meat handlers may be unaware of their role in spreading infection to others.

This study found disagreement between knowledge/attitudes and practices in several circumstances. For example, while nearly all of the respondents (93.4%) agreed that meat handlers with hand injuries should not touch or handle meat, more than half admitted to doing so. Similarly, while the vast majority (98.2%) believed that wearing protective clothing improved food safety, only a minority few reported wearing protective clothing, aprons, or gloves properly. These findings are consistent with other studies in Ethiopia, where a sizable proportion of meat handlers reported handling meat while suffering from hand injuries and/or not wearing gloves (Tegegne *et al.*, 2017; Haileselassie *et al.*, 2013; Yenealem *et al.*, 2020).

Training was the only modifiable factor among the several factors identified in multivariable analysis as with a major factor in improving food safety KAP. That is, food hygiene training that specifically addresses the gaps identified in this study could potentially improve food safety in Ethiopian slaughterhouses and retail meat shops. This may be especially important in retail meat shops, where food safety practices are lax and training is non-existent, according to respondents in this study. There are initiatives to train staff regarding meat cleanliness, but they are not on a regular basis and they are not sufficient, according to personal communications with the abattoir managers and retail meat shop owners. Additionally, there was a strong correlation between knowledge and meat handlers' attitudes toward food safety. This finding suggests that training that increases knowledge will also increase meat handlers' attitudes toward food safety. When it comes to attitudes and understanding about food safety, Cameroonian slaughterhouse employees showed a similar positive correlation (Matchawe *et al.*, 2019).

It is noteworthy that in this study, good food safety procedures were not independently connected with good food safety knowledge or favorable attitudes. Other researchers have shown results that are comparable. For instance, a UK survey of food handlers found that 63% of those polled said they did not always exhibit the associated positive behavior despite having adequate awareness of food safety measures (Clayton *et al.*, 2002). Time restrictions and a staffing shortage were identified in that study as obstacles to good food safety standards. Additional hurdles in Ethiopia may include weak regulatory frameworks and their implementation, managers' and coworkers' negative attitudes, and structural issues

such a dearth of facilities and supplies. Indeed, informal observations made during the interviews revealed a number of elements that probably influenced poor practices, including a shortage of hot water baths for hand washing, dipping knives and other equipment, and inadequate chilling facilities. Another study that used direct observation and a checklist of best procedures for handling beef in slaughterhouses and beef retail stores in the same region also showed similar findings (Gutema *et al.*, 2021). As a result, while it is obvious that Ethiopia has to increase the caliber and scope of food safety training, it is also necessary to address these other issues in order to lessen consumer risk.

This study also attempted to detect *E.coli* O157:H7 in abattoirs and retail meat shops. Hence, the overall prevalence of *E. coli* O157:H7 was 3.97%, which is consistent with Ethiopia's national prevalence estimate 4.0% (Assefa, 2019). This finding was slightly higher than 2.4% (Atnafie *et al.*, 2017), however, slightly lower than 5.4% (Sebsibe & Asfaw, 2020), and 8.6% (Atnafie *et al.*, 2017). *E. coli* O157:H7 was prevalent in 5.95% of samples from the slaughterhouses; consistent with the findings of Bekele *et al.*, (2014) which is 5.7%. A lower prevalence than the present finding was reported in Ethiopia (Atnafie *et al.*, 2017), the United Kingdom (De Boer & Heuvelink, 2000), and Ireland (Carney *et al.*, 2006), 2.7%, 3.2%, and 3.0%, respectively. The variation in prevalence could be attributed to differences in slaughterhouse standards, worker hygienic practices, sampling and isolation methodology, season, geographical origins, and sample size (Varela-Hernández *et al.*, 2007).

In a previous study, Bekele *et al.*, (2014) found a higher prevalence of *E. coli* O157:H7 (13.3%) in butcher shop samples collected from central Ethiopia. Similar prevalence was recorded in butcher shops in Ambo (Tadese *et al.*, 2021) and Bishoftu (Gutema *et al.*, 2021). The variation in the prevalence of *E. coli* O157:H7 in butcher shops could be attributed to the butcher shops' hygiene and sanitation practices, sample size, and sampling techniques. A study by Sebsibe & Asfaw (2020) reported isolation of the *E. coli* O157:H7 from various items such as, for example from the knife (1/30), cutting board (3/30), and protective clothing (1/30). However, the pooled sampling method, which was used in this study, recovers only one (1/92), demonstrating the importance of sampling methods.

E. coli O157:H7 occurrence was significantly higher at the municipal slaughterhouse (8.3%) than at the private slaughterhouse (3.5%). There is clear difference among slaughterhouses in terms of the goals; hence, private and export abattoirs are intended for international markets and should fulfill the importing country requirements. Facilities, origin of animals, personnel hygiene and standard operating procedures

might have contributed to the differences in the occurrences of the pathogen. Mummé & Webb, (2015) supplemented that public slaughterhouses have poorer management and facilities than private abattoirs.

In terms of sample type, the detection of the pathogen was higher in carcass and carcass contact surface swabs followed by fecal content and beef samples. Gutema *et al.*, (2021), Sebsibe & Asfaw, (2020) and Atnafie *et al.*, (2017) also detected the pathogen in fecal, swab, and beef samples. Even though lower prevalence is detected in this study, a substantial amount of the pathogen is detected fecal, carcass and carcass in contact surface with wastewater, and beef sample. This demonstrates that there is carcass cross-contamination during the slaughtering process, which reflects the overall unsanitary conditions among employees, utensils, unsanitary operating procedures and environmental sanitation of the slaughterhouse. Similarly, Beyi *et al.*, (2017) detected *E. coli* O157:H7 from the surface of wooden cutting boards. An attempt was also made to detect *E. coli* O157:H7 from slaughterhouse wastewater which is an alarming situation and emphasis should be given to the significance of establishing treatment facilities for slaughterhouse waste water efflux.

An observational survey was also used in this study to evaluate the hygiene and sanitary practices of slaughterhouses and butcher shops, as well as the overall slaughtering operations. Hygienic practices in beef production, processing, and distribution are critical for developing preventive measures to reduce meat's contribution to foodborne diseases (Havelaar *et al.*, 2012). However, it was observed that the lairage was dirty and during hiding there were no space between the operation activities so that fecal contamination is inevitable. Previous research found that the main source of beef carcass contamination was dairy cattle/hide entering processing facilities (Fegan *et al.*, 2009). Employees dressed in plastic gowns that covered their backs. The hygienic status of these protective garments, however, was not up to the standard. During meat processing, employees at slaughterhouses did not wear clean aprons, boots, or hair caps. This could explain why *E. coli* O157:H7 is detected in beef in butcher shops. In this study, evisceration was carried out without regard for the spillage of fecal material into the carcass. Microbial contamination of carcasses occurred most likely during evisceration (Wambui *et al.*, 2017). However, In terms of hygiene and sanitation, it is well documented that a lack of food safety education and training can contribute to unsanitary meat handling, processing, and display at slaughterhouses and butcher shops (Jeffer *et al.*, 2021; Tegegne & Phyto, 2017). The slaughterhouses and butcher shops were not equipped with necessary infra-structure to maintain general hygienic practices. There was no hot water for hand washing or knife dipping, no clean towels, no foot bath, and no separate rooms for processing rumen and

intestine (municipal slaughterhouse). Above all, an insufficient supply of tap water posed one of the most significant challenges to maintaining hygiene.

The findings of this study showed all *E. coli* O157:H7 isolates were susceptible to three antimicrobials, namely azithromycin, cefotaxime, and chloramphenicol, which were consistent with Dulo *et al.*, (2015). In contrast, all isolates were found to be resistant to tetracycline, which is consistent with the findings of Iwu *et al.* (2017). The probable reason for the development of resistance could be emanating from the fact that tetracycline is one of the most commonly used drugs both in animals and humans. Moreover, it is also common to see drugs including tetracycline being sold in the markets without prescription and along the roads by informal vendors. Mayrhofer *et al.*, (2006) also disclosed that this is not surprising given that tetracycline is frequently used as a first-line antimicrobial for disease prevention and treatment in food animals, and its widespread use is likely to have contributed to high resistance rates. Disassa *et al.*, (2017) and Ababu and Fesseha (2020) also reported tetracycline resistance isolates from raw milk.

Antimicrobial resistance to multiple antibiotics is becoming more common in *E. coli* O157:H7 isolates (Ahmed & Shimamoto, 2015). Multidrug resistance to three or more antimicrobial classes was also found in 85.7% of the isolates in this study. Resistance to three, four, five, and six antimicrobial classes was also found in 42.8%, 28.6%, 7.1%, and 7.1% of the *E. coli* O157:H7 isolates, respectively. A similar finding of multiple antimicrobial resistances on STEC strains has been documented in Ethiopia (Shecho *et al.*, 2017) and other parts of the world (Amézquita-López *et al.*, 2016, Maal-Bared *et al.*, 2013). Multiple antimicrobial resistance profiles were observed in fecal samples, carcass and carcass contact swabs, beef, and water and wastewater samples. The difference in sample source, variability of the resistant gene within isolates, and the type of antimicrobials used in the study may explain the higher multiple antimicrobial resistance seen in this study. This finding, particularly the multi-drug resistant wastewater isolates, suggests that slaughterhouse effluent could become a source of resistance pathogens in the environment.

The study that dealt with the KAP of AMR and AMU has identified some critical gaps that may contribute to AMR. Accordingly, 84.2% of non-health science students have failed to correctly answer knowledge questions about AMU and AMR. However, students in the health-related fields (both human (70.0%) and animal health (72.0%)) had better knowledge toward AMU and AMR. Hence, this study showed field of studies has an impact on the level of knowledge and attitude among students. Our finding showed that

90.7% of students irrespective of their field of studies were aware that antibiotics are used to treat bacterial infections. Strikingly, 80.6% of VM students reported that viral infections can be treated with antibiotics. Pogurschi *et al.* (2022) reported that antibiotics are ineffective against viruses, however, 37.5% of respondents believing that antibiotics treat viral infections. Similar confusions have been reported by previous researchers (Alqarni and Abdulbari, 2019; Yusef *et al.*, 2018). Seid & Hussen (2018) reported that approximately 28.0% of the participants believed that antibiotics could kill both viruses and bacteria. Our findings were consistent with a study conducted in Portugal, in which more than 60.0% of participants agreed that antibiotics should be prescribed for viral illnesses (Azevedo *et al.*, 2009). Encouraging results were also recorded in this study that a large proportion of students had heard about AMR, and frequent use of antibiotics reduces treatment efficacy antibiotics. Jairoun *et al.* (2019) reported that large proportion of respondents has similar agreements. This implies that a rigorous assessment of University students' knowledge would aid in the development of an educational program.

In terms of student attitudes, 63.4% of students have correctly answered attitude related questions toward antimicrobial resistance and antimicrobial usage. Similar results (66%) were reported from India (Sadasivam *et al.*, 2016). In this study, almost all students claimed that antimicrobial resistance is viewed as a major global issue including Ethiopia. This is an interesting research finding; in addition to the issue being acknowledged by students in the context of Universities, antibiotic resistance is also a topic that receives extensive coverage and discussion in the news, on television, and in other media, making it simple to raise public awareness. Khan *et al.* (2013) disclosed that media and internet in particular, have emerged as significant informational resources and platforms for educating people of all ages about a range of social issues. Similarly, Higuaita-Gutiérrez *et al.* (2020), Seid & Hussen (2018) and Patel *et al.* (2016) revealed that 97%, 70% and 92% of their respondents believed antimicrobial resistance is both a local and global problem, respectively.

In this study, 72.2% (HS), 81.1% (NHS), and 62.0% (VM) students reportedly stopped using antibiotics after symptom improvement. This tendency was reported by previous studies in the UAE (Jairoun *et al.*, 2019) (44.5%), Pakistan (Limaye *et al.*, 2019) (28%), and Lebanon (Mouhieddine *et al.*, 2015) (51.5%). Self-medication could be a potential factor in the medication's discontinuance. Medical students appear to disregard proper antibiotic use, which sets a poor example for the general public (Sylvia, 2019). Antibiotics were among the top three medications used for self-medication among undergraduate healthcare students in Nigeria, who believed they had acquired the necessary medical knowledge of what

to use for a specific condition treated (Akande-Sholabi & Ajamu, 2021). However, Akande-Sholabi & Ajamu (2021) reported that the majority of participants (91%) always completed the entire course of treatment. Saving money on expensive consultation fees and the inconvenience of making a doctor appointment for a mild illness are two possible justifications for storing antibiotics for potential respiratory illnesses and using leftover antibiotics.

Overall, AMR and AMU practice is poor (54.6%); hence, the appropriate use of antibiotics was not demonstrated in their daily lives. This is demonstrated by the proportion of respondents who used antibiotics to treat cough, sore throat, and common cold. A majority of students reported using antibiotics to treat a common cold, sore throat, when coughing up yellow-green, and flu with fever. Similar studies in Nigeria and Rwanda found the use of antibiotics to treat common cold and sore throat (Akande-Sholabi & Ajamu, 2021; Nisabwe *et al.*, 2020). This inappropriate use of antibiotics could be attributed to a lack of knowledge about antimicrobial usage. A systematic review of antibiotic usage for the common cold and acute purulent rhinitis, on the other hand, found that antibiotics have no benefit for the common cold, sore throat, and cough (Kenealy & Arroll, 2013). Moreover, a majority of students reported using antibiotics without a doctor's prescription. In a similar study from Nigeria, however, the majority of participants always consulted a doctor before beginning an antibiotic regimen. According to Kalungia *et al.* (2019) and Fadare *et al.* (2019), reinforcing antimicrobial stewardship in all health facilities would raise antimicrobial resistance awareness among healthcare professionals.

This study also revealed that more than half of the students claimed to have obtained antibiotics without a prescription from vendors or drug stores. Reynolds & Rodin (2009) supplemented to our finding that due to relatively lax antibiotic regulations, it is not difficult to obtain antibiotics without a proper prescription (over-the-counter acquisition) in China. Personal observation also revealed that purchasing antibiotics without a prescription is common in Ethiopia. This indicates a lack of stringent and strict enforcement of laws and regulations governing how antibiotics are prescribed and dispensed in retail pharmacies. Lukovic *et al.* (2014) revealed that the prevalence of non-prescription drug use is quite high and common among medical students. Personal observation also revealed that there is an indiscriminate abuse of prescription drugs for non-prescription drugs among several pharmaceutical shops in different areas of the country. As far as someone can afford the prices, several pharmaceutical shops can sell them to their customers. Alnasser *et al.*, (2021) stressed that the public should be educated on the effectiveness of these medications and how they should be used in order to reduce AMR. Age, educational level, family

attitudes, drug manufacturer advertising, laws governing the dispensing and sale of pharmaceuticals, prior experiences with the symptoms or condition, and significance attached to the disease are factors that have been found to influence how often people self-medicate (Zhu *et al.*, 2016), prescription medicines kept at home (Klemenc-Ketis and Kersnik, 2010) and financial circumstance of responders (James *et al.*, 2006). It has to be noted that there is a risk of overprescribing antibiotics in retail pharmacies and among vendors if antimicrobial susceptibility tests are not performed. Hence, the findings this study supports strict enforcement of laws and regulations governing how antibiotics are prescribed and dispensed in retail pharmacies.

In this study, approximately 60.6% of University students reported using antibiotics at least once annually, and a similar finding was also reported (Sakr *et al.*, 2020). In another study conducted in Lebanon, 68.3% of the respondents claimed to use antibiotics 1-3 times annually (Mouhieddine *et al.*, 2015). Our finding is higher than previous studies from Ethiopia (Jifar & Ayele, 2018) (53.5%) and Tesfaye, (2017) (35.9%). However, it was significantly lower than what was discovered in Namibia 80% (Pereko *et al.*, 2015). Some of the possible reasons for the variations could be related to the prevalence of diseases and the affordability to purchase drugs.

6.1. Conclusion and recommendations

The study findings have highlighted the existence of knowledge gaps, and a low level of the desired attitude regarding the transmission of Zoonoses among farmers which is implied by the high-risk self-reported behavioral practices. This study also revealed good knowledge among meat handlers, which was reflected in a favorable attitude toward food safety, but which was surprisingly not effectively converted into practice. Gaps in the usage of personal protective equipment and other sanitary procedures that are required to lower the hazards associated with food contamination during handling were in particular identified. The study results have also demonstrated the occurrence of *E. coli* O157:H7 in retail meat shops and abattoirs and the existence of various profiles of antibiotic resistance among the isolates; highlights the meat processing and distribution practice was unhygienic. Moreover, this study showed critical gaps regarding AMU and AMR among University students. However, students majoring in health-related fields (both human and animal health) had better knowledge (higher percentages of correct answers) in almost all knowledge and attitude-related questions. In general, our results found knowledge gaps and high risky behavioural practices toward transmission of Zoonoses and, low levels of adherence to food safety standards, and critical gaps regarding AMU and AMR. In general, our study showed the

interconnection and critical gaps in knowledge, attitude and practices about transmission of Zoonoses, food-borne infections, and development of antimicrobial resistance along the meat value chain. Afterwards, the information acquired could be used to create effective awareness and education initiatives. Hence, the following points are recommended:

- Meat handlers should receive continuous education as well as hands-on training in food safety which can help to improve appropriate food safety measures through better awareness and a more upbeat attitude.
- Strengthening awareness of farmers and veterinary supervision of animal husbandry aimed towards the transmission of Zoonoses among farmers.
- Slaughterhouses and meat retailer shops need to adopt good hygienic and manufacturing practices for the best control of pathogens such as *E. coli* O157:H7.
- Interventions to raise awareness about AMR and AMU should target students majoring in fields other than health sciences and the public in general.
- Veterinary public health researchers are encouraged to incorporate social science studies into their research agenda so that to augment their research findings towards the mediation of behavioral factors in affecting food safety along the meat value chain in Ethiopia
- Further research is recommended to detect the circulating drug resistant genes in pathogenic microbials at the interface of human-animal-environment.
- Being human element involved in various professionals under health condition, the glob including Ethiopia shall practice one health regardless of profession
- It is timely to incorporate ‘ONE HEALTH’ course for different education levels

6.2. Limitations of the study

As a cross-sectional study, it cannot document causal relationships because of the current survey's cross-sectional nature. Because our study was questionnaire-based, there is a possibility of recall bias as well. Because of the use of multiple-choice answers, response bias is unavoidable, though it may have been mitigated in the construction of the knowledge score due to some stringent requirements. An intervention-based study strategy may be able to overcome these constraints. However, the Cronbach's alpha results indicate that our questionnaire had good internal consistency. Specifically, lack of sufficient female respondents in the food safety and zoonotic disease KAP make comparison of male and male meaningless. It may have inflated the odds ratio and led to erroneous results for gender in logistic

regression models. Additionally, a lot of practices were evaluated using self-report. Participants likely over reported good practices because they frequently had positive attitudes and good knowledge about food safety. Additionally, some KAP questions were based on recalls of the respondents which may also depends on the cognitive of the person. Throughout the study, informal personal observations were made, and some differences between what persons claimed to do and what was seen were documented. The bulk of the respondents' answers did, however, corroborate our observations at the meat establishments.

Similarly, in the survey of AMU and AMR, as the measurements were transversal the associations found do not indicate causality. It is a self-administered questionnaire; thus, the use of antimicrobials was basically self-reported, and the frequency of antimicrobial use may be overestimated or underestimated due to recall bias. Moreover, the results are only representative of students from ten public Universities in the country. The results obtained in this study were dependent on the recall ability and honesty of the respondents and hence, underreporting or over reporting could not be ruled out. Moreover, a conventional way of pathogen detection was followed in the case of *E.coli*, O157:H7 detection. It could have been better if molecular detection the pathogen was employed.

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8. APPENDICES

Appendix 1. Ethical clearances



Central University Research Ethics Committees

10 August 2021

Dear Dr Mor,

I am pleased to inform you that the University is willing to accept the ethical review provided by the external ethics committee. Details and conditions of the approval can be found below.

Reference: 9935
 Project Title: Comparative study on the Assessment of Knowledge, Attitude and Practices (KAP) towards Zoonotic diseases, Antimicrobial Resistance/Use and Food Safety/Hygiene in Ethiopia: A One Health Approach
 Principal Investigator: Dr Siobhan Mor
 Co-Investigator(s):
 Student Investigator(s):
 Department: Livestock & One Health
 Approval Date: 10/08/2021
 Approval Expiry Date: Five years from the approval date listed above

The application was **APPROVED** subject to the following conditions:

Conditions

Please note: this approval is subject to the University's research restrictions during the pandemic, as laid out on the [research ethics webpages](#). Therefore, wherever possible, research should be conducted via remote means which avoid the need for face-to-face contact with human participants during the pandemic. The process for requesting an exemption to these restrictions is described on the [research ethics webpages](#).

- All serious adverse events must be reported to the Committee within 24 hours of their occurrence, via the Research Integrity and Ethics Officer (ethics@liverpool.ac.uk).
- Any amendments or changes to the study must be approved by the external ethics committee and the University of Liverpool ethics committee.
- If you wish to extend the duration of the study beyond the research ethics approval expiry date listed above, a new application should be submitted.
- If you wish to make an amendment to the research, please create and submit an amendment form using the research ethics system.
- If the named Principal Investigator or Supervisor leaves the employment of the University during the course of this approval, the approval will lapse. Therefore it will be necessary to create and submit an amendment form using the research ethics system.
- It is the responsibility of the Principal Investigator/Supervisor to inform all the investigators of the terms of the approval.

Kind regards,

Central University Research Ethics Committees

ethics@liverpool.ac.uk

Central-review

Appendix - Approved Documents

(Relevant only to amendments involving changes to the study documentation)

The final document set reviewed and approved by the committee is listed below:

Document Type	File Name	Date	Version
Other Committee Terms Of Reference	AAU_CHS_IRB_ToR		
Other Committee Approval Letter	Ethics Clearance Fufa Abunna Ethiopia	20/04/2021	-
Participant Consent Form	aspect 1 - consent form	20/04/2021	-
Participant Consent Form	aspect 3 - consent form	20/04/2021	-
Participant Consent Form	aspect 2 - consent form	20/04/2021	-
Other Committee Application Form	PhD Proposal_ HORN OH for funding Fufa Abunna	17/06/2021	-
Fieldwork Risk Assessment	F2F exemption request _FUFA ABUNNA KURRA HoD Approval final	17/06/2021	-
Research Tools	F2F exemption request _FUFA ABUNNA KURRA HoD Approval final	17/06/2021	-
Other Committee Approval Letter	IRB CHS AAU_ FUFA ABUNNA	17/06/2021	-
Participant Information Sheet	Participant Information sheet (PIS)_ Fufa_ Ethiopia	19/06/2021	-



**ADDIS ABABA UNIVERSITY, COLLEGE OF HEALTH SCIENCES (IRB)
Institutional Review Board**

ANNEX 3
Form AAUMF 03-008

IRB's Decision

Meeting No: 04/2021

Meeting Date: April 28,2021

Protocol number: 031/21/External

Protocol Title: Comparative study on the Assessment of Knowledge, Attitude and Practices (KAP) towards Zoonotic diseases, Antimicrobial Resistance/use and Food Safety/Hygiene in Ethiopia: A One Health Approach	
Principal Investigator:	Dr. Fufa Abunna
Institute:	College of Health Sciences, AAU
Elements Reviewed (AAUMF 01-008)	<input checked="" type="checkbox"/> Attached <input type="checkbox"/> Not attached
Review of Revised Application <input type="checkbox"/> Yes <input type="checkbox"/> No	Date of Previous review:
Decision of the meeting:	<input checked="" type="checkbox"/> Approved <input type="checkbox"/> Approved with Recommendation <input type="checkbox"/> Resubmission <input type="checkbox"/> Disapproved

I. Elements approved-

1. Protocol Version No: 2
2. Protocol Version Date:
3. Informed consent Version No. 2
4. Informed Consent Version Date:

II. Obligations of the PI-

1. Should comply with the standard international & national scientific and ethical guidelines
2. All amendments and changes made in protocol and consent form needs IRB approval
3. The PI should report SAE within 10 days of the event
4. End of the study, including manuscripts and thesis works should be reported to the IRB
5. The PI should report non-compliance and unanticipated events

III. TO NERC

Institution Review Board (IRB) Approval: Period from: June 16, 2021 to June 15, 2022

Follow up report expected in

3 Months _____ 6 months _____ 9 months one year _____

Chairperson, IRB
Dr. Adamu Addissie

Signature _____

Date: June 16, 2021





Lakk/Ref.NO BEP/1HB/FH/116/520
Guyyaa/Date 22/07/2013 E.C

To: Fufa Abunna Kurra, Principal Investigator

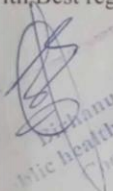
Addis Ababa, Ethiopia

Subject: Ethical approval

The research project titled ‘‘*Comparative study on the Assessment of Knowledge, Attitude and Practices (KAP) towards Zoonotic diseases, Antimicrobial Resistance/use and Food Safety/Hygiene in Ethiopia: A One Health Approach*’’ is going to conduct in Oromia Region. Based on the principal investigator’s request for ethical approval, the Oromia Regional Health Bureau research ethics review board has reviewed the aforementioned research protocol in an expedited manner. We are writing to advise you that Oromia Regional Health Bureau research ethics review board has granted full approval and decided to give this ethical approval letter internalizing the existing problem and the study eventually come up with possible solutions.

We, therefore, request you as your esteemed organization to ensure the commencement and conduct of the study accordingly and wish for the successful completion of the study. Finally, we would like to inform the PIs to submit the result of the study up on completion to Oromia Regional Health Bureau for implementation purpose.

With Best regards,


Yohannu Qanante
Public health research team
Coordinator



011-371-72-77 | befokom2008@gmail.com or ohbhead@telecom.net.et | Tin NO 0001298

011-371-72-27 | Oromia Regional Health Bureau | 24341 | www.orhb.gov



Lakk/Ref.NO BEDA/HBTFFH/146/13/1
Guyyaa/Date 22/07/2013 E.C

To:

- HORN OH project
- West Shoa zone Health office
- East Hararge zone Health office
- Arsi zone Health office
- Jimma zone Health office
- East Shoa zone Health office
- East Wollega zone Health office

Subject: Research Ethical Clearance

Fufa Abunna Kura has requested our Bureau regarding a Research Ethical Clearance for a research title '**Comparative study on the Assessment of Knowledge, Attitude and Practices (KAP) towards Zoonotic diseases, Antimicrobial Resistance/use and Food Safety/Hygiene in Ethiopia: A One Health Approach**'.

The above indicated research project is acceptable from the ethical perspective, relevance, originality and technical competence point of view. Hence the project is allowed to be executed.

Regards,

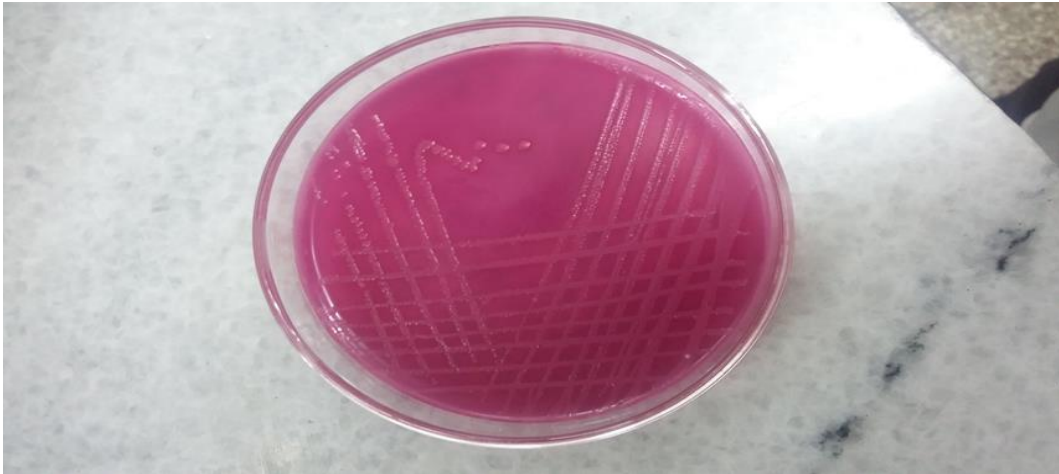
CC.

Dr Fufa Abunna Kurra

(Signature)
Public Health research team
Coordinator



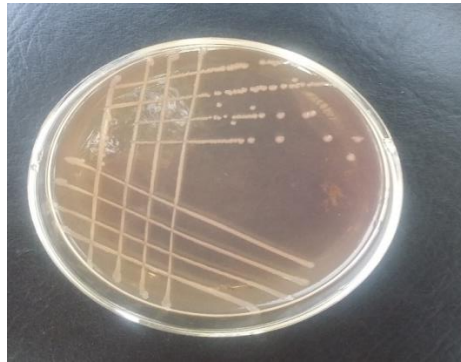
Appendix 2. Biochemical test results of *E.coli* O157:H7



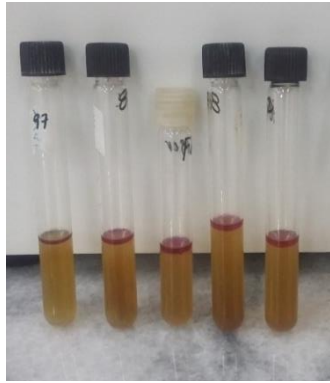
Lactose fermenting pink colonies on MacConkey agar



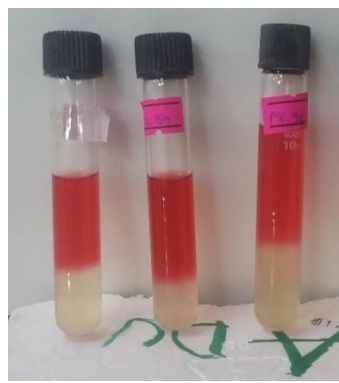
Metallic green sheen colonies
Eosin Methylene Blue (EMB) Agar



Colorless colonies on Sorbitol MacConkey Agar (CT- on
SMAC)



Indole positive (red ring)



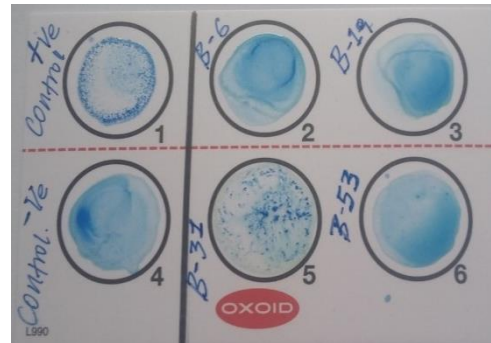
Methyl Red positive



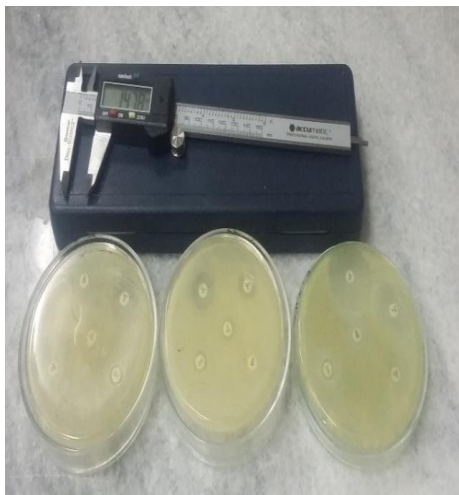
H₂S negative on TSI test



Latex positive (F-40, Card No.-6)



Latex positive (B-31, Card No. 5)



Antimicrobial susceptibility test



Carcass swab sample collection

Appendix 3. Microbiological media preparation

Buffered Peptone Water (Cat. 1402.00, CONDA, Madrid, Spain)

Composition (g/l): Enzymatic digest of casein 10.0g; Sodium chloride 5.0g; disodium phosphate dodecahydrate 9.0g and potassium dihydrogen phosphate 9.0g

Preparation: suspend 20 grams of components in 1000ml of distilled water. Mix well and distribute into universal bottle of suitable capacity to obtain the portions necessary for the test and sterilize in autoclave at 121 °C for 12 minutes. Final PH is 7.0 + 0.2 at 25°C.

Modified Tryptone Soya Broth (TSB) (CM089, Oxoid Basingstoke, UK)

Composition (g/l):: Enzymatic digest of casein (17.0 g), Enzymatic digest of soya (3.0 g), sodium chloride (5.0 g), Bile salt no.3(1.5g) (Di-Base potassium phosphate(k₂ HPO₄ (4.0 g), Glucose (2.5 g)

Preparation: suspend 33g grams of components in 1 liter of distilled water. Mix thoroughly in universal bottle and sterilize in autoclave at 121 °C for 15 minutes. Final PH is 7.0 + 0.2 at 25°C.

Novobiocin Solution

Composition (g/l): Novobiocin 0.45g

Preparation: dissolve Novobiocin in 100ml of water and sterilized by filter paper **MacConkey Agar (CM0007B, Oxoid, England)**

Composition (g/l): Peptone 20.0 Lactose 10.0, Bile salts, 5.0, Sodium chloride, 5.0, Neutral red, 0.075, Agar, 12.0, pH 7.4 ± 0.2

Preparation: Suspend 52g in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Dry the surface of the gel before inoculation

Eosin Methylene Blue Agar (Oxoid, England)

Composition (g/l): Peptone (10.0g), Lactose (10.0g), Dipotassium hydrogen phosphate (2.0g), Eosin (0.4g), Methylene blue, (0.065g), Agar, (15.0g), pH 6.8 ± 0.2

Preparation: Suspend 37.5g in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 60°C and shake the medium in order to oxidise the methylene blue (i.e. restore its blue color) and to suspend the precipitate which is an essential part of the medium.

Cefixime Tellurite Sorbitol MacConkey agar(CT-SMAC) (Oxoid Basingstoke, England)

Composition (g/l):: Enzymatic digest of casein (17.0 g), Enzymatic digest of animal tissue (3g), sorbitol (1g), Bile salts no.3 (1.5g), Sodium chloride (5.0g), (Neutral red 0.03g), crystal violet(0.001g).

Preparation: 50 g of the powder was suspended in 1 liter of distilled water. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121°C for 15 minutes. Then Potassium tellurite (2.5 mg/l) and Cefixime (0.05mg/l) were added on the prepared base media tempered at 50-55OC. gently shaken and poured into Petri dishes.

Nutrient Agar (CM 0003, OXOID, Basingstoke, England)

Composition (g/l): peptic digest of animal tissue 5.00; sodium chloride 5.00; beef extract 1.5; yeast extract 1.5; agar 15.

Preparation: suspend 28 grams in 100ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour in to sterile petridishes. Final PH (at 25°C): 7.4 + 0.2.

Triple sugar agar (CM 0277, OXOID, Basingstoke, England)

Preparation: suspend 65 grams in 1000ml of distilled water. Bring to boil to dissolve completely. Mix well and distribute in to containers. Sterilize by autoclaving at 121°C for 25 minutes. Allow the set as slope with 2.5 cm butts. PH: 7.4 + 0.2 at 25°C.

Composition (g/l): 'lab-lemco' powder 3.0; peptone 20.0; sodium chloride 5.0; lactose 10.0; sucrose 10.0; glucose 1.0; ferric citrate 0.3; sodium thiosulfate 0.3; phenol red 0.025; agar 12.0

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

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

Composition (g/l): magnesium sulphate 0.20; ammonium dihydrogen phosphate 1.0; dipotassium phosphate 1.00; sodium citrate 2.00; sodium chloride 5.00; bromothymol blue 0.08; agar 15.00.

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

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

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

Tryptone soya agar (Oxoid, England)

Composition (g/l): Pancreatic digest of casein (15.0g) Enzymatic* digest of soya bean (5.0g), Sodium chloride (5.0g) Agar, (15.0g) PH: 7.3 ± 0.2 @ 25°C

Preparation: Dissolve the component in the water by boiling if necessary. Sterilize by autoclaving at 121°C for 15 minutes. PH: 7.3 + 0.1 at 25°C

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

Composition (g/l): beef, dehydrated infusion 300; casein hydrolysate 17.5; starch 1.5; agar 17.00

Preparation: suspend 38 grams in 1000ml of distilled water. Bring to boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes. PH: 7.3 + 0.1 at 25°C.

0.5 McFarland standards

Composition: 1.17% BaCl₂·2H₂O solution and 0.36N of 1% sulfuric acid (H₂SO₄).

Preparation: Add approximately 85ml of 1% H₂SO₄ to a 100ml of volumetric flask, using a 0.5ml pipette add 0.5ml of 1.17% BaCl₂·2H₂O drop wise to the H₂SO₄ while constantly swirling the flask. Bring to 100ml with 1% H₂SO₄. Place a magnetic stirring in the flask and place on the magnetic stirrer for approximately three to five minutes. Examine solution visually to make certain it appears homogeneous and free of visible clumps. Dispense three to seven ml, cub tube tightly and seal with paraffin and keep at dark and room temperature.

Appendix 4. Chemicals and reagents for *E.coli* O157: H7

Kovac's reagent

Preparation: Dissolve 10g P-dimethylamino benzaldehyde (Sigma, Steinheim, Germany) in 150 ml ethanol alcohol, and slowly add 50ml concentrated hydrochloric acid while constantly stirring the mixture. Finally, pale color was formed and stored in brown bottle at refrigerator.

Methyl Red reagents

Preparation: Dissolve (0.1g) methyl red in 300ml alcohol and finally add 200ml-distilled water. Finally, red color was formed and stored in brown bottle at refrigerator.

Voges-Proskauer (VP) reagent

Preparation: a) VP-reagent-1: Dissolve 5g α -Naphthol (Sigma, Steinheim, Germany) in small amount of ethyl alcohol and bring to 100ml in flask. Alcohol should be color less. Then store in Brown bottle and in refrigerator. b) VP-reagent-2: Add less than 100ml distilled water to 40g pellets of KOH in cold water bath to prevent overheating and bring to 100ml. Finally 40% solution that store in polyethylene bottle at refrigerator.

Appendix 5. Questionnaires



Knowledge-, Attitudes, and Practices regarding Food safety/Hygiene among Meat handlers in Ethiopia: One Health approach

Dear participant of this study,

Greetings,

I am a PhD student in the Veterinary Public Health Program at the College of Veterinary Medicine and Agriculture of Addis Ababa University. I would like to ask your participation in this study by filling this questionnaire format based on your voluntariness. The aim of this study is to assess the Knowledge, Attitude and Practices (KAP) of Food safety/hygiene in Ethiopia. There is no apparent risk on you. However, the result of this study will help us improve actions taken in response to the wellbeing of the public. Filling of this questionnaire format may take 15-20 minutes.

Do you agree to participate in this study? Yes No

If yes, please indicate X above and continue filling the remaining questionnaire. If not, don't do anything and kindly return the format.

If you have any questions, please contact Dr Fufa Abunna, at fufa.abunna@aau.edu.et, +251-911899435

By taking part, you are agreeing that you have read and understood the information about the study described above.

Tick your answer with X in the box or write your answers on the space provided

Thank you!!!

Basic Demographic Data

Sex Female Male

1. Marital status Single Married Divorced/separated

2. Age (years) _____

3. Level of study Illiterate Read and write

Elementary School High School College and University

4. Year of services (Years) _____

Meat handlers' food safety Knowledge in abattoirs and retail meat shops

5. Improper handling of meat could pose health hazards to consumers

Yes No

6. Regular washing of hands before and during meat processing reduces risk of contamination

Yes No

7. Using gloves while handling meat reduces the risk of contamination Yes No

8. Proper cleaning and sanitization of knives and hooks reduce the risk of meat contamination.

Yes No

9. Eating and drinking in the work place increase the risk of meat contamination Yes No
10. Washing and disinfection of working surfaces and tools are important for safety of meat
Yes No
11. Regular rotation of disinfectants for cleaning can reduce the risk of meat contamination from working surfaces and cutting tools Yes No
12. Insects and pests could be a source of contamination to raw meat Yes No
13. Diarrhea can be transmitted by food Yes No
14. *E.coli* is one of the food-borne pathogens Yes No
15. Hepatitis A virus is one of the food-borne pathogens Yes No
16. Staphylococcus is one of the food-borne pathogens Yes No
17. Microbes are on the skin, nose and mouth of healthy meat handlers Yes No
18. Clean is same as sanitized Yes No
19. Cross contamination is when microorganisms from a contaminated meat are transferred by the meat handler's hands or utensils to another Yes No
20. The ideal place to store raw meat is in the refrigerator Yes No
21. Freezing kills all the bacteria that may cause food-borne illness Yes No
22. High temperature or freezing is a safe method to destroy bacteria Yes No
23. Contaminated meat always have some change in color, odor or taste Yes No
24. People with open skin injury, gastroenteritis, and ear or throat diseases should not be allowed to handle meat Yes No
25. The health status of workers should be evaluated before employment. Yes No

MEAT HANDLERS' FOOD SAFETY ATTITUDE IN ABATTOIRS AND RETAIL MEAT SHOPS

- 1) Meat handlers with wounds, bruises or injuries on their hands must not touch or handle meat
Yes No
- 2) Using watches, earrings and rings will increase the risk of meat contamination)
Yes No
- 3) Improper meat storage is dangerous to health
Yes No
- 4) Hand washing before handling meat reduces the risk of contamination Yes No
- 5) Regular training could improve meat safety and hygiene practices Yes No
6. Safe meat handling to avoid contamination and diseases is part of meat handler job responsibilities
Yes No
7. Keeping working surfaces and utensils clean reduces the risk of illness Yes No
8. Using different knives and cutting boards for meat and offal is worth Yes No
9. It is unsafe to leave meat out of the refrigerator for more than 2 hour. Yes No
10. Inspecting meat for freshness and wholesomeness is valuable Yes No
11. Surfaces and equipment should be clean before re-using for meat processing
Yes No
12. After processing meat, any leftovers should be kept in a cool place within Yes No
13. Raw meat is healthier and nutritious than cooked Yes No

14. Knives, hooks and cutting boards can be a source of food contamination

Yes No

15. Knives and cutting boards should be properly sanitized to prevent cross contamination Yes No

16. The same towel can be used to clean many places Yes No

17. Sneezing or coughing without covering our noses or mouth could contaminate the meat Yes No

18. Wearing protective clothing and shoes could help improve work safety and hygiene practices Yes No

19. Putting on hair cover on the head is a good practice in food industry Yes No

20. It is important to use potable water to wash working surfaces and cutting tools after disinfection Yes No

Food safety practices questions for meat handlers in abattoir and retail meat shops

26. Do you eat or drink at your work place? Yes No

27. Do smoke inside meat processing areas? Yes No

28. Do you use gloves while handling meat? If no, go to question no. Yes No

29. Do you wash your hands properly before or after using gloves? Yes No

30. Do you wash your hands before and after handling meat? Yes No

31. Do wash hands after handling waste/garbage? Yes No

32. Do wash hands after using toilet? Yes No

33. Do you wash your hand after smoking, sneezing or coughing? Yes No

34. Do you wear an apron while working? Yes No

35. Do you wash your aprons after each day's work? Yes No

36. Do you wear a mask while working? Yes No

37. Do you wear a hairnet or a cap while working? Yes No

38. Do you wear nail polish when handling meat? Yes No

39. Do you properly clean the meat storage area before storing new products?

Yes No

40. Do you use the sanitizer when washing service utensils (knives, hooks and cutting boards)? Yes No

41. Do you replace knives or sterilize them after each meat processing? Yes No

42. Do you remove your work equipment when using toilets? Yes No

43. Do you remove your personal stuffs such as rings, necklaces, watch etc. while processing meat? Yes No

44. Do you handle/process meat when you are ill? Yes No

45. Do you handle/process meat when you have cuts, wounds, bruises or injuries on your hands? Yes No

46. Do you know ONE HEALTH? Yes No

If yes, describe _____



በኢትዮጵያ በስጋ አቅራቢዎች መካከል የምግብ ደህንነት / ንፅህናን አስመልክቶ ዕውቀት ፣ አመለካከቶች እና ልምምዶች-አንድ የጤና አቀራረብ

ውድ የዚህ ጥናት ተሳታፊ ፣

ሰላምታ

በአዲስ አበባ ዩኒቨርሲቲ የእንሰሳት ህክምና ኮሌጅ እና እርሻ ኮሌጅ የእንሰሳት ጤና ጥበቃ ሀብረተሰብ ፕሮግራም ውስጥ የፔሌቸዲ ተማሪ ነኝ ። በፈቃደኝነት ላይ በመመርኮዝ ደህንን መጠይቅ ቅርጾች በመሙላት በዚህ ጥናት ውስጥ ተሳትፎዎን መጠየቅ እፈልጋለሁ ። የዚህ ጥናት ዓላማ በኢትዮጵያ ውስጥ የምግብ ደህንነት / ንፅህናን ዕውቀት ፣ አመለካከት እና ልምምዶች (KAP) መገምገም ነው ። ለጥያቄዎቹ ሁሉ የሚሰጡዎት እውነተኛ ምላሾች እጅግ በጣም አስፈላጊ ናቸው ። በዚህ ምርምር ውስጥ በመሳተፍ ምክንያት ለእርስዎ ወይም ለድርጅትዎ ምንም ዓይነት የግል ጥቅም ወይም አደጋ የለም ። ሆኖም ከዚህ በላይ እንደተገለጸው ጥናቱ የህዝቡን ደህንነት በመጠበቅ ረገድ የሚወሰዱ እርምጃዎችን ለማሻሻል የምግብ ደህንነትን በተመለከተ የእውቀት ደረጃን በመረዳት ረገድ በጣም አስፈላጊ ነው ብለን እናምናለን ። የእርስዎ ተሳትፎ ሙሉ በሙሉ በፈቃደኝነት ነው። እንደ ጥሩ የምርምር ተግባር አካል እርስዎ መልስዎ በሚስጥር እንደሚቆይ እና ስለ የግል ማንነትዎ እና ስለድርጅትዎ ማንነት የሚሰበሰብ ማንኛውም መረጃ ለሶስተኛ ወገን እንደማይጋራ እና ረገጥጥልዎታለን ። ሪፖርቱ ለዚህ ጥናት ሲዘጋጅ የተወሰኑ ጥያቄዎችን እና መልሶችን በተመለከተ የድርጅትዎ ማንነት ወይም ማንነት ለሰው አይገለጽም ። በስምምነትዎ መሠረት በሪፖርቱ ውስጥ የተዘረዘሩትን ድርጅትዎን ማስገባት እንችላለን ። በአንተ ላይ ምንም ግልጽ አደጋ የለም ። የዚህን መጠይቅ ቅርጾች መሙላት ከ15-20 ደቂቃዎች ሊወስድ ይችላል።

በዚህ ጥናት ውስጥ ለመሳተፍ ተስማምተዋል? አዎ አይ

አዎ ከሆነ እባክዎን ከላይ ያመልክቱ እና የቀረውን መጠይቅ መሙላትዎን ይቀጥሉ። ካልሆነ ምንም ነገር አያድርጉ እና ቅርጹን በደግነት ይመልሱ። ማንኛውም ጥያቄ ካለዎት እባክዎን ዶ / ሮ ፉፋ አቡናን በ fufa.abunna@aau.edu.et ፣ + 251-911899435 ወይም chs.irb@aau.edu.et እና 01118961396 ያነጋግሩ

ተካፋይ በመሆንዎ ከላይ ስለተገለጸው ጥናት መረጃውን አንብበው እንደተረዱ እየተስማሙ ነው ።

አመሰግናለሁ!!!

መሰረታዊ የስነ-ህዝብ መረጃ

1. ፆታ ሴት ወንድ
2. የጋብቻ ሁኔታ ነጠላ ጋብቻ በፍቺ / በመለያየት
3. ዕድሜ (ዓመታት) _____
4. የጥናት ደረጃ መሃይም ማንበብ እና መፃፍ

የመጀመሪያ ደረጃ ትምህርት ቤት ሁለተኛ ደረጃ ትምህርት ቤት ኮሌጅ እና ዩኒቨርሲቲ

5. የአገልግሎት ዓመት (ዓመታት) _____

ስጋ አስተናጋጆች የምግብ ደህንነት በአዳዎች እና በችርቻሮ ሥጋ ሱቆች ውስጥ ዕውቀት

- 6. ተገቢ ያልሆነ የስጋ አያያዝ ለተጠቃሚዎች የጤና አደጋ ያስከትላል። አዎ አይደለም
- 7. ከስጋ ማቀነባበሪያው በፊት እና ወቅት አዘውትሮ እጅን መታጠብ የብክለት አደጋን ይቀንሳል። አዎ አይደለም
- 8. ስጋን በሚይዙበት ወቅት ጓንት መጠቀም የብክለት ተጋላጭነትን ይቀንሳል። አዎ አይደለም
- 9. ቢላዎችን እና መንጠቆዎችን በትክክል ማፅዳትና ማጽዳት የስጋ ብክለት አደጋን ይቀንሳል። አዎ አይደለም
- 10. በሥራ ቦታ መብላት እና መጠጣት የስጋ መበከል ተጋላጭነትን ይጨምራል። አዎ አይደለም
- 11. የሥራ ቦታዎችን እና መሣሪያዎችን ማጠብ እና ማጽዳት ለሥጋ ደህንነት አስፈላጊ ናቸው። አዎ አይደለም
- 12. ለጽዳቶች አዘውትሮ መዘሪያዎችን ማጽዳት ለሥራ ከሚሠሩ ቦታዎች እና ከመቁረጫ መሳሪያዎች የስጋ ብክለት አደጋን ሊቀንስ ይችላል። አዎ አይደለም
- 13. ነፍሳት እና ተባዮች ጥሬ ሥጋን የመበከል ምንጭ ሊሆኑ ይችላሉ። አዎ አይደለም
- 14. ተቅማጥ በምግብ ሊተላለፍ ይችላል። አዎ አይደለም
- 15. ኢኮሊ ከምግብ ወለድ በሽታ አምጪ ተህዋሲያን አንዱ ነው። አዎ አይደለም
- 16. ሄፓታይተስ ኤ ቫይረስ ከምግብ ወለድ በሽታ አምጪ ተህዋሲያን አንዱ ነው። አዎ አይደለም
- 17. ስታፊሎኮከስ ከምግብ ወለድ በሽታ አምጪ ተህዋሲያን አንዱ ነው። አዎ አይደለም
- 18. ረቂቅ ተሕዋስያን ጤናማ የሥጋ አሠሪዎች በቆዳ ፣ በአፍንጫ እና በአፍ ላይ ናቸው። አዎ አይደለም
- 19. ንፅህና ከተጣራ ጋር ተመሳሳይ ነው። አዎ አይደለም
- 20. የመስቀል ብክለት ከተበከለ ስጋ የሚመጡ ረቂቅ ተህዋሲያን በስጋ ጠባቂው እጆች ወይም ዕቃዎች ወደ ሌላ ሲተላለፍ ነው። አዎ አይደለም
- 21. ጥሬ ሥጋን ለማከማቸት ተስማሚ ቦታ በማቀዝቀዣ ውስጥ ነው። አዎ አይደለም
- 22. ማቀዝቀዣ በምግብ ወለድ በሽታ ሊያስከትሉ የሚችሉትን ባክቴሪያዎች ሁሉ ይገድላል። አዎ አይደለም
- 23. ባክቴሪያዎችን ለማጥፋት ከፍተኛ ሙቀት ወይም ማቀዝቀዣ አስተማማኝ ዘዴ ነው። አዎ አይደለም
- 24. የተበከለው ስጋ ሁልጊዜም በቀለም ፣ በመዳዘ ወይም በጣዕም የተወሰነ ለውጥ አለው። አዎ አይደለም
- 25. ክፍት የቆዳ ጉዳት ፣ የጨጓራ እና የጆሮ ወይም የጉሮሮ በሽታዎች ያሉባቸው ሰዎች ስጋን እንዲይዙ አይፈቀድላቸውም። አዎ አይደለም
- 26. የሰራተኞች የጤና ሁኔታ ከቅጥር በፊት መገምገም አለበት። አዎ አይደለም

የሥጋ አስተናጋጆች ምግብ ደህንነት ሁኔታ እና የችርቻሮ ሥጋ ሱቆች

- 1) በእጃቸው ላይ ቁስሎች ፣ ቁስሎች ወይም ቁስሎች ያሉባቸው የስጋ አስተናጋጆች ሥጋን መንካት ወይም ማስተናገድ የለባቸውም። አዎ አይደለም
- 2) ሰዓቶችን ፣ ጉትቻዎችን እና ቀለበቶችን በመጠቀም የስጋ መበከል ተጋላጭነትን ከፍ ያደርገዋል። አዎ አይደለም
- 3) ተገቢ ያልሆነ የስጋ ክምችት ለጤና አደገኛ ነው። አዎ አይደለም
- 4) ስጋን ከመያዝ በፊት እጅን መታጠብ የብክለት አደጋን ይቀንሳል። አዎ አይደለም
- 5) መደበኛ ስልጠና የሥጋ ደህንነትን እና የንፅህና አጠባበቅ ልምዶችን ሊያሻሽል ይችላል። አዎ አይደለም
- 6) ብክለትን እና በሽታዎችን ለማስወገድ ደህንነቱ የተጠበቀ የስጋ አያያዝ የስጋ አስከባሪ የሥራ ኃላፊዎች አካል ነው። አዎ አይደለም
- 7) የሥራ ቦታዎችን እና ዕቃዎችን በንጹህና መጠበቁ የበሽታዎችን ተጋላጭነት ይቀንሳል።አዎ አይደለም
- 8) የተለያዩ ቢላዎችን እና የመቁረጫ ቦርዶችን ለስጋ እና ለ offal መጠቀም ዋጋ አለው። አዎ አይደለም

- 9) ስጋን ከ 2 ሰዓት በላይ ከማቀዝቀዣ ውስጥ መተው ጤናማ አይደለም። አዎ አይደለም
- 10) ስጋን ለአዲስ እና ለጠቅላላ ማንነት መመርመር ዋጋ አለው። አዎ አይደለም
- 11) ለስጋ ማቀነባበሪያ እንደገና ከመጠቀም በፊት ቦታዎች እና መሳሪያዎች ንፁህ መሆን አለባቸው። አዎ አይደለም
- 12) ስጋን ከተቀነባበሩ በኋላ ማንኛውም ተረፈ በውስጣቸው በቀዝቃዛ ቦታ መቀመጥ አለበት። አዎ አይደለም
- 13) ጥሬ ሥጋ ከበሰለ የበለጠ ጤናማና ገንቢ ነው። አዎ አይደለም
- 14) ቢላዎች ፣ መንጠቆዎች እና የመቁረጥ ሰሌዳዎች የምግብ መበክል ምንጭ ሊሆኑ ይችላሉ። አዎ አይደለም
- 15) የመስቀል ብክለትን ለመከላከል ቢላዎች እና የመቁረጥ ቦርዶች በትክክል መፀዳት አለባቸው። አዎ አይደለም
- 16) ተመሳሳይ ፎጣ ብዙ ቦታዎችን ለማፀዳት ሊያገለግል ይችላል። አዎ አይደለም
- 17) አፍንጫችንን ወይም አፋችንን ሳንሸፍን በማስነጠስ ወይም በመሳል ስጋውን ሊበክል ይችላል። አዎ አይደለም
- 18) መከላከያ ልብሶችን እና ጫማዎችን መልበስ የስራ ደህንነት እና የንፅህና አጠባበቅ ልምዶችን ለማሻሻል ይረዳል። አዎ አይደለም
- 19) በፀጉር ሽፋን ላይ ራስ ላይ ማድረግ በምግብ ኢንዱስትሪ ውስጥ ጥሩ ተግባር ነው። አዎ አይደለም
- 20) በፀረ-ተባይ በሽታ ከተያዙ በኋላ የሚሰሩ ቦታዎችን እና የመቁረጥ መሳሪያዎችን ለማጠብብ የመጠጥ ውሃ መጠቀም አስፈላጊ ነው። አዎ አይደለም

በሥጋ ማስቀመጫ እና በችርቻሮ ሥጋ ሱቆች ውስጥ ለስጋ አሠሪዎች የምግብ ደህንነት ልምዶች ጥያቄዎች

- 27. በሥራ ቦታዎ ይመገባሉ ወይም ይጠጣሉ? አዎ አይደለም
- 28. በስጋ ማቀነባበሪያ ቦታዎች ውስጥ ያጩሳሉ? አዎ አይደለም
- 29. ስጋን በሚይዙበት ጊዜ ዳንት ይጠቀማሉ? አዎ አይደለም
- 30. ዳንት ከመጠቀም በፊት ወይም በኋላ እጅዎን በትክክል ይታጠባሉ? አዎ አይደለም
- 31. ስጋን ከመያዝ በፊት እና በኋላ እጅዎን ይታጠባሉ? አዎ አይደለም
- 32. ቆሻሻ / ቆሻሻን ካስተናገዱ በኋላ እጅ ይታጠባሉ? አዎ አይደለም
- 33. መጸዳጃ ቤት ከተጠቀሙ በኋላ እጅ ይታጠባሉ? አዎ አይደለም
- 34. ከማጫስ ፣ በማስነጠስ ወይም ከሳል በኋላ እጅዎን ይታጠባሉ? አዎ አይደለም
- 35. በሚሰሩበት ጊዜ መደረቢያ ይለብሳሉ? አዎ አይደለም
- 36. ከእለት ተእለት ስራ በኋላ መደረቢያዎን ይታጠባሉ? አዎ አይደለም
- 37. በሚሰሩበት ጊዜ ጭምብል ይለብሳሉ? አዎ አይደለም
- 38. በሚሰሩበት ጊዜ የፀጉር መረብ ወይም ኮፍያ ይለብሳሉ? አዎ አይደለም
- 39. ስጋን በሚይዙበት ጊዜ የጥፍር ቀለም ይለብሳሉ? አዎ አይደለም
- 40. አዳዲስ ምርቶችን ከማከማቸት በፊት የስጋ ማስቀመጫ ቦታውን በትክክል ያፀዳሉ? አዎ አይደለም
- 41. የአገልግሎት እቃዎችን (ቢላዎች ፣ መንጠቆዎች እና የመቁረጥ ሰሌዳዎች) ሲታጠቡ የንፅህና ሰራተኛውን ይጠቀማሉ? አዎ አይደለም
- 42. ከእያንዳንዱ የስጋ ማቀነባበሪያ በኋላ ቢላዎችን ይተካሉ ወይም ያፀዳሉ? አዎ አይደለም
- 43. መጸዳጃ ቤቶችን ሲጠቀሙ የሥራ መሣሪያዎን ያስወግዳሉ? አዎ አይደለም
- 29. ስጋን በሚይዙበት ጊዜ ዳንት ይጠቀማሉ? ካልሆነ ፣ ወደ ጥያቄ ቁጥር ይሂዱ ። አዎ አይደለም
- 30. ዳንት ከመጠቀም በፊት ወይም በኋላ እጅዎን በትክክል ይታጠባሉ? አዎ አይደለም

- 31. ስጋን ከመያዝዎ በፊት እና በኋላ እጅዎን ይታጠባሉ? አዎ አይደለም
- 32. ቆሻሻ / ቆሻሻን ካስተናገዱ በኋላ እጅ ይታጠባሉ? አዎ አይደለም
- 33. መጻዳጃ ቤት ከተጠቀሙ በኋላ እጅ ይታጠባሉ? አዎ አይደለም
- 34. ከማጨስ ፣ በማስነጠስ ወይም ከሳል በኋላ እጅዎን ይታጠባሉ? አዎ አይደለም
- 35. በሚሰሩበት ጊዜ መደረቢያ ይለብሳሉ? አዎ አይደለም
- 36. ከእለት ተእለት ስራ በኋላ መደረቢያዎን ይታጠባሉ? አዎ አይደለም
- 37. በሚሰሩበት ጊዜ ጭምብል ይለብሳሉ? አዎ አይደለም
- 38. በሚሰሩበት ጊዜ የፀጉር መረብ ወይም ኮፍያ ይለብሳሉ? አዎ አይደለም
- 39. ስጋን በሚይዙበት ጊዜ የጥፍር ቀለም ይለብሳሉ? አዎ አይደለም
- 40. አዳዲስ ምርቶችን ከማከማቸትዎ በፊት የስጋ ማስቀመጫ ቦታውን በትክክል ያፀዳሉ? አዎ አይደለም
- 41. የአገልግሎት አቃዎችን (ቢላዎች ፣ መንጠቆዎች እና የመቁረጥ ሰሌዳዎች) ሲታጠቡ የንፅህና ሰራተኛውን ይጠቀማሉ? አዎ አይደለም
- 42. ከእያንዳንዱ የስጋ ማቀነባበሪያ በኋላ ቢላዎችን ይተካሉ ወይም ያፀዳሉ? አዎ አይደለም
- 43. መጻዳጃ ቤቶችን ሲጠቀሙ የሥራ መሣሪያዎን ያስወግዳሉ? አዎ አይደለም
- 44. ስጋ በሚሰሩበት ጊዜ እንደ ቀለበት ፣ የአንገት ጌጥ ፣ ሰዓት ወዘተ ያሉ የግል ዕቃዎችዎን ያስወግዳሉ? አዎ አይደለም
- 45. በሚታመምበት ጊዜ ስጋን ያስተናግዳሉ / ያካሂዳሉ? አዎ አይደለም
- 46. በእጆቻዎ ላይ ቁስሎች ፣ ቁስሎች ፣ ቁስሎች ወይም ቁስሎች ባሉበት ጊዜ ስጋን ያስተናግዳሉ / ያስኬዳሉ? አዎ አይደለም
- 47. አንድ ጤናን ያውቃሉ? አዎ አይ
- 48. አዎ ከሆነ ይግለጹ _____



Knowledge, Attitudes, and Practices regarding Transmission of Zoonoses among smallholder farmers of Ada’a district, Oromia, Ethiopia

Dear participant of this study,

Greetings,

This is a PhD research work in the Veterinary Public Health Program at the College of Veterinary Medicine and Agriculture of Addis Ababa University. I would like to ask your participation in this study by filling this questionnaire format based on your voluntariness. The aim of this study is to assess the Knowledge, Attitude and Practices (KAP) of Zoonotic Diseases in Ethiopia. There is no apparent risk on you. However, the result of this study will help us improve actions taken in response to the wellbeing of the public. Filling of this questionnaire format may take 15-20 minutes.

Do you agree to participate in this study? Yes No

If yes, please indicate ‘X’ above and continue filling the remaining questionnaire. If not, don’t do anything and kindly return the format.

If you have any questions, please contact Dr Fufa Abunna, at fufa.abunna@aau.edu.et, +251-911899435

By taking part, you are agreeing that you have read and understood the information about the study described above.

Tick your answer with ‘X’ in the box or write your answers on the space provided

Thank you!!!

Section 1. BASIC DEMOGRAPHIC DATA

- 1) Profession of the respondent: _____
- 2) Gender of the respondent: Male Female
- 4) Age (years) _____
- 5) Marital status Single Married Widowed Divorced
- 6) Type of toilet used Open field Ordinary with septic tank

Section 2. KNOWLEDGE

1. Do you know what a zoonotic disease is?
 Yes No
If yes, please describe: _____
2. When animals are sick in your flock, you can get the same sickness.
 True False
3. Many animal diseases can be transmitted from animals to humans
 True False
4. Identify the name of diseases which can be transmitted from animals to humans

5. Please list at least one symptom for any one zoonotic disease in animals

-
6. Animal disease can be transmitted via different routes to humans Yes No
7. Eating uncooked meat can transmit diseases from animals to human True False
8. Drinking of raw milk can transmit diseases from animals to human Yes No
9. Close contact with sick/dead animal can transmit diseases to human Yes No
10. You can get infection from environment contaminated from secretions of sick animals Yes No
11. Insect bite can transmit animal diseases to human Yes No
12. Animal bites can transmit diseases to human Yes No
13. Animal abortion can cause a serious economic and public health problem Yes No
14. Which zoonotic diseases are common in your area?

-
15. For you, what are the available sources of information about zoonotic diseases
- Daily or weekly newspapers Conversations with family, colleagues and friends Radio stations Celebrities and social media influencers Papers (newspapers, posters) Videos (channels, YouTube) Other sources,

16. In which of the following ways, animals can transmit infections to humans?

- a) Drinking raw milk from animals Yes No Don't know
- b) Drinking yoghurt from animals Yes No Don't know
- c) Eating cheese from animals Yes No Don't know
- d) Eating meat from animals Yes No Don't know
- e) Being in close contact with animals Yes No Don't know
- f) Being bitten by an animal Yes No Don't know

17. Which of the following diseases in which humans contract from consumed milk?

- a) Bovine tuberculosis Yes No Don't know
- b) Anthrax Yes No Don't know
- c) Brucellosis Yes No Don't know

18. Which of the following diseases can be transmitted to humans via meat consumption?

- a) Bovine tuberculosis Yes No Don't know
- b) Anthrax Yes No Don't know
- c) Cysticercosis Yes No Don't know
- d) Brucellosis Yes No Don't know

Section 3: ATTITUDES ON MAJOR ZOOONOTIC DISEASES

19. Do you agree with consuming or using the following products from sick or dead animals?

- a) Meat Yes No Don't know
- b) Milk Yes No Don't know
- c) Yoghurt Yes No Don't know
- d) Cheese Yes No Don't know
- e) Offal Yes No Don't know
- f) Hide/skin Yes No Don't know
- g) Wool Yes No Don't know

20. Do you agree with doing the following activities?

- a) Do you agree with eating meat from a regularly aborting sheep/goat? Yes No Don't know

- b). Do you agree with eating raw or undercooked meat of animals? Yes No Don't know
- c) Do you agree with eating raw or undercooked meat of animals? Yes No Don't know
- d) Do you agree with drinking raw milk? Yes No Don't know
- e) Do you agree with drinking raw milk? Yes No Don't know
- f) Do you agree with drinking raw Yoghurt? Yes No Don't know
- g) Do you agree with eating raw cheese? Yes No Don't know
- h) Do you agree with touching stray dogs Yes No Don't know

PRACTICE ON MAJOR ZOO NOTIC DISEASES

21. Do you drink raw milk? Yes No Don't know
22. Do you wash your hands after having contact with animals? Yes No Don't know
23. Do you prefer walking bare feet at home? Yes No Don't know
24. Do you prefer walking bare feet at farm or garden? Yes No Don't know
25. Have you ever done deworming of your animal? Yes No Don't know
26. Have you ever tested your animals for Brucellosis? Yes No Don't know
27. Have you ever tested your animals for T.B.? Yes No Don't know
28. Do you know ONE HEALTH? Yes No

If yes, what is it?



Bekumsa, ilaalcha fi raawwatamaa barsiisoota Yunivarsiitiifi qotee bulaa wa'ee dhibee horii irraa gara namatii darbuu: Qorannoo jiddu-seenuu akkaataa Fayyaa Tokkummaa Unka Gaaffii

Kabajamoo hirmaataa qorannoo kanaa, Nagaan isiniif haa ta'u, Aanii Barataa PhD Yuniversiitii Finfinnee irraa qorannoo waa'ee dhibee daddaarboo horii irraa gara namaattii daddaarbaa, waa'ee qulqullinnaa nyaataa, waa'ee ittii fayyadama qoriicha irrattii geggeessaan jirra. Kaayyon qorannoo kun, Bekumsa, Ilaalcha fi Raawwatamaa ilaala. Unka gaaffii kana guutuun, fedhii keessanin qorannoo kana keessatti akka hammatamtan kabajaan isin gaafanna. Hirmachuun keessanin kan ka'e rakkina isin irratti hin fidu, dabalataanis bu'aa addas isinii hin fidu. Garuu bu'a qorannoon kun raawwii deebii-gaaffii ilaallatee geggeefama jiru gargaara. Unka kana guutuuf daqiiqaa 15-20 isin fudhachuu danda'a.

Qorannoo kana keessatti hirmaachuuf fedhii qabduu?

Eyyeen Lakki Eyyeen yoo ta'e, unka kana guutuu itti fufi. Lakki yoo ta'e, dhiisiti qorattotatti deebisi. Gaaffii dabalataa yoo qabaattaniif, Dr Fufa Abunna, E-mail: fufa.abunna@aau.edu.et, Telefona: +251-911899435 qunnamaa.

Gaffii keessan sanduqa keessa X mallatto kaa ykn gaaffii keessan bakka duwwaa jirutti barreessa.

Galatoomaa

Kutaa 1. Wa'ee dhimmaa dhuunffaa

1. Ogummaa keessaani: _____
2. Saala Dhiraa Dubarttii
3. Umurii (wagaa) _____
4. Haalaa fuudhaafii eerumaa Hinherumeera/hinfuudheeraaarried Najala du'ee/duutee
Herumeera/fuudheeraaarried Gaahilaa digee jiraa
5. Haala mana fincaanii Hurufaa Hamayyaa kaan septik tankii kaan qabuu

Kutaa 2. Beekumssaa

7. Dhibee Zoonosiis jedhaamuu beektaa? Eeeyyeen Lakkii Yoo eeeyyeen jettee ibsii: _____
8. Yoo horriin dhukkubsattu, atiis dhukkubsachuu danddeessaa. Eeeyyeen Lakkii
9. Dibee baayyeen horriirra namaatii dadaarbuu Eeeyyeen Lakkii
10. Dhibee horii irraa namaatii daddarbaani ibsii _____
11. Maloo mallaattoo dhibee horii irraa namaatii darbaa ibsii Eeeyyeen Lakkii
12. Dibee horii haalaa ada addaatiin namattii darbaa Eeeyyeen Lakkii
13. Foon hinbilchannee nyaachuun dhibee fidaa Eeeyyeen Lakkii
14. Annaan hindanfinee dhuguun dhibee fidaa Eeeyyeen Lakkii
15. Horii dhukkubu tutuquun dhibee fidaa Eeeyyeen Lakkii
16. Dhnagala'aa qaamaa horii keessaa bahuun dhibeedhaan qabamuun nidanda'amaa Eeeyyeen Lakkii
17. Bookeedhaan hidamuun dhibee fidaa Eeeyyeen Lakkii 18. Ciniinnaa horiitiin dhibeen nidhufa
Eeeyyeen Lakkii
19. Gataachuun saawwaa kufaattii qabeeynaafii fayyaa fidaa Eeeyyeen Lakkii

20. Dibee kaamfaatuu jiraa naannoo keessaan? _____
21. Odeefaannoo eessaa argaattuu? Gaazzeexxaa Maattii Radioo stations Midiyyaa Hawassumma
22. Haalootaa kamiin horiin dhibee dabarssu?
- a. Anaan otoo hindanfinfiinnnee dhuguu Eeyyeen Lakkii hinbeekuu
- b. Urgoo dhuguu Eeyyeen Lakkii Hinbeekuu c) Chiizzii nyaachuu Eeyyeen Lakkii Hinbeekuu
- d) Foon nyaachuu Eeyyeen Lakkii Hinbeekuu
- e) Horii tutuqquu Eeyyeen Lakkii Hinbeekuu
- f) Horii ciniinamuu Eeyyeen Lakkii Hinbeekuu
23. Dhibee kaam yoo aannaan otoo hindanfisiin nuttii dhufaa? a. Gatachiisaa Eeyyeen Lakkii Hinbeekuu b. Abbaa sangaa Eeyyeen Lakkii Hinbeekuu c. Daranyoo sonbaa Eeyyeen Lakkii Hinbeekuu
24. Dhibee kaam yoo foon nyaachuun nuttii dhufaa? a. Gatachiisaa Eeyyeen Lakkii Hinbeekuu b. Abbaa sangaa Eeyyeen Lakkii Hinbeekuu c. Koosoo Eeyyeen Lakkii Hinbeekuu d. Daranyoo sonbaa Eeyyeen Lakkii Hinbeekuu

Kutaa 3: Ilaalchaa

25. Horii dhukubsata irraa warreen kanaan gadii fayyadamuu jalattaa?
26. Foon Eeyyeen Lakkii
27. Aannaan Eeyyeen Lakkii Hinbeekuu
28. Urgoo Eeyyeen Lakkii Hinbeekuu
29. Chiizzii Eeyyeen Lakkii Hinbeekuu
30. Xeerii Eeyyeen Lakkii Hinbeekuu
31. Gogaafii kallee Eeyyeen Lakkii Hinbeekuu
32. Rifeensaa Eeyyeen Lakkii Hinbeekuu
33. Waan kaneen dalaguu jalattaa? Eeyyeen Lakkii Hinbeekuu
34. Foon re'ee/hoolaa yeeroo baayyee gatatu irraa nyaachuu? Eeyyeen Lakkii Hinbeekuu
- b). Foon/Aannaan hin bilchannee Eeyyeen Lakkii Hinbeekuu
36. Aannaan hindanfinee dhuguu? Eeyyeen Lakkii Hinbeekuu
37. Urgoo dheedhii dhuguu? Eeyyeen Lakkii Hinbeekuu
38. Chiizzii dheedhii nyaachuu? Eeyyeen Lakkii Hinbeekuu
39. Saree kara deemttuu qabachuu Eeyyeen Lakkii Hinbeekuu
40. Fayyaa tokkoo beektaa? Eeyyeen Lakkii Hinbeekuu
- Eeyyeen yoojettee maalii hinnii?



Knowledge, Attitudes, and Practices regarding Antimicrobial Resistance and Use among University students in Ethiopia: One Health approach

Dear participant of this study,

Greetings,

This is a PhD research work in Veterinary Public Health Program at the College of Veterinary Medicine and Agriculture of Addis Ababa University. I would like to ask your participation in this study by filling this questionnaire format based on your voluntariness. The aim of this study is to assess the Knowledge, Attitude and Practices (KAP) of Antimicrobial Resistance and Usage in Ethiopia. There is no apparent risk on you. However, the result of this study will help us improve actions taken in response to the wellbeing of the public. Filling of this questionnaire format may take 15-20 minutes.

Do you agree to participate in this study? Yes No

If you have any questions, please contact Dr Fufa Abunna, at fufa.abunna@aau.edu.et, +251-911899435

By taking part, you are agreeing that you have read and understood the information about the study described above.

Thank you!!!

Demographic information

1. Sex Female Male
2. Age (years)
3. Study year 1 2 3 4 5 6 PG
4. Major/discipline of study Health science Veterinary Non-health science

KNOWLEDGE OF ANTIBIOTICS USE AND ANTIMICROBIAL RESISTANCE IN HUMANS

5. Microorganisms can become resistant to antimicrobial True False Don't know
6. Antibiotics can be used to treat which of the following conditions? (Please check all that apply).
 HIV/AIDS Gonorrhoea Urinary tract infection (UTI) Cold and flu Fever
Malaria Measles Skin or wound infection Sore throat Bodyaches
Headaches
7. Are there bacteria in the human body that are good for our health? Yes No
8. Can antibiotics be used to cure infections caused by bacteria? Yes No
9. Can antibiotics be used to cure infections caused by virus? Yes No
10. Do you think the use of antibiotics speeds up recovery from colds, coughs, and other diseases? Yes
No
11. Are Antibiotics obtainable without a prescription at pharmacies? Yes No
12. Have you heard of resistance of bacteria? Yes No
13. Do you think frequent use of antibiotics will decrease their efficacy when used again? Yes No

14. Is efficacy better if the antibiotics are newer and the price higher? Yes No
15. Is Amoxicillin is an antibiotic? Yes No
16. Penicillin is an antibiotic. Yes No
17. Tetracycline is an antibiotic. Yes No
18. Bacteria causing diseases in animals can become resistant to antibiotics?
 True False Don't know
19. Treatment of sick animals should be carried out by? (Please check all that apply).
 Animal attendants Pet owners Farm managers Veterinarians
20. Antibiotics are better sourced from drug stores (over the counter) based on prescription
 Yes No Don't know
21. Antibiotics should be used as additives to compound animal feeds Yes No Don't know
22. Are you aware that antibiotics used to treat animals can remain within their tissues?
 Yes No Don't know
23. What is the earliest time (days) food animals treated with antibiotics be sold for consumption?
 Your answer
24. Antibiotics are potent against which of these? (Please check all that apply).
 Non pathogenic bacteria Pathogenic bacteria Viruses Fungi
 Prions Parasites Toxins

FACTORS CONTRIBUTING TO ANTIMICROBIAL RESISTANCE

25. The following contribute to the problem of antimicrobial resistance: (Please check all that apply).
- ✓ Proliferation of various antimicrobial agents in circulation
 - ✓ Use of antimicrobials in livestock and food production as growth promoter
 - ✓ Use of antibiotics to prevent diseases in healthy animals
 - ✓ Poor disease prevention and control strategies
 - ✓ Under dosage of antibiotics prescription
 - ✓ Too long durations of antimicrobial therapy
 - ✓ Poor hygiene and sanitation
 - ✓ Inadequacy of drug regulation agencies
 - ✓ Non availability of new antibiotics
 - ✓ Environmental factors and climate change

ATTITUDE TO ANTIMICROBIAL RESISTANCE

26. Antimicrobial resistance will affect you and your family's health.
 Yes No
27. Antimicrobial resistance will affect animal health and production Yes No
28. I have sufficient knowledge on antibiotics use for future clinical practice Yes No
29. It is necessary to give more education to clinical level students about antimicrobial resistance.
 Yes No
30. Inappropriate use of antimicrobials causes antimicrobial resistance. Yes No
31. Poor infection control practices by veterinarians and other health care professionals will cause spread of antimicrobial resistance. Yes No

32. Clinical level students should get special training on the appropriate ways of prescribing antimicrobials before graduation. Yes No
33. You have to follow existing guidelines on the use of antimicrobials in the future. Yes No
34. Currently, antimicrobial resistance is a major problem in the world as well as in Ethiopia. Yes No
35. Prescribing antibiotics should be more closely controlled. Yes No
36. Dispensing antibiotics without prescription or over-the-counter should be more closely controlled. Yes No
37. People's socioeconomic status has an effect on the risk of being affected by antibiotic resistance. Yes No
38. The consequences of antimicrobial resistance will affect your future work as a health professional when caring for patients with bacterial infections. Yes No
39. Students can contribute to the work being done to control antimicrobial resistances. Yes No
40. Research on antimicrobial resistance is necessary and students should be involved. Yes No
41. There is a link between human, animal and environmental health in terms of antimicrobial resistance. Yes No

QUESTIONS ON THE PRACTICE OF ANTIBIOTIC USE

Practice of use antibiotics:

42. For fever Yes No
43. I stop antibiotics as soon as complaints lessen Yes No
44. Prescribed by doctors Yes No
45. I follow prescription after choosing antibiotic Yes No
46. I use antibiotics without doctor's instructions Yes No
47. I ask doctor to prescribe antibiotics for common cold Yes No
48. For common cold Yes No
49. For acute bronchitis Yes No
50. For pneumonia Yes No
51. When coughing up yellow/green phlegm Yes No
52. I use antibiotics for sore throat Yes No
53. For cough with fever Yes No
54. When did you take antibiotics last? If "Never" skip the next 6 questions.
 the last month In the last 6 months In the last year More than a year ago
 Can't remember
55. On that occasion, did you get the antibiotics from a doctor's prescription? Yes No
 can't remember
56. On that occasion, did you get advice from a doctor, nurse or pharmacist on how to take them? Yes
 No Can't remember
57. On that occasion, where did you get the antibiotics? Stall or hawker Friend or family member
 I had them saved up from previous time Medical store or Pharmacy Can't remember
58. When do you think you should stop taking antibiotics once you've begun treatment?
 When you feel better When you've taken all of the antibiotics as directed Don't know
59. "It's okay to use antibiotics that were given to a friend or family member, as long as they were used to treat the same ill True False

60. "It's okay to buy the same antibiotics, or request these from a doctor if you are sick and they helped you get better when you had the same symptoms before" True False
61. Do you know ONE HEALTH? Yes No
62. If yes, what is it?
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