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Prevalence and Antimicrobial Resistance of *Salmonella enterica* in Cow Milk and Cottage Cheese in Major Milk Shades of Oromia Region, Ethiopia.

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Table of Contents

List of Tables iv
List of Figures v
List of Abbreviations vi
Acknowledgments vii
Abstract viii
1. INTRODUCTION 1
1.1. Background and Justification 1
1.2. Statement of the problem 4
1.3. Objectives of the study 5
1.3.1. General Objective 5
1.3.2. Specific Objectives 5
2. LITERATURE REVIEW 6
2.1. Food hygiene and food safety 6
2.2. Risk factors potentially associated with foodborne illnesses 8
2.3. History of *Salmonella* 9
2.4. Virulence factors and pathogenesis 11
2.5. Clinical manifestations 13
2.5.1. Enteric fever 13
2.5.2. Gastroenteritis 14
2.5.3. Bacteraemia and other extraintestinal complications 14
2.5.4. Chronic carrier state 15
2.6. Sources and transmission of *Salmonella* 15
2.7. Epidemiology of Salmonellosis 16
2.8. *Salmonella* characterization 17
2.8.1. Isolation and Identification of *Salmonella* 18
2.8.1.1. Conventional Culturing Method 18
2.8.1.2. Molecular Based Detection 19
2.9. Antibiotic resistance in *Salmonella* Spp. 20
2.9.1. Mechanisms of resistance 21

2.9.2. Prevention and control measures 22
3. MATERIALS AND METHODS 23
3.1. Description of the study areas 23
3.2. Study Design and Approach 24
3.3. Sample size determination 24
3.3.1. Sampling methods 25
3.4. Microbial analysis of *Salmonella enterica* 25
3.4.1. Primary Enrichment 25
3.4.1.1. Fluid Milk 25
3.4.1.2. Cheese 25
3.4.2. Secondary Enrichment: 26
3.4.3. Plating onto Selective Agar 26
3.4.4. Detection: 27
3.5. DNA extraction 28
3.6. PCR confirmation 28
3.6.1. Primers set 28
3.6.2. PCR amplification program 29
3.6.2.1. *invA* PCR 29
3.6.2.2. Multiplex PCR for the detection of serotypes 30
3.7. Electrophoresis of PCR products: 31
3.8. Antimicrobial susceptibility testing 32
3.9. Survey Data Collection 32
3.10. Statistical analysis 33
3.11. Ethical consideration 33
4. RESULTS 34
4.1. Selective Plate Results on XLD and HE agars 34
4.2. Overall prevalence of *Salmonella enterica* by *invA* PCR 35
4.3. Prevalence of *Salmonella* serotypes by multiplex PCR 36
4.4. Assessment of risk factors 38
4.4.1. Factors associated with milk production 38

4.4.2. Factors Associated with milk collectors 40
4.4.3. Factors associated with milk processors 42
4.4.4. Factors associated with milk retailors 44
4.4.5. Factors Associated with cottage cheese production 45
4.4.6. Characteristics and practices of cottage cheese retailers 47
4.5. Antimicrobial susceptibility results 49
4.5.1. Mono-drug resistance result 49
4.5.2. Multi-drug resistance patterns 51
4.5.3. Antimicrobial Resistance of the serotypes 53
5. DISCUSSION 55
6. CONCLUSION AND RECOMMENDATION 61
7. REFERENCES 62
ANNEXES 86

List of Tables

Table 1. The proportion of raw milk, pasteurized milk, and cottage cheese samples collected... 25

Table 2. Primer sets used to detect *Salmonella enterica* and its serotypes.................................. 29

Table 3. PCR mix per 25 µl PCR reaction for detection of *S. enterica* 30

Table 4. PCR mix per 25 µl PCR reaction for the detection of *S. enterica* serotypes 31

Table 5. Culture positivity rates of *Salmonella spps* on XLD and HE media across the value chains and study sites... 34

Table 6. Prevalence of *S. enterica* across the value-chains, sites, and sample types.................... 35

Table 7. Assessment of risk factors associated with contamination of raw milk by *S. enterica* during milk production 39

Table 8. Attribution of statistically significant variables on the apparent prevalence of *S. enterica* 40

Table 9. Assessment of milk collection centers with the apparent prevalence of Salmonella 41

Table 10. The attribute of gender and milk filtration towards the prevalence of *S. enterica* 42

Table 11. Assessment of milk processors towards the prevalence of Salmonella........................ 43

Table 12. The attribute of statistically significant variables assessed in milk processors 43

Table 13. Assessment of milk retailers with the apparent prevalence of *S. enterica* 45

Table 14. Assessment of cottage cheese with the apparent prevalence of *S. enterica* 46

Table 15. Assessment of Cottage cheese retailers with the apparent prevalence of *S. enterica*... 48

Table 16. Attribution of production system towards the prevalence of *S. enterica*...................... 49

Table 17. The number and percent of the level of resistance of bacterial isolates to different antimicrobials.. 51

Table 18. Antimicrobial resistance patterns of the Salmonella isolates 52

Table 19. The attribution of AMR and serotypes 54

List of Figures

Figure 1. Map of the study sites in Oromia Regional (ENSURE E-Dairy Project Ethiopia)....... 23
Figure 2. The overall isolation procedure of S. enterica demonstrates the protocol starting from enrichment to selective (ENSURE E-Dairy Project Ethiopia) 27
Figure 3.Representative gel of invA PCR.. 36
Figure 4. Representative results of multiplex PCR based serotyping Salmonella isolates........... 37
Figure 5. The three serotypes distribution assessed in the study 37
Figure 6. Percent of the level of resistance of 71 bacterial isolates to different antimicrobials. .. 50
Figure 7. Salmonella species colony morphology on XLD agar (A) and HE agar (B) 86
Figure 8. Pure colonies of Salmonella spps on BHI agar 86
Figure 9. Disc Diffusion Test for salmonella positive isolates 87

List of Abbreviations

BAM – Bacteriological Analytic Manual

BGA – Blue Green agar

BHI – Brain-heart infusion

BPW – Buffered Peptone Water

BSA – Bovine Serum Albumin

CDC – Centers for Disease Control & Prevention

CFU – Colony-forming units

CLSI – Clinical and Laboratory Standards Institute

ELISA- Enzyme-Linked Immunosorbent Assay

FAO- Food and Agricultural Organization

FDA - Food and Drug Administration

HACCP- Hazard Analysis and Critical Control Points

HE – Hectoen Enteric

invA – invasion gene of salmonella

ISO- International Standard Organization

MDR- Multidrug Resistance

MHA – Muller-Hinton Agar

MKTTn – Mueller-Kauffman Tetrathionate

NABRC-National Agricultural Biotechnology Research Center

NTS – Nontyphoidal Salmonella

PCR – Polymerase Chain Reaction

RES – Reticuloendothelial system

RVS – Rappaports Vassiliads Soy

SOP – Standard Operating Procedure

SPI – Salmonella Pathogenicity Island

USDA – United States Department of Agriculture

XLD – Xylose Lysine Deoxycholate

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Abstract

Foods of animal origin are major vehicles of Salmonella infections and a serious public health problem with increasing concern in the world, particularly for developing countries. There has been a median of 6% in raw milk and dairy products but most prior works in this area have focused on biochemical confirmation from dairy isolates. Almost all report of the prevalence of Salmonella spp. in dairy products as well as raw milk was highly varied. To address the limitations of earlier research, molecular methods and the country's milk and dairy value chain were tested. Risk factors related to pathogen prevalence, antibiotic resistance testing, and PCR-based pathogen serotyping were performed. A cross-sectional study was conducted on milk and cottage cheese in major milk shades of the Oromia region of Ethiopia from December to March 2020 to determine the prevalence and of *S. enterica.* A total of 480 samples (384 milk and 96 cottage cheese) were collected using simple random techniques from producers, collectors, processors, and retailer value chains. The samples were tested for *Salmonella* using Iso 6579-1: 2008 methods. The risk factor for contamination of these dairy products across the dairy value chain was done using pre-tested questionnaires. Selective plating reveals a higher prevalence of *S. enterica* among 480 samples, with 67.5% (324/480) for Hektoen enteric agar and 40% (192/480) for Xylose lysine desoxycholate agar while 14.8% (71/480) molecularly by detecting the presence of a highly conserved region of the *invA* gene. Raw milk accounted for 21.3% (41/192) of the overall prevalence, whereas pasteurized milk accounted for 12.5% (24/192), and cottage cheese accounted for 6.25% (6/96). The collector value chain received 20.8% (20/96), whereas Bishoftu received 23.3% (28/120). *Salmonella* infection was found to have a statistically significant relationship with access to training and the water temperature with which udder was washed in producers; gender and milk filtration in collectors; sieving in milk processors; and production system in cottage cheese retailer value chain respectively The greatest serotype was S. Heidelberg (59.1%), and clindamycin showed full resistance among 10 antimicrobials, whereas ceftriaxone showed 81.7% susceptibility. The results of this investigation show that there were problems relating to the safety of milk and cottage cheese in the area. Therefore, strict hygienic approaches and quality control measures should be applied to improve the safety of the products. Awareness creation should be required for producers, retailers, processors, and consumers regarding the quality and safety of milk and other dairy products.

Keywords: Cottage cheese, PCR, prevalence, *Salmonella enterica*, serotyping

1. INTRODUCTION

1.1. Background and Justification

Milk, as one of the most common animal products, is the lacteal product of mammary glands and plays an important function in human feeding universal, where it aids body tissue development and survival. It is the foremost plentiful animal food commodity, providing substantially more essential nutrients than other food (Amentie *et al.,* 2016). Cows, camels, goats, and sheep provide milk, but the most commonly employed agricultural animal used in a dairy farm is a cow. Cow's milk has been deemed a highly healthy, precious human food for an extended time and is eaten across the globe by millions daily. Daily ingestion of a liter of cow's milk has been shown to supply a mean person with about all fat, calcium, phosphorus, and riboflavin, 33.33% with A, a water-soluble vitamin, and thiamine, 25% of the calories, and every mineral consumed daily except iron, copper, manganese, and magnesium (Amentie *et al.,* 2016). However, its fluid or semi-fluid nature and chemical composition render it to be one of the ideal culture media for microbial growth and multiplication (Bereda *et al.*, 2012). As a result, milk produced, handled, and processed under unhygienic conditions harbors a variety of foodborne pathogens and consequently can cause diseases in humans, if consumed without further treatment (Oliver *et al.*, 2009).

Even if milk from the mammary gland of mammals is sterile (Alnakip *et al.*, 2014), microbial contamination begins from the mammalian udder, insufficient milking activity, dairy atmosphere (polluted soil, animal excreta), milking utensils, bad handling methods (freeze procedure, heating, and other properties) and lack of cold chain transportation (Kitinoja, 2013; Getabalew *et al.*, 2020). These microorganisms are indicators of both manners of handling milk from milking to consumption and the quality of the milk (Hadrya *et al*., 2012; Shunda *et al*., 2013: Tegegne and Tesfaye, 2017;).

Thus, raw milk is an excellent medium for cultivating various forms of microbes as a complete nutritious product. This may be a medium for the propagation of a large range of pathogens. The ability of raw or processed milk to support the growth of several spoiling or pathogenic microorganisms can lead to infections and intoxications of consumers (Abdissa *et al.*, 2017). Such pathogens include *Salmonella* spp., *Klebsiella* spp., *Enterobacteria* spp., and *Escherichia*

coli, which have been identified in milk products in Ethiopia (Yilma *et al.*, 2007). Gram-positive pathogens such as *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes,* and *Enterococcus* spp. have also been frequently isolated from milk (Adugna and Asresie, 2015).

In addition, tuberculosis, typhoid, brucellosis, diphtheria, and anthrax are common bacterial diseases that can be spread by milk ingestion from diseased cows and bad hygiene practices. From an economic point of view because it is highly endangered, of limited shelf life, and the low output of goods will contribute to wastage or/and quality of raw milk (Amentie *et al.,* 2016; Pal *et al.,* 2016); Pérez-Carrera *et al.,* 2016).

Milk-borne pathogens cause human diseases ranging from gastrointestinal disturbances characterized by diarrhea and vomiting that could pose life-threatening foodborne illnesses (Oliver *et al.,* 2009b). In addition to causing serious economic losses in dairy cattle production, it poses a major barrier for the trade of animals and animal products, and this could seriously impair socio-economic progress especially in developing countries like Africa (Herrero *et al*., 2013). Furthermore, the level of awareness among farmers about the economic and public health importance of zoonotic diseases in most of these countries is low, and this further stifles efforts to control these diseases (Mosalagae *et al*., 2011; Girma, 2012).

The safety of dairy products with regards to foodborne disease is of great concern around the world and it is especially true in developing countries where the production of milk and various milk products take place under unsanitary conditions and poor production practices (Karshima *et al.*, 2013; Mossie, 2019).

Many countries have milk quality regulations including limits on the total number of bacteria in raw milk to ensure the quality and safety of the final product. However, hygienic quality control of milk and milk products in Ethiopia is not usually conducted on a routine basis. There is little information on the microbial quality of raw milk (Bereda *et al*., 2012) especially in the area of central highlands of Ethiopia, where milk production plays a significant role in the diet and business of the community (Tegegne *et al.*, 2013; Gizaw *et al.*, 2016).

Evidence indicates low-income and minority communities experience higher rates of foodborne disease, and these populations could be at higher risk of being exposed to foodborne pathogens

in the farm-to-fork spectrum. High incidence of pathogens such as *Salmonella*, *Shigella*, and *Campylobacter* infections do not appear to be due to a single source of food; Hence, individuals themselves and their food safety-related awareness and habits are a decent initial spot to look. The food retailers or food service units are other options where minority communities may experience higher risks of foodborne disease (Zenk *et al.*, 2005; Henley *et al.*, 2012; Quinlan, 2013).

Among the most common foodborne pathogens, *Salmonella* is considered the most prevalent pathogen worldwide (Sanchez *et al.,* 2007). It is the most frequently isolated foodborne pathogen and is predominantly found in poultry, milk, and other dairy products (Yalew, 2020). *Salmonella* is a global public health concern accounting for more than 93.8 million foodborne illnesses, and 155,000 deaths per year worldwide (Majowicz *et al.,* 2010; Wang *et al.*, 2015). It poses a significant economic impact on humans and animals, predominantly in developing countries (Ejo *et al.* 2016).

Milk and dairy products are major sources of *Salmonella* infection particularly for consumers who preferred to consume raw milk and milk products. Contamination of milk and other products by *Salmonella* species can occur from production to consumption at different value chains (Oliver *et al*., 2005; Nada *et al.,* 2012). Many factors such as improper hygienic conditions in the farm, improper food storage, poor personal hygienic practices, inadequate cooling, and reheating of food items are the sources of *Salmonella* infections (Karshima *et al.*, 2013; Ejo *et al.*, 2016).

Salmonella infection continues to be a decisive global public health pestilence, leading to an economic impact on both industrialized and underdeveloped nations. This outcome is associated with charges related to the surveillance, detection of an outbreak, and disease control (Eng *et al.*, 2015). Gastroenteritis is the most common manifestation of *Salmonella* infection worldwide, followed by bacteremia and enteric fever (Majowicz *et al.*, 2010; Ngogo *et al*., 2020).

The foodborne pathogen predicament is growing globally because these pathogens are more virulent and develop resistance to antibiotics from time to time. It leads to an increase in the death rate of infected people (Chiu *et al.*, 2002; Liyuwork *et al.,* 2013). *Salmonella* spp. antibiotic resistance to a single antibiotic was reported first in the early 1960s (Eng *et al.,* 2015).

For then, the incidence of isolation of antibiotic-resistant *Salmonella* strains has increased in Saudi Arabia, United States, United Kingdom, and other countries around the world (Afolami and Onifade, 2018). The resistance towards the traditional first-line antibiotics such as ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole defined multidrug resistance (MDR) in *S. enterica* (Marchello *et al.*, 2020).

1.2.Statement of the problem

Milk is an important component of the world's diet (FAO, 2013). It is among the oldest foodstuffs in Ethiopia, and many people rely on milk products as their nutritional source. The main difference between traditionally produced and industrially produced products is the way of production which could have an impact on the quality and safety of the final products. It is estimated that 68% of the total milk produced in Ethiopia is used for human consumption in the form of raw milk, butter, cheese, and yogurt, while the rest is fed to calves and wasted during milk processing (Land O'Lakes, 2010). Milk is nutritious and when contaminated can support the growth of spoilage and pathogenic microorganisms (Solomon and Ketema, 2011).

The handling and safety of milk and milk products are of great concern around the world and particularly in developing countries where the production takes place under unsanitized conditions and poor production practices (Mossie, 2019). In addition, there are challenges with continuous maintenance of the cold chain. Limited knowledge on the hygienic handling of milk and milk products, un-sanitized processing of milk by the smallholder of milk and milk products can be considered as indicators for the sub-standard production of milk and milk products in the country (Yilma, 2012). Therefore, improvement in the production and processing of milk is necessary (Negash *et al.*, 2012). Thus, farmers, collectors, processors and retailers, and all who handle milk before sale must consider the health of consumers (Bereda *et al.,* 2014). In addition, in Ethiopia, there is no standard hygienic recommendation for food producers, cooperatives, and unions during milk production and marketing. Hence, unhygienic production and processing practices that compromise milk safety remain to be a challenge that has not been effectively addressed to date (Yilma *et al.*, 2011).

If milk safety and quality standards are not in place, the high nutritional composition and neutral pH of milk may cause many outbreaks associated with contaminated milk, and thereby constitute a public health risk to the consumers (LeJeune and Rajala-Schultz, 2009). Raw milk continues to

be used by a significant number of farm families and workers. Besides, many people believe that raw milk is safe (healthy) and, its health consequence may get impaired due to the application of heat (pasteurization) (Zeinhom and Abdel-Latef, 2014). Inversely, several milk-borne pathogens can persist and transmit in the population asymptomatically and cause growth faulting in children (Mandefero and Yeshibelay, 2018).

In Ethiopia, almost all previous studies on the prevalence of *Salmonella* spp. in dairy products were limited to biochemical confirmation of suspected isolates that have high uncertainty to confirm and take no consideration of the dairy value chain to identify the essential point of contamination. To overcome the drawbacks of previous studies, molecular techniques and dairy value chains in the country were both used to confirm the prevalence of *S. enterica*.

1.3. Objectives of the study

1.3.1. General Objective

The general objective of the present study is:

- To determine the prevalence of *S. enterica* in milk and cottage cheese of cows in the study area.

1.3.2. Specific Objectives

- To isolate and identify *S. enterica* in milk and cottage cheese of cows in the major milk shades of the Oromia region
- To investigate the risk factors associated with the prevalence of *S. enterica* in milk and cottage cheese of cows in the study area.
- To determine the antimicrobial resistance pattern of the positive isolates in the study area.
- To assess the prevalent *S. enterica* serotypes in milk and cheese by multiplex PCR and determine their antimicrobial resistance.

2. LITERATURE REVIEW

2.1. Food hygiene and food safety

In both developed and developing countries, foodborne diseases continue to be a persistent and pervasive problem, causing great human suffering and extraordinary budgetary losses (FAO/WHO, 2010; Mehlhorn, 2015). More than 420,000 people die every year from food-borne diseases across the world. About 600 million – almost 1 out of 10 – suffer from tainted food intake. Since contaminated food is still a big cause of disease and death, food safety is still a critical issue. Changes in global eating patterns meanwhile impact public health and economic development (FAO, 2019).

The predicament is severe in developing countries due to complications in settling optimal hygienic food handling practices. In developing countries, up to 70% of cases of diarrheal disease are associated with the consumption of contaminated foods. This does not mean that the risk for foodborne diseases would be eliminated but there is room for improvement to reduce the risks if basic hygiene and sanitary practices are implemented in all places where food is produced, processed, prepared, and consumed (Teferi, 2020).

Guaranteeing food safety in the changing world today is a difficult job that can only be accomplished by a collective effort from all sectors including government, user, and enterprise. Assuring the consumer's trust in the food system and creating a sound regulatory framework for domestic and foreign trade in food, promoting economic growth, can be done utilizing successful food protection systems. The Sanitary and Phyto-sanitary Agreement (SPS) requires countries to take legal action to protect the lives and welfare of consumers, animals, and plants, provided these steps can be scientifically justified and not improperly hinder trade (FAO, 2005).

Food protection issues vary across regions due to variations in income levels, diets, local environments, and government facilities. Changes in livestock farming, growth in international trade and tourism, changes in food or agricultural technologies, and behavioral changes and demands on the consumer are common phenomena that will increase food safety in both developing and developed countries (Tomaska, 2004; DeWaal and Robert, 2005).

Therefore, food safety is a pinpoint for public health, and achieving a safe supply of food poses challenges for national food safety. The changing global patterns of food production,

international trade, technology, public health expectations, and many other factors have created an increasingly demanding environment in which food safety systems operate. A variety of food-borne hazards, both familiar and new, pose health risks and barriers to international food trade. These risks should be determined and controlled to meet the growing and more complex national objective goals (FAO and WHO, 2007; FAO, 2017).

At the country level, both food shortages and insufficient infrastructure for food security are challenges that have become barriers to the growth of Ethiopia and public health. Although the country has tremendous potential for agricultural and industrial food production, its global market competitiveness is still very limited (Ayalew *et al*., 2013; Teferi, 2020).

Food is a significant vector for the dissemination of infectious agents that cause severe morbidity and mortality. Food managers play an important part in maintaining food safety. However, the proportion of licensed food handlers and their carrier status is not well studied in developed countries such as Ethiopia. The hands of food service workers may be vectors in the transmission of foodborne pathogens due to inadequate personal hygiene or cross-contamination. Lack of basic infrastructure, lack of knowledge of hygiene, absence of potable water, lack of proper storage facility, and unsuitable environments for food operations (such as proximity to sewers and garbage dumps) can contribute to the poor microbial quality of foods (Kibret and Abera, 2012; Saba *et al.*, 2001).

Milk supply chains in Ethiopia are typically categorized into three divisions based on geographical location: agricultural, peri-urban, and urban. In rural areas, milk is mainly processed for household consumption, and leftovers are sold in local informal markets. Rural systems can be further subdivided into production by pastoralists (limited to lowlands), agro-pastoralists, and mixed-crop farmers. The rural system has been reported to account for 98% of the country's milk production. A minority of milk has been reported to be produced in peri-urban systems, including smallholder and commercial farms in suburban areas near cities with grazing land available. Finally, a limited proportion of milk is produced in urban areas which are restricted to farms near cities and which do not have access to pasture land (Tadesse and Yilma, 2018; Aemneh *et al.,* 2019).

Even though Ethiopia can increase its dairy production, due to the necessary geographical and climatic conditions, in particular in the highlands, the low milk yield per animal (currently 1.54 L

per day), management of infectious and parasitic diseases of cattle, access to pasture land and poor milk handling practices leading to poor microbiological milk content are postal constraints. Therefore the development of good hygiene and processing practices and milk microbiology quality could theoretically increase the growth of the milk supply chain through improvement not only of its quality and therefore of its shelf life and accessibility but also its milk welfare (Keba *et al.*, 2020).

2.2. Risk factors potentially associated with foodborne illnesses

Even if we know that the majority of foodborne diseases are preventable, these are surprising figures for the 21st century. Some of the underlying conditions are known: unsafe water used for cleaning and processing food, poor food production processes, inadequate storage, and food handling practices, including infected food workers and cross-contamination of food. These may be coupled with inadequate or poorly enforced regulatory standards and industry compliance. The knowledge of these, however, is not enough (Todd, 2020).

Prevention and control practices require a suite of interlinked actions. These include finding the root cause of outbreaks, determining conditions in which pathogens grow and multiply. Understanding human behavior is key to understanding human behavior in food processing and its preparation, the CDC says (Uçar *et al.*, 2016; Todd, 2020). The human element is now known to be critical in applying safe practices to prevent food-borne illnesses, but it is much more difficult to influence positive change, both from the culture of an organization and individual backgrounds and preferences (Doyle *et al.*, 2015).

Microbial contamination is the main and most widespread concern for the overall safety of milk: the presence of infectious bacteria or viruses. Pathogens commonly found in milk include *Salmonella* species, *Campylobacter jejuni*, Shiga-toxin produced by *Escherichia coli* (STEC), and *Listeria monocytogenes*. These bacteria are also naturally found in the environment. Cows may be exposed to environmental sources of microbes on the farm that may cause mastitis, an udder infection that may spread pathogens during milking (LeJeune and Rajala-Schultz, 2009). Fecal contamination from the cows during milking can also allow high amounts of pathogenic microbes to enter the milk (Davis *et al.,* 2014).

Therefore, assessment of risk factors associated with milk production systems is central to ensuring the quality and safety of milk and milk products (Tigabu *et al.*, 2015). Besides, there is a need for epidemiologic studies and surveillance programs to fill the gap where data for these models are not available, as well as to provide an independent assessment of the sources of illnesses (Doyle *et al.*, 2015).

2.3. History of *Salmonella*

The genus *Salmonella* is a motile, gram-negative, non-spore-forming, and aerobic to anaerobic bacillus family *Enterobacteriaceae*, designated in honor of Daniel Elmer Salmon. The beginning to observe *Salmonella*, in 1880, was Karl Joseph Eberth from samples of patients with typhoid fever (from the Greek-typhōdes), which he used to label *Eberthella typhosa*. This bacillus (later defined as *Salmonella* Typhi) was successfully isolated from patients with typhoid disease in 1884 and confirmed Eberth's findings. Shortly after American bacteriologist, *Salmonella* Choleraesuis was isolated from swine by his assistant Theobald Smith, who mistakenly believed that this sprout was the causative agent of hog cholera. Later in appreciation of Salmon's efforts, Joseph Lignières, a French bacteriologist, suggested the genus designation *Salmonella (*Eng *et al*., 2015).

The *Salmonella* genus is now known as two species, due to variations in the sequence analysis 16S rRNA (Jajere, 2019), as *S. enterica* and *S. bongori*, covering 2,659 serotypes based on somatic O and H flagellar antigen as defined under the Kauffmann–White–Le Minor scheme. *S. enterica* is classified into 6 subtypes: *enterica, salamae, arizonae, diarizonae, houtenae,* and *indicae*. This zoonotic pathogen is undoubtedly still one of the world's most urgent concerns. It creates several conditions within many hosts and a lot of its ongoing evolution needs to be studied and deciphered (Liyuwork *et al*., 2013; Gossner *et al*., 2016).

Salmonella cells are moving through a peritrichous flagellum. They are 2–5 µm long by 0.5–1.5 µm wide, depending on the serotype, their genome ranges from 4460 to 4857 kb. *Salmonella* is a lactose fermenter (some subspecies) and a hydrogen sulfite producer and is oxidase-negative and catalase-positive. It hydrolyzes urea, uses citrate, and decarboxylates lysine as its sole carbon source (Gut *et al.*, 2018).

Salmonella is a phenotypically and biochemically homogeneous entity of facultative anaerobe, non-spore-forming, oxidase-negative, catalase-positive Gram-negative rod-shaped bacteria, and

though long filaments may be formed. Most strains are motile owing to peritrichous flagella and ferment glucose with acid and gas production. *Salmonella* is typically either slow or non-lactose fermenter, although it is fermented quickly by certain strains; adonitol, sucrose, salicin, and 2-ketogluconates are not fermented; tryptophan and phenylalanine are not deaminated, acetoin is not produced; thiosulphate produces hydrogen sulfide (H2S) and lysine and ornithine are decarboxylated. Most systems for the detection of an organism are based on these properties (Tadesse, 2006; Smith *et al*., 2015;). Certain strains create a biofilm matrix of complex carbohydrates, celluloses, and proteins. The capacity to make biofilm can be a sign of dimorphism which is the ability for a single genome, in response to environmental changes, to produce several phenotypes (Hall-Stoodley *et al.*, 2008).

Many genetic studies of *Salmonella*, in particular serovar Typhimurium, have been carried out (Baker and Dougan, 2007). The chromosome of its molecular DNAs made of around 4 x x 10^6 base pairs with a molecular weight of 4 x 10^9 and a total length of around 1.4 mm, is very similar to that of the *Escherichia coli* molecule. Numerous of these genes were mapped (Yap *et al*., 2014; Fraser *et al*., 2010). Different selective plating media were developed according to the biochemical reactions and growing conditions.

The use of selective and differential plating media is a simple method for the isolation of *Salmonella* spp. A wide variety of selective and differential media has been developed for this purpose, including xylose lysine desoxycholate agar (XLD), Hektoen enteric (HE) agar, and bismuth sulfite (BS) agar (Cooke *et al.*, 1999). XLD and HE agar are the most popular media for isolating *Salmonella* spp., and their differentiation abilities rely on characteristics of *Salmonella*, such as hydrogen sulfide production and the non-fermentation of lactose (Rambach, 1990). However, these characteristics are shared with other microorganisms, such as Proteus and Citrobacter (Sang *et al.*, 2012). Thus, numerous false-positive results are observed on these media which require further confirmation testing, a time-consuming and labor-intensive activity (Zadernowska, 2012). BS agar is the medium of choice for the isolation of *Salmonella enterica* serovar Typhi, and it is used for the isolation of atypical *Salmonella*, such as those which ferment the lactose (Cox, 1993). However, BS agar has several disadvantages, such as low sensitivity and long incubation time for the development of the characteristic colony morphology (Park *et al*., 2012). *Salmonella* grows red colonies on Brilliant green agar (BGA).

Salmonella can grow within the range of 2 to 54°C, although growth below 7°C has largely been observed only in bacteriological media, not in food, while growth above 48°C is confined to mutants or tempered strains (Jajere, 2019). The optimum temperature for growth is 37°C. The natural ecology of most *Salmonella* strains of concern to public health is in the gastrointestinal tract of warm-blooded animals. The optimum pH for the growth of *Salmonella* is within a range of 6.5-7.5, in liquid nutrient media strains can grow at pH values up to 9.5 and down to 4.05 (Betancourt, 2019). While growth occurs down to or close to the minimum pH with non-volatile organic acids such as citric acid or mineral acid such as hydrochloric acid, growth stops at higher pH values when volatile fatty acids are used. *Salmonella* grows at aw (water activity) values between 0.999 and 0.945 in laboratory media, down to 0.93 in foods, with an optimum of 0.995 (Mattick *et al.*, 2000).

2.4. Virulence factors and pathogenesis

The pathogenesis of *Salmonella* spp. is defined by virulence factors encoded by genes organized into blocks on the chromosome, classified as "pathogenicity islands" or SPI (Salmonella Pathogenicity Island) and constituting an essential feature of the virulence of *Salmonella*.

Five SPIs have been well characterized (Bonny *et al.,* 2011). SPI1 and SPI2 encode type III secretion systems, which provide the main virulence characteristics of *S. enterica* (Boko *et al.,* 2013). SPI1 is involved in the invasion of host cells and inflammation in particular of phagocytic or non-phagocytic cells of the intestinal mucosa (Brisabois, 2001). It hosts the invasion *invA* gene, which is found in most *Salmonella* strains. SPI2 is needed to encode the proteins involved in intracellular survival and replication within phagocytes. It also contributes to the systemic spread of *Salmonella*. SPI2 contains the *spiC* gene that encodes the structural components and secretion and helps to initiate the production of mediators with considerable function in the virulence of *Salmonella*. *spiC* is also involved in the expression of flagella filament components and plays an important role in *Salmonella* infection (Brenner *et al.,* 2000). SPI3 is present in all lineages. However, distributions of SPI4 and SPI5 have not been established even though their role is known. SPI4 intervenes in the initial interaction with the intestinal epithelium and contributes to long-term persistence (Bonny *et al.,* 2011). It contains the *orfL* gene, which is necessary for intramacrophage survival and possibly carries a system involved in the secretion of toxins (Boko *et al.,* 2013). SPI5 is involved in the realization of several pathogenic processes

during infection. Its first gene, *pipD* SPI5, has a target on the surface or inside the host cells (Carip *et al.,* 2008). *Salmonella* has other virulence factors that are not located in the SPI. They can be observed on mobile genetic elements such as plasmids. The *Salmonella* virulence plasmid, composed of five genes (spvRABCD) potentiates the systemic spread of the pathogen and helps to replicate in extra-intestinal sites (Brisabois, 2001).

The severity of infections with *Salmonella* in humans varies depending on the serotype engaged and the state of health of the human host. Children under 5 years of age, elderly people, and immunosuppressive patients are more susceptible to infection with *Salmonella* than healthy individuals. Almost all *Salmonella* strains are pathogenic as they can invade, replicate, and survive in human host cells, resulting in potentially fatal diseases (Eng *et al*., 2015).

During its invasion of non-phagocytic human host cells, *Salmonella* exhibits a remarkable characteristic that induces its phagocytosis to gain access to the host cell (Hansen-Wester *et al.*, 2002). The impressive genetics influencing this ingenious tactic is found in the *Salmonella* Pathogenicity Islands (SPIs), the gene clusters sited in the large chromosomal DNA region, and the encoding of the structures involved in the invasion process (Siriken, 2013).

When bacteria invade the digestive tract through contaminated water or food, they strive to permeate the epithelial cells that line the intestinal wall. SPIs encode multi-channel protein secretion systems that allow *Salmonella* to administer its effectors into the cytoplasm through the intestinal epithelial cell membrane. The bacterial effectors then unlock the signal transduction pathway and set off the reorganization of the actin cytoskeleton of the host cell, resulting in the outward extension or respiration of the epithelial cell membrane to engulf the bacteria. The morphology of the membrane ruffle is similar to the phagocytosis process (Takaya *et al.*, 2003).

For pathogenesis, the capacity of *Salmonella* strains to persist in the host cell is crucial, as strains lacking this capacity are non-virulent (Jajere, 2019). Following the engulfment of *Salmonella* into the host cell, the bacterium is enclosed in a membrane compartment called a vacuole, which consists of the host cell membrane. The presence of a bacterial foreign body would activate the immune response of the host cell under normal circumstances, resulting in the fusion of the lysosomes and the secretion of digestive enzymes to degrade the intracellular bacteria. *Salmonella*, however, uses the type III secretion system to inject other effector proteins into the vacuole, causing the compartment structure to be altered. The redesigned vacuole blocks

lysosome fusion, allowing intracellular survival and replication of the bacteria in the host cells. The capacity of the bacteria to sustain within macrophages enables them to be carried through the reticuloendothelial system (RES) (Monack *et al.*, 2004).

2.5. Clinical manifestations

Based on clinical trends in human salmonellosis, the strains of *Salmonella* may be categorized into typhoid *Salmonella* and non-typhoid *Salmonella* (NTS). In human infections, enteric fever, gastroenteritis, bacteremia, and other extraintestinal problems and the chronic carrier state are the four distinct clinical manifestations (Darby and Sheorey, 2008).

2.5.1. Enteric fever

Salmonella Typhi is an aetiological agent for typhoid fever, while paratyphoid fever is induced by S. Paratyphi A, B, and C. Since the clinical symptoms of paratyphoid fever are inseparable from typhoid fever, the term 'enteric fever' is used collectively for both fevers and both S. Typhi and S. Paratyphi are referred to as typhoid *Salmonella* (Gibani *et al.*, 2018).

Humans are the only reservoir for the two strains of typhoid *Salmonella*. The organisms are transmitted through the ingestion of food or water contaminated with the waste of infected people. Enteric fever is characterized by an incubation period of one week or more, with prodromal symptoms such as headache, abdominal pain, and diarrhea (or constipation) followed by fever (Trawinski *et al*., 2020). Diarrhea is more frequently seen in children, while sufferers with immunosuppression are more prone to developing constipation (Getto *et al.*, 2011). If the patient is left untreated, the fever may persist for a month or more (Al-Bayati and Al-Khafaji, 2020). In addition to fever, myalgia, bradycardia, and hepatomegaly (growth of the liver), splenomegaly (swollen spleen), and rose patches on the chest and abdomen may also be developed in infected patients (Eng *et al.,* 2015b). Hemorrhage is a major gastrointestinal complication caused by the Payers' patch perforation of the lymph nodules on the ileum endpoint. Around 15% of infected patients experience gastrointestinal problems in endemic areas (Humphries and Linscott, 2015).

2.5.2. Gastroenteritis

Salmonella strains other than *S.* Typhi and *S.* Paratyphi are referred to as NTS and are predominantly found in animal reservoirs. NTS infections are characterized by gastroenteritis or 'stomach flu', an inflammatory disease of the gastrointestinal tract followed by symptoms such as non-bloody diarrhea, vomiting, nausea, headache, abdominal cramps, and myalgia, are characterized by NTS infections. Symptoms such as hepatomegaly and splenomegaly in patients infected with NTS are less frequently seen (Pegues *et al*., 2006; Gal-Mor *et al*., 2014). NTS infections have a lesser incubation period (6–12 h) and are usually self-limiting and last for only 10 days or less. Cholecystitis, pancreatitis, and appendicitis are gastrointestinal complications of NTS infections. Particularly prone to NTS diseases, babies, young children, elderly people, and immunocompromised patients experience more serious symptoms than average individuals (Eng *et al*., 2015; Awofisayo-Okuyelu *et al*., 2018).

2.5.3. Bacteraemia and other extraintestinal complications

Bacteremia from *Salmonella* is a disease in which bacteria enter the bloodstream after the intestinal barrier is invaded. Almost all *Salmonella* serotypes can cause bacteria, while S. Dublin and S. Choelaraesuis are two invasive strains that are largely correlated with the manifestations of bacteremia (Woods *et al*., 2008). Like an enteric fever, high fever is a common bacteremia symptom, albeit without the forming of rose spots in enteric fever patients. Bacteremia can cause a septic shock, with a high mortality rate, to the immune response in extreme conditions. Bacteremia is more often seen in clinical presentations of NTS infections than in typhoid *Salmonella*.

The variation in clinical manifestation is expected to be associated with the involvement, through genetic study, of the *spv* (*Salmonella* plasmid virulence) gene in STNs causing non-typhoidal bacteremia (Guiney and Fierer, 2011). Although the gene mechanisms for improving the virulence characteristics of NTS remain unknown, gene expression is important for prolonging apoptotic cell death, allowing bacteria to stay in the host cells for longer periods. Around 5% of NTS patients experience bacteremia, often with extraintestinal complications, the most frequently affected organ being the lung. Cellulitis, urinary tract infections, diarrhea, endocarditis, and meningitis are other extra-intestinal complications. (Guiney, 2005; Eng *et al*., 2015).

2.5.4. Chronic carrier state

The chronic carrier status is characterized as the release into stools of bacteria for more than one year following acute *Salmonella* infection. The carriers S Typhi and S Paratyphi are responsible for the spread of enteric fever into endemic areas as they are only the human reservoir of typhoid *Salmonella* because the typical transmission route is water or food intake that is polluted by the feces of Chronic carriers (Trawinski *et al.*, 2020). Nearly 4% of enteric fever patients can become chronic carriers, mainly in children, elderly people, and women (Gonzalez-Escobedo *et al.*, 2011). The carrier status of NTS in patients with non-typhoidal salmonellosis in comparison is less common and the incidence rates are 0.1 %. The largest reservoir of NTS is animals, rather than humans (Pegues *et al.*, 2006).

2.6. Sources and transmission of *Salmonella*

The primary habitat of the species *Salmonella* is in the intestinal tract of animals such as birds, reptiles, farm animals, and occasionally insects and humans (Andino and Hanning, 2015). It may also be present in fresh fruits, vegetables, spices, and nuts, and water body. Once the organisms are infected, a person can act as a shed of the organism, usually through feces, but unnoticed (Jensen *et al.,* 2006). These distributions of *Salmonella* in the environment are easy to spread, resulting in an enormous impact on medicine and public health and on the economies of the world due to their increasing prevalence in the global food chain (Jajere, 2019). Such distribution of *Salmonella* in the environment, its increased prevalence in the global food chain, and its virulence and adaptability properties make it easy to transmit, resulting in enormous medical, public health, and economic impact worldwide. *Salmonella* spreads through the fecal-oral route and can be transmitted through food and water, through direct contact with animals, rarely from person to person. An estimated 94 % of salmonellosis is transmitted through food. Humans usually become infected by eating food contaminated with feces from infected animals. As a result, the food concerned is often of animal origins, such as beef, poultry, milk, and eggs (Ngogo *et al*., 2020).

2.7. Epidemiology of Salmonellosis

Salmonella serovars can be classified into three groups based on their interaction with specific host populations. *Salmonella* serotypes, which are almost exclusively associated with a particular host species, are called host-restricted serotypes (e.g. human Salmonella Typhi and poultry Salmonella Pullorum). Serotypes that are predominant in a single host species but may cause disease in other host species are host-adapted serotypes (e.g. Salmonella Dublin causes disease in cattle but can also infrequently cause disease in other mammalian hosts). The last group of serotypes is unregulated or broad-range serotypes capable of causing disease in a wide range of unrelated host species (e.g. Salmonella Typhimurium and Salmonella Enteritidis) (Rasschaert, 2007).

When the bacteria of *Salmonella* are swallowed, they pass into the stomach of a human and colonize the small and large intestines. The bacteria enter the intestinal mucosa and proliferate there. The bacteria can invade the lymphoid tissues of the gastrointestinal tract and spread to the bloodstream (Finn *et al.,* 2013). Blood circulation relies on host conditions and the virulence of the strain of *Salmonella* and occurs in less than 5% of infections. If the infection spreads to the bloodstream, any organ can become infected (e.g., liver, gallbladder, bones, or meninges). The incubation time for salmonellosis is roughly 12–72 hours, but can be longer. *Salmonella* gastroenteritis is characterized by a rapid onset of diarrhea (sometimes blood-tinged), stomach cramping, fever, and occasionally nausea and vomiting. The disorder typically lasts 4–7 days. If the virus progresses to the bloodstream and distant tissues, the condition will escalate in length and intensity and will generally have signs and symptoms related to the infected organ. A limited percentage of individuals infected with *Salmonella* experience reactive arthritis as a long-term sequel to infection (Ngogo *et al*., 2020).

An approximate 535,000 (95% uncertainty range 409000–705000) cases of non-typhoid *Salmonella* invasive disease occurred in 2017, with the largest prevalence in sub-Saharan Africa (34.5 [26.6–45.0] cases per 100,000 person-years) and in children younger than 5 years (34.3 [23.2–54.7] cases per 100,000 person-years). 77,500 (46,400–123,000) deaths were estimated in 2017, of which 18,400 (12,000–27,700) were attributed to HIV. The remainder 59,100 (33,300–98,100) non-HIV-related deaths accounted for 4.26 million (2.38–7.38) DALYs in 2017. The mean all-age fatality incidence was 14.5 % (9.2–21.1), with higher figures for children younger

than 5 years (13.5 % [8.4–19.8]) and elderly (51.2 % [30.2–72.9] for those ≥ 70 years of age), HIV infection (41.8 % [30.0–54.0]) and low socio-demographic growth (e.g. 15.8 % [10.0–22.9] in sub-Saharan Africa) (Stanaway *et al*., 2019).

2.8. *Salmonella* characterization

Typing techniques should be able to type all the isolates of the sample (high type-ability) and to differentiate at an acceptable level against such isolates (discriminatory power). The ability of a typing system to discriminate between unrelated strains is referred to as a discriminatory index (DI). A high degree of reproducibility of the process would offer accurate outcomes of the method, making it easier to be used in libraries and evaluated by a computer. The index should be 1.00 to be considered optimal, but it should be at least 0.95 in the experimental analysis (van Belkum *et al*., 2007; Petersen *et al*., 2011; Ferrari *et al*., 2017).

Classical typing techniques include phenotype-based approaches such as serotyping, phage typing, biochemical profiling, and antimicrobial resistance profiles. Organisms clustered due to their similarities as a consequence of the representation of their genotypes provide a functional description of phenotyping approaches. Antigenic classification of the organism by the recognition of flagellar (H) and somatic (O) antigens by complex antiserum reactions is called serotyping (Herikstad *et al*., 2002; Eriksson *et al*., 2005).

Multiple scholars have long suggested alternative molecular typing to replace traditional *Salmonella* serotyping isolates, e.g. repeated sequence-based PCR, microarrays, pulsed-field gel electrophoresis, and multilocus sequence typing. However, all these techniques require further confirmation before they can be adopted globally serologically. In the field of clinical microbiology, antibiogram typing has been regarded as an election procedure for the detection of potential causes of bacterial cross-transmission in healthcare institutions for many years. Beyond the serotype stage, both antimicrobial resistance profiling and phage typing are standard methods of *Salmonella* phenotyping (Fang *et al*., 2010; Wattiau *et al*., 2011; Achtman *et al*., 2012; Ranieri *et al*., 2013; Zou *et al*., 2013).

2.8.1. Isolation and Identification of *Salmonella*

2.8.1.1. Conventional Culturing Method

The isolation and identification of *Salmonella* spp in food can be performed using the current ISO horizontal method recommended by International Organizations for Standardization (ISO, 2017; (Andrews *et al.*, 2018). Conventional cultural methods for the detection of foodborne *Salmonella* species generally consist of five distinct and successive. These are pre-enrichment in nonselective media and selective enrichment in broth media, plating on differential agar, biochemical screening, and serological confirmation (Karalis, 2000; Wallace *et al.,* 2009; Anvarinejad *et al.,* 2016; Andrews *et al.*, 2018)

Salmonella may be present in small numbers in environmental samples, feces from apparently healthy animals, and in animal feed which are often accompanied by considerably larger numbers of other *Enterobacteriaceae* or other families (Davies and Wales, 2013). Therefore, the first step in traditional detection methods for most food samples is usually a pre-enrichment culture in a non-selective liquid medium such as buffered peptone water incubated at 37°C for 18 hours (Amagliani *et al.*, 2012).

Success in isolating *Salmonella* is usually enhanced by the inoculation of incubated pre-enrichment broth into selective enrichment media (Daquigan *et al*., 2016). Three main families of selective enrichment media for *salmonella* are recognized including selenite, tetrathionate (TT), and Rappaport-Vassiliadis (RV). Rappaport Vassiliadis is currently recommended for *Salmonella* recovery from low and highly contaminated foods (Rall *et al*., 2005).

The ISO method specifies the XLD agar and one optional selective medium. A variety of alternatives are available, including Bismuth Sulphite agar, Brilliant Green agar, and Hektoen Enteric agar. Typical *Salmonella* colonies on selective agar are sub-cultured onto non-selective media before confirmatory testing. From each enrichment medium, plating onto agar media plates (one of which is Xylose-Lysine Desoxycholate (XLD) agar) is carried out after 24 h and 48 h of incubation. Up to five colonies per plate have to be confirmed, which may potentially involve the confirmation of up to 40 presumptive colonies (EAC, 2008; Mainar-Jaime *et al*., 2013).

It can take at least three to five days to obtain a result using traditional methods of detection for *Salmonella* species. For this reason, a substantial number of alternative rapid screening methods

have been developed to produce results more quickly for food and environmental samples. *Salmonella* rapid test and screening kits utilize several different technologies, including novel culture techniques, immunomagnetic separation, EIA and ELISA-based assays incorporating fluorescent or colorimetric detection, and simple lateral flow assays incorporating immuno-chromatographic technology. Some methods can be automated to screen large numbers of samples (Oliveira *et al*., 2003; Paniel and Noguer, 2019).

Preliminary identification based on colony appearance on chromogenic and other selective agar media is traditionally confirmed using classical biochemical and serological testing. Key biochemical tests are the fermentation of glucose, negative urease reaction, lysine decarboxylase, negative indole test, H2S production, and fermentation of Dulcitol. Serological confirmation tests typically use polyvalent antisera for flagellar (H) and somatic (O) antigens (Kauffmann, 1972; Ratiner *et al*., 2003; Joseph and Carlos., 2012).

Rapid immunological identification and confirmation tests based on latex agglutination, enzyme immunoassay (EIA), and enzyme-linked immunosorbent assay (ELISA) have been developed for *Salmonella*, and simple-to-use lateral flow test strips using immune chromatographic technology have also been developed into commercial products by several manufacturers.

2.8.1.2. Molecular Based Detection

Molecular methods are also available, notably DNA hybridization and PCR assays for the identification of *Salmonella enterica*. However, these are generally designed for use as part of a method for rapid detection and screening rather than for confirmation. A range of DNA-based typing techniques has also been developed for use by specialist laboratories involved in the investigation of food-borne disease outbreaks (Koczula and Gallotta, 2016; ÇAM, 2020).

Genotyping methods, such as the restriction enzyme digestion-based methods (i.e., (Pulsed-field Gel Electrophoresis) PFGE) and DNA sequence-based methods, i.e., multi-locus variable-number tandem repeat analysis (MLVA); multi-locus sequence typing (MLST); ribotyping; clustered regularly interspaced short palindromic repeats (CRISPR) (Mojica *et al.*, 2000); and, whole-genome sequencing (WGS), are technologies that study the bacterial DNA instead of their phenotypic characteristics *(*Foley *et al*., 2006). The genotypic analysis also involves molecular genetic approaches using Restriction Fragment Length Polymorphism (RFLP), Ribotyping,

Amplified Fragment Length Polymorphism (AFLP), and Random Amplification of Polymorphic DNA (RAPD) are used for these purposes (Liu *et al*., 2011; Almeida *et al*., 2017).

Currently, due to the stability of the generated profiles, the discriminatory power, and the reproducibility of the results, PFGE is considered the golden standard for genotyping of *Salmonella* and it is the only universal molecular method appropriate for all *Salmonella* serotypes. In contrast, MLVA is serotype-specific and was developed for Sequence Type (ST). This method has a higher discrimination power and is widely used for surveillance and outbreak studies of ST in comparison with PFGE (EFSA, 2012; Ferrari *et al*., 2017).

2.9. Antibiotic resistance in *Salmonella* Spp.

Antimicrobial-resistant *Salmonella* is increasing due to the use of antimicrobial agents in food animals at sub-therapeutic level or prophylactic doses which may promote on-farm selection of antimicrobial-resistant strains and markedly increase the human health risks associated with consumption of contaminated meat products. Cattle have been implicated as a source of human infection with antimicrobial-resistant *Salmonella* through direct contact with livestock and the isolation of antimicrobial-resistant *Salmonella* from raw milk, cheddar cheese, and hamburger meat traced to dairy farms. Antimicrobial use in animal production systems has long been suspected to be a cause of the emergence and dissemination of antimicrobial-resistant *Salmonella* (Addis *et al.* 2011).

According to Bauer *et al*. (1996), antimicrobial resistance is currently the greatest challenge to the effective treatment of infections globally. For instance, more than 80% of food poisoning bacteria such as *Salmonella* are reported as antibiotic-resistant to at least one type of antimicrobial and more than 50% as resistant to two or more (Dabassa and Bacha, 2012). Globally, the three main causes of antimicrobial resistance have been identified as the use of antimicrobial agents in agriculture, over-prescribing by physicians, and misuse by patients (Dabassa and Bacha, 2012).

2.9.1. Mechanisms of resistance

Antimicrobial resistance mechanisms fall into four main categories: (1) limiting uptake of a drug; (2) modifying a drug target; (3) inactivating a drug; (4) active drug efflux. Intrinsic resistance may make use of limiting uptake, drug inactivation, and drug efflux; acquired resistance mechanisms used may be drug target modification, drug inactivation, and drug efflux (Reygaert, 2018).

Resistances are genetically encoded and can vary from mutations in endogenous genes to horizontally acquired foreign resistance genes carried by mobile genetic elements (MGEs) like plasmids. Both point mutations and horizontally acquired genes can encode all three categories of resistance. Point mutations in a promoter or operator can result in the overexpression of endogenous genes such as an antimicrobial inactivation enzyme like the AmpC β-lactamase gene, or an efflux system like the *mar* locus (Frye and Jackson, 2013). Point mutations in genes encoding antimicrobial targets can result in a resistant target, such as mutations to the gyrase gene leading to the expression of a fluoroquinolone-resistant gyrase enzyme (Ruiz, 2003).

Exogenous resistance genes encoded on plasmids, integrons, phage, and transposons can be horizontally transmitted by transformation, conjugation, or transduction and these foreign genes can encode all three mechanisms of resistance. This includes genes encoding enzymes that inactivate the antimicrobial, such as β-lactamases that cleave the four-membered ring in β-lactams, genes which encode efflux systems like *tet*(A), genes encoding a modified version of the enzyme that is the target of the antimicrobial, such as *dfrA*, or genes encoding an enzyme that modifies the antimicrobial target like a ribosomal RNA methylase, such as *ermB* (Munita and Arias, 2016).

Analysis of these resistance mechanisms can then be used to determine the genetic relationship between resistances found in isolates from animals and humans. Because of the diversity of genetic elements that lead to AR, it may be possible to determine if resistances seen in bacterial isolates from human infections are closely related to those found in animal isolates, thus identifying animal sources of resistant bacteria in human infections that can be targeted to reduce human disease (Omulo *et al.,* 2016; Frye and Jackson, 2013).

The mechanisms encoded by antimicrobial resistance determinants include antimicrobial modification and inactivation, alteration of the antimicrobial target site, efflux pumps, and membrane impermeability. These protect the bacteria from being attacked by antibiotics. Bacteria that are resistant to the beta-lactam class of antibiotics produce beta-lactamase enzyme which destroys the beta-lactam ring thus deactivating the antibiotic. Macrolide repressor protein produced by *Salmonella* spp. inactivates azithromycin and erythromycin. Resistance to quinolones is achieved by mutations in DNA gyrase coding genes (*gyrA* and *gyrB*) thus modifying the target site of the antibiotic. Efflux pumps transport substances out of the cell, including detergents, dyes, and antibiotics. *Salmonella* spp. uses the well-studied AcrAB-TolC efflux pump to extrude antibiotics such as tetracycline, chloramphenicol, and quinolones. Bacteria resist antibiotic entry into the cell by reducing or modifying porin channels in the outer membrane which are used by antibiotic molecules to enter the bacterial cell to reach their targets. Genes that encode for antibiotic resistance are either located on the chromosome or plasmids within a bacterial cell and are mobilized by transposons and integrons during conjugation or phages through transduction (Mthembu *et al*., 2019).

2.9.2. Prevention and control measures

To reduce the risk of salmonellosis, it is very important to improve the biosecurity and management practices in animal and poultry flocks. Trials for the protection of poultry flocks against different species of *Salmonella* infections using locally prepared autogenous bacterins were done with successful and promising results. Intervention strategies including *Salmonella* monitoring programs along the farm-to-table continuum should be planned. Lohiniva *et al.* (2008) advocated frequent, thorough, and proper hands and body washing as well as improving the water supply and sanitary conditions. It is very crucial to regulate the application of antimicrobials in the country's Veterinary field and study the antibiotic sensitivity profile to reduce the problem of MDR *Salmonella* and consequently alleviate the serious public health hazard. Symptomatic treatment of especially in very young and elderly patients using electrolyte therapy and antibiotics. Inactivated and live vaccines for the prevention and control of typhoid fever have been developed (Abd El-gany, 2020).

3. MATERIALS AND METHODS

3.1. Description of the study areas

Milk production is market-based in the highlands of Ethiopia. This is because, water is ideal for forages, with precipitation, temperatures, and soil types. The administrative regions of the country do have not equal potential for milk production, according to CSA results (CSA, 2018; Yilma *et al*., 2017). In Ethiopia, Oromia produces 52% of its milk production, whereas 23%, 20%, and 5%, SNNP, Amhara, and Tigray, respectively (Gizaw *et al.,* 2017).

A cross-sectional study was carried out from January to May 2020 in four selected dairy potential areas of Oromia Regional State, namely, Walmara (located 40 km West of Addis Ababa on the main road of Addis to Ambo), Bishoftu (located at 47 km from the capital of the country South-East, of Finfinne), Asela (which is located 120 km southeast), and Fiche (which is located 109 km northwest of Addis Ababa), respectively, (Figure 1). These four areas of research in Oromia were selected based on the high potential for milk production. In these selected areas, four milk value chains, i.e., producers, processors, factories, and, retailers were traced for collecting the samples.

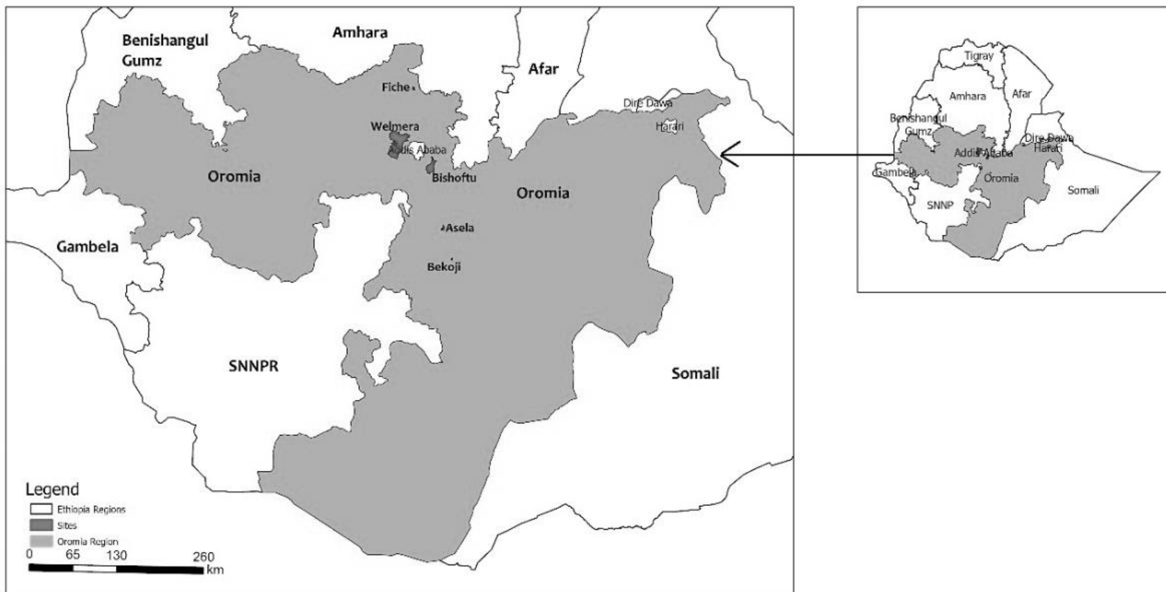


Figure 1. Map of the study sites in Oromia Regional (ENSURE E-Dairy Project Ethiopia)

3.2. Study Design and Approach

A cross-sectional study was used to identify the prevalence of *S. enterica*, to check similar risk factors along the value chains, and to assess antimicrobial resistance in cow milk and Cottage Cheese quality in the preceding microbial prevalence in the samples. In addition, a semi-structured questionnaire approach was developed to collect data on practices of milking, handling, processing, and storage in the study areas.

3.3. Sample size determination

In estimating the sample size, the minimum sample size was determined using statistical formula (Daniel, 1991):

$$N = \frac{\left(Z_{\frac{\alpha}{2}}\right)^2 p(1-p)}{d^2}$$

Where N is the minimum sample size required, Z is 1.96 at a 95% confidence interval, D is the margin of sampling error, and P is an estimate of the prevalence rate for the population. Since PCR-based studies are limited in the study area, the sample size was estimated by taking 50% expected prevalence, and a total of 384 samples were collected, 192 raw milk, 192 pasteurized milk.

$$N = \frac{(1.96)^2 0.5(1-0.5)}{0.0025} = 384$$

Besides, a cottage cheese sample (n=96) had sampled for the detection of *S. enterica.* This much sample of cottage cheese had taken in the proportion of its production in the Ethiopian dairy industry, which accounts for about 25% (Yilma *et al.,* 2007).

A total of 480 samples comprising: 192 raw milk, 192 pasteurized milk, and 96 cottage cheese samples were collected from the study sites according to the list indicated in the table (Table 1).

Table 1. The proportion of raw milk, pasteurized milk, and cottage cheese samples collected

Sites	Dairy value chain						Total
	Raw milk		Pasteurized milk		Cottage cheese		
	Farmer	Collector	Processor	Retailer	Farmer	Retailer	
Welmera	24	24	24	24	12	12	120
Asella	24	24	24	24	12	12	120
Bishoftu	24	24	24	24	12	12	120
Fiche	24	24	24	24	12	12	120
Total	**96**	**96**	**96**	**96**	**48**	**48**	**480**

3.3.1. Sampling methods

The whole specimen was collected, labeled, and transported as fast as possible in sterile wide-mouthed jar tanks to the Holeta Dairy Research Laboratory and NABRC, EIAR to carry out the isolation and identification of *Salmonella*. The analyses of specimens were carried out within 6-12 hours after being delivered to the laboratory.

3.4. Microbial analysis of *Salmonella enterica*

S. enterica was detected in samples following the ISO 6579-1:2018 and FDA / BAM protocols (Wallace *et al*., 2009; Mooijman, 2018).

3.4.1. Primary Enrichment

3.4.1.1. Fluid Milk

The milk sample (25 ml) was measured aseptically using a sterile serological pipette and added to a sterile plastic whirl-pack bag. Then the measured sample was homogenized with 225 ml of Buffered Peptone Water (BPW) (Oxoid, CM 0509). Finally, the mixture was incubated at 35 °C± 2 °C for 24±2 hours.

3.4.1.2. Cheese

The cheese sample (25g) was measured aseptically and added to a sterile whirl-pack bag. Next, it was homogenized in 225 ml of BPW and mixed well (hand stomaching). After that, it was let to

stand at room temperature for 60 minutes. Then the mixture was mixed well by swirling and the pH was determined with test paper. Finally, the mixture was incubated at 35 °C ± 2 °C for 24 ± 2 hrs.

3.4.2. Secondary Enrichment:

Rappaport-Vassiliadis with soya (RVS) broth (HIMIDIA) and Muller-Kauffman Tetrathionate-novobiocin (MKTTn) broth (HIMIDIA) were both adjusted to room temperature. Then the incubated primary enrichment sample was mixed well by hand massaging for at least 10 s. After that 0.1 ml aliquot was transferred and added to 10 ml of RVS and 1 ml aliquot was transferred and added to 10 ml of MKTTn. Finally, the tubes were vortexed and RVS was incubated at 41.5 °C ± 2 °C for 24 ± 2 hrs while MKTTn is at 37 °C ± 2 °C for 24± 2 hrs.

3.4.3. Plating onto Selective Agar

Xylose lysine deoxycholate (XLD) agar (HIMIDIA) and Hektone enteric (HE) agar (HIMIDIA) were adjusted to room temperature. Then the secondary enrichment tubes were vortexed. Samples from each secondary enrichments, were streaked, using a 10 µl loop, onto one XLD and one HE plate. A positive control *Salmonella* isolates and a negative control *Escherichia coli* (or another negative control isolate of choice) was streaked and finally, the agar plates were incubated at 35°C± 2°C for 24± 3 hrs.

Thus, there were a total of four agar plates per sample: two XLD plates (1 streaked from RVS and 1 streaked from MKTTn) and two HE plates (1 streaked from RVS and 1 streaked from MKTTn). The general microbiological analysis of *Salmonella* is depicted in Figure 2.

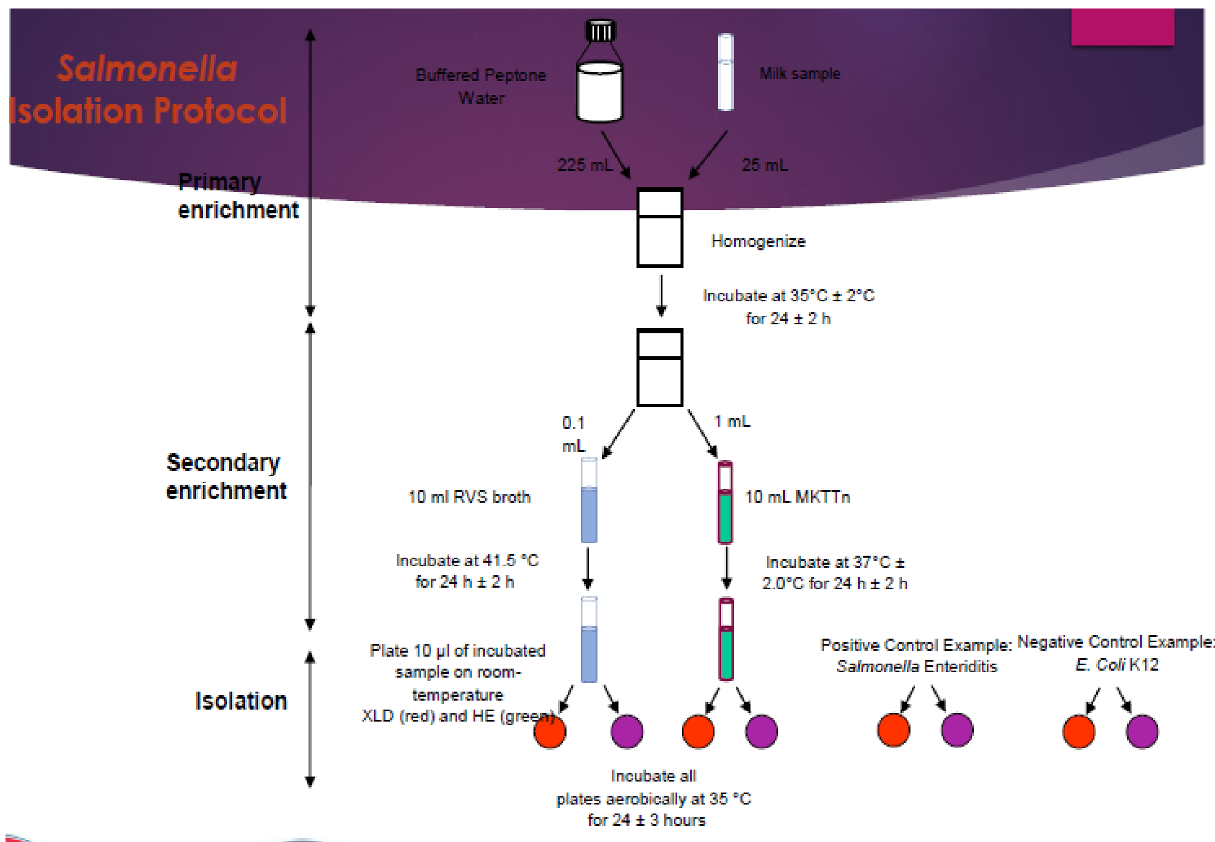


Figure 2. The overall isolation procedure of S. enterica demonstrates the protocol starting from enrichment to selective (ENSURE E-Dairy Project Ethiopia)

Passing through all the non-selective enrichment (BPW) and selective enrichments (RVS and MKTTn broths), the isolates were cultured on selective plates (XLD and HE agars). Then, the presumptive colonies were selected visually: pink colonies with or without black centers on XLD agar and blue-green to blue colonies with or without black centers on HE agar were identified as *Salmonella* spp presumptively (Annex 1).

3.4.4. Detection:

After the recommended incubation time is passed, the selective-differential agar plates and control plates were examined for the presence of colonies meeting the description for suspected *Salmonella* colonies.

HE agar: Blue-green to blue colonies with or without black centers. Many cultures of *Salmonella* may produce colonies with large, glossy black centers or may appear as almost completely black colonies.

XLD agar: Pink colonies with or without black centers. Many cultures of *Salmonella* spp. may produce colonies with large, glossy black centers or may appear as almost completely black colonies (Park *et al*., 2012).

For presumptive positive isolation, the colonies with large, glossy black centers (or may appear as almost completely black colonies) were selected using an isolation needle and streaked onto Brain Heart Infusion (BHI) agar (BBL^{TM}). Then they were incubated at 35° ± 2.0° C for 24 ± 2.0 hours. Finally, pure *Salmonella* colonies were selected for further analysis; some of them were used for molecular analysis while the rest were cryopreserved at a temperature of -80°C in a BHI agar containing 20% glycerol.

3.5. DNA extraction

All presumptively identified isolates were grown in BHI agar overnight at the optimal temperature (Annex 2). DNA was extracted using the boiling method: A single colony was picked up with a 10 µl loop and resuspended in nuclease-free water by swirling the loop in an Eppendorf tube and the tube was vortexed for 30 s. The tube was then placed in a heated thermal block and incubated at 95 – 100°C for 10 min. The tube was cooled down for 2 min at ambient temperature and centrifuged in a mini centrifuge at the highest speed for 5 min. Then 50 µl of the supernatant was transferred to a fresh tube (carefully by avoiding the pellet). The supernatant was then used as template DNA and stored at -20 °C for as long as needed. Later, up to 5 µl of the collected supernatant per 50 µl PCR reactions or up to 2.5µl per 25µl PCR reaction was used (El-baz *et al.,* 2017*;* Reischl and Wittwer, 2001).

Finally, the Gel electrophoresis system and NanaoDrop (Spectrophotometry) were utilized to check the quality and quantity of the extracted DNA.

3.6. PCR confirmation

3.6.1. Primers set

PCR confirmation was carried out using two PCR reactions using gene-specific primers as listed in Table 2.

Table 2. Primer sets used to detect *Salmonella enterica* and its serotypes

Salmonella	Primer	5* - 3*	PCR product, size	Reference
***invA* gene (*Salmonella* spps.)**	Salm3	GCTGCGCGCGAACGGCGAAG	389	(Ferretti *et al.*, 2001
	Salm4	TCCCGGCAGAGTTCCCATT		
***Salmonella* genus**	SG_F	TTTGGCGGCGCAGGCGATTC	423	Park and Ricke, 2015
	SG_R	GCCTCCGCTTCATCAATCCG		
***Salmonella* Subsp. I**	SS-I_F	GGTGGCCTCGATGATTCCCG	137	>>
	SS-I_R	CCCACTTGTAGCGAGCGCCG		
***Salmonella* Enteritidis**	SE_F	GCCGAGCTTGATGACAAACCTG	171	>>
	SE_R	GCGCTTCGCTTTTCCAACTGCC		
***Salmonella* Heidelberg**	SH_F	TGTTTGGAGCATCATCAGAA	216	>>
	SH_R	GCTCAACATAAGGGAAGCAA		
***Salmonella* Typhimurium**	ST_F	AACAACGGCTCCGGTAATGAGATTG	310	>>
	ST_R	ATGACAAACTCTTGATTCTGAAGATCG		

3.6.2. PCR amplification program

The reaction of the PCR Master Mix (Gnei, Bangalore) and other components are stated in Table 3. below. Accordingly, two PCR conditions were settled: *invA* PCR and serotype PCR.

3.6.2.1. *invA* PCR

The PCR reaction for *invA* amplification was held according to Table 3 below. The DNA was used as a template for the amplification of the highly conserved region of the *invA* gene using the primers Salm3 (5´-GCTG CGCG CGAA CGGC GAAG-3') and Salm4 (5´-TCCC GGCA GAGT TCCC ATT-3´) which amplify a 389 base pairs fragment of the conserved *invA* gene sequence of *Salmonella enterica* (Ferretti *et al.*, 2001).

Table 3. PCR mix per 25 µl PCR reaction for detection of *S. enterica*

No:	Components	Volume
1	PromegaGoTaq Green Master Mix	12 µl
2	Primer Salm 3	0.81 µl
3	Primer Salm 4	0.87 µl
4	Nuclease Free Water	8.82 µl
5	2.5 µl DNA template (each)	

Amplification was conducted in a Thermocycler (*BIO-RAD T100^{TM}*, Singapore) using the following cycle conditions: An initial incubation at 95 °C for 5 min followed by 35 cycles of amplification (denaturation at 95°C for 90 sec, annealing at 60°C for 60 sec, and elongation at 72°C for 90 sec), ending with a final extension at 72°C for 7 min.

3.6.2.2. Multiplex PCR for the detection of serotypes

The multiplex PCR reaction for *Salmonella* serotypes was held according to Table 4 below. The primers used in serotyping of *S. enterica* were those which detect *Salmonella* genus (SG_F (TTTGG CGGCG CAGGC GATTC) and SG_R (GCCTC CGCCT CATCA ATCCG)) that produce an amplicon with an expected length of 423 bp), *Salmonella* subsp. I (SS_F (GGTGG CCTCG ATGAT TCCCG) and SS_R (CCCAC TTGTA GCGAG CGCCG), that produce an amplicon with expected length of 137 bp), Salmonella Enteritidis (SE_F (GCCGA GCTTG ATGAC AAACC TG) and SE_R(GCGCT TCGCT TTTCC AACTG CC), that produce an amplicon with expected length of 171 bp), Salmonella Heidelberg (SH_F (TGTTT GGAGC ATCAT CAGAA) and SH_R (GCTCA ACATA AGGGA AGCAA), that produce an amplicon with expected length of 216 bp) and Salmonella Typhimurium (ST_F (AACAA CGGCT CCGGT AATGA GATTG) and ST_R (ATGAC AAACT CTTGA TTCTG AAGAT CG) that produce an amplicon with expected length of 310 bp) (Park and Ricke, 2015).

Table 4. PCR mix per 25 µl PCR reaction for the detection of *S. enterica* serotypes

No:	Components	Volume
1	PromegaGoTaq Green Master Mix	12 µl
2	Primer SG_F	0.68 µl
3	Primer SG_R	0.68 µl
4	Primer SS-I _F	0.68 µl
5	Primer SS-I _R	0.68 µl
6	Primer SH_F	1.25 µl
7	Primer SH_R	1.25 µl
8	Primer SE_F	0.5 µl
9	Primer SE_R	0.5 µl
10	Primer ST_F	0.5 µl
11	Primer ST_R	0.5 µl
12	Nuclease Free Water	3.28 µl
13	2.5 µl DNA template (each)	

Serotype PCR thermal cycling condition was as follows. The initial denaturation of 5 min step at 94°C, followed by 40 amplification cycles that consisted of a 30-sec denaturing step at 94°C, a 30-sec annealing step at 56°C, and a 1 min elongation step at 72°C, and a final extension step of 5 min (Beaubrun *et al*., 2017).

3.7. Electrophoresis of PCR products:

The amplified DNA products from *Salmonella* specific-PCR were analyzed with electrophoresis on 1.2% agarose w/v gels (1.5 g of agarose was mixed with 100 ml of 1 x TAE buffer in a glass flask) stained with gel red and visualized by UV illumination. A current of 120 V was applied to each gel. Eight microliters of PCR product mixed with 3 microliters of 6 X-loading dye (B7025S, New England), was loaded onto an agarose gel. The DNA ladder used as a marker for PCR products was 100 bp (product no. N32315) (Shanmugasamy *et al.,* 2011; El-baz *et al.,* 2017).

3.8. Antimicrobial susceptibility testing

The experiment of antibiotic resistance test for the isolates was done by disk diffusion method (Kirby & Bauer method) with the following antimicrobials (OXOIDs): Tetracycline (30 µg), Ampicillin (10 µg), Ceftriaxone (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Clindamycin (2 µg), Penicillin (10 units), Trimethoprim (W) (2 µg), Streptomycin (S) (10 µg) and Oxy-tetracycline (OT) (30 µg) (CLSI, 2013; Callan and Westblade, 2020).

From each PCR confirmed isolate, a loop full of well-grown colonies (3-5) on BHI agar was transferred with a sterile loop into sterile tubes containing 2ml of normal saline solution (0.85% NaCl). The inoculated colonies were mixed well with saline solution by vortex until the smooth suspension was formed. Saline solution (if the suspension is more turbid) or colonies (if the suspension is less turgid) was added to the suspension until it achieved the 0.5 McFarland turbidity standards. Then sterile cotton swab was dipped into the suspension and the bacteria were swabbed uniformly over the entire surface of the Muller Hilton Agar (HIMEDIA) plate.

The plates were held at room temperature for 3 minutes in a biosafety cabinet of the microbial biotechnology laboratory of NABRC to allow drying. Ten antimicrobial disks with a known concentration of antimicrobial were placed on the Muller Hinton Agar plate; nine of them in a circular pattern and one at the center and the plates were incubated for 22 hrs at 37ºC (Annex 3). The diameters of clear zone of inhibition produced by diffused antimicrobial on lawn inoculated bacterial colonies were measured to the nearest mm using caliper. All zone of inhibition against the ten antimicrobial agents for each isolate were recorded and compared with standards and interpreted as resistant, intermediate, or susceptible according to the published interpretive chart (CLSI, 2013) (Annex 6).

3.9. Survey Data Collection

The socio-demographical characteristic data on handling, storing, transporting, and package procedures of milk and cottage cheese were obtained using the pretested semi-structured questionnaire approved by the Institutional Review Board (IRB) of Addis-Abeba University in the Kobo toolbox (annex 7).

3.10. Statistical analysis

Data from the milk samples and surveys were gathered and documented in an excel sheet. Samples were processed using bacterial and molecular assays. This information was further optimized and analyzed using SPSS version 20.0 (SPSS IBM). The relationship of the multiple risk factors with the apparent prevalence of *Salmonella enterica* was analyzed utilizing logistic regression. The Odds Ratio (OR) was used to signify the degree of association with the apparent prevalence of *S. enterica* between the multiple risk factors. WHONET 5.6 program evaluated the antimicrobial resistance test findings. P values below 0.05 were statistically relevant in all analyzes.

3.11. Ethical consideration

The study was approved and ethical clearance was obtained from the Institutional Review Board (IRB) of the College of Natural and Computational Sciences, Addis Ababa University (CNS-IRB 42/2019). Moreover, both informed and written consent was obtained from the human subjects. Confidentiality of the data was assured by giving a unique personal identification code to each participant and sample.

4. RESULTS

4.1. Selective Plate Results on XLD and HE agars

In a total of 480 samples of raw milk, pasteurized milk, and cottage cheese cultured individually on both XLD and HE selective media, 40 percent (192/480) and 67.5 percent (324/480) isolates were suspected as *Salmonella* positive on XLD and HE agar plates, respectively (Table 5). However, 36.8 percent (177/480) of the isolates were grown on both agar plates.

In all three samples (e.g. raw milk, pasteurized milk, and cottage cheese) all of the value chains are found presumptive colonies of *Salmonella* species. The raw milk samples had the greatest *Salmonella* species colony: 79.7 % (153/192) and 52.6 % (101/192) on HE and XLD agar plates, respectively. Raw milk samples obtained from milk producers and collectors resulted in 80.2 % (77/96) growing on HE agar plates and 59.4 % (57/96) on XLD agar plates, respectively (Table 5).

Table 5. Culture positivity rates of *Salmonella spps* on XLD and HE media across the value chains and study sites

Value chains	Culture media type	Culture positivity rate from the collection sites (N=192, n=324)				
		Asela n(%)	Bishoftu n(%)	Fiche n(%)	Wolmera n(%)	Total (%)
Milk producers	XLD	11/24 (45.8)	18/24 (75.0)	4/24 (16.7)	11/24 (45.8)	44/96 (45.8)
	HE	19/24 (79.2)	23/24 (95.8)	16/24 (66.7)	19/24 (79.2)	77/96 (80.2)
Milk collectors	XLD	14/24 (58.3)	22/24 (91.7)	6/24 (25.0)	15/24 (62.5)	57/96 (59.4)
	HE	20/24 (83.3)	24/24 (100)	16/24 (66.7)	16/24 (66.7)	76/96 (79.2)
Milk processors	XLD	12/24 (50.0)	1/24 (4.2)	7/24 (29.2)	12/24 (50.0)	32/96 (33.3)
	HE	23/24 (95.8)	10/24 (41.7)	24/24 (100)	16/24 (66.7)	73/96 (76.0)
Milk Retailers	XLD	7/24 (29.2)	0/24 (0.0)	8/24 (33.3)	7/24 (29.2)	22/96 (22.9)
	HE	9/24 (35.7)	12/24 (50.0)	21/24 (87.5)	16/24 (66.7)	58/96 (60.4)
Cottage Cheese farm	XLD	8/12 (66.7)	3/12 (25.0)	7/12 (58.3)	4/12 (33.3)	22/48 (45.8)
	HE	10/12 (83.3)	5/12 (41.7)	11/12 (91.7)	4/12 (33.3)	30/48 (62.5)
Cottage Cheese Retailers	XLD	9/12 (75.0)	0/12 (0.0)	4/12 (33.3)	2/12 (16.7)	15/48 (31.2)
	HE	8/12 (66.7)	0/12 (0.0)	11/12 (91.7)	11/12 (91.7)	30/48 (62.5)

N=isolates suspected as Salmonella positive on XLD, n= isolates suspected as Salmonella positive on HE agar plate

The colonies with the least suspicion were recovered from cottage cheese samples cultured on XLD and HE agar plates (38.5 % and 62.5 %, respectively). Overall, samples from Asella had the greatest rates of culture-positive, followed by Fiche, while Welmera had the lowest (Table 4.1.).

4.2. Overall prevalence of *Salmonella enterica* by *invA* PCR

The *invA* PCR was used to confirm *S. enterica*, which had previously passed through the selective mediums (HE and XLD). From 480 samples (raw milk (n=192), pasteurized milk (n=192), and Cottage cheese (n=9)) examined 14.79% (71/480) were found to be *S. enterica* positive. From the overall prevalence in dairy products,14.79% (71/480), raw milk, pasteurized milk, and cottage cheese were found to have prevalence rates of 21.35% (41/192), 12.5% (24/192), and 6.25% (6/96), respectively. Among these dairy products, a relatively higher prevalence of *S. enterica* was shown in raw milk followed by pasteurized milk (Table 6). Fig 3 shows the PCR product of *S. enterica* detected using *invA* gene primers (Salm3 and Salm4).

Table 6. Prevalence of *S. enterica* across the value-chains, sites, and sample types

Variable	Observation	N	n(%)	95% CI of A.P	OR	χ^2	P-value
Value Chain	Producer	144	24(16.67)	3.10 – 10.98	**2.2**	**8.48**	**0.037***
	Collector	96	20(20.83)	13.21 - 30.32	**2.9**		
	Processor	96	15(15.63)	9.02 – 24.46	**2.0**		
	Retailer	144	12(8.33)	4.38 – 14.10	**1**		
	Total	**480**	**71(14.79)**	**11.73 – 18.29**			
Site	Welmera	120	8(6.67)	2.92 – 12.71	**1**	**15.38**	**0.002****
	Bishoftu	120	28(23.33)	16.09 – 31.93	**4.3**		
	Asella	120	14(11.67)	6.53 – 18.80	**1.8**		
	Fiche	120	21(17.5)	11.17 – 25.50	**2.9**		
	Total	**480**	**71(14.79)**	**11.73 – 18.29**			
Sample Type	Raw milk	192	41(21.35)	15.78 – 27.83	**4.1**	**13.60**	**0.001****
	Pasteurized milk	192	24(12.5)	8.17 – 18.03	**2.1**		
	Cottage Cheese	96	6(6.25)	2.33 – 13.11	**1**		
	Total	**480**	**71(14.79)**	**11.73 – 18.29**			

Key: N=Number of samples, n=number of positive isolates, *Significant at p<0.5, **Significant at p<0.01

The level of exposure was triple in collectors and is half as high in producers and processors as in the value chain of retailers (χ^2 = 8.48, p = 0.037). Raw milk and pasteurized milk were four times and nearly twice exposed to the pathogen as cottage cheese (χ^2 = 15.38, p = 0.002). The exposures in Asella, Fiche, and Bishoftu were two, three times, and four times, respectively, in comparison with Welmera (χ^2 = 13.60, p = 0.001) (Table 6).

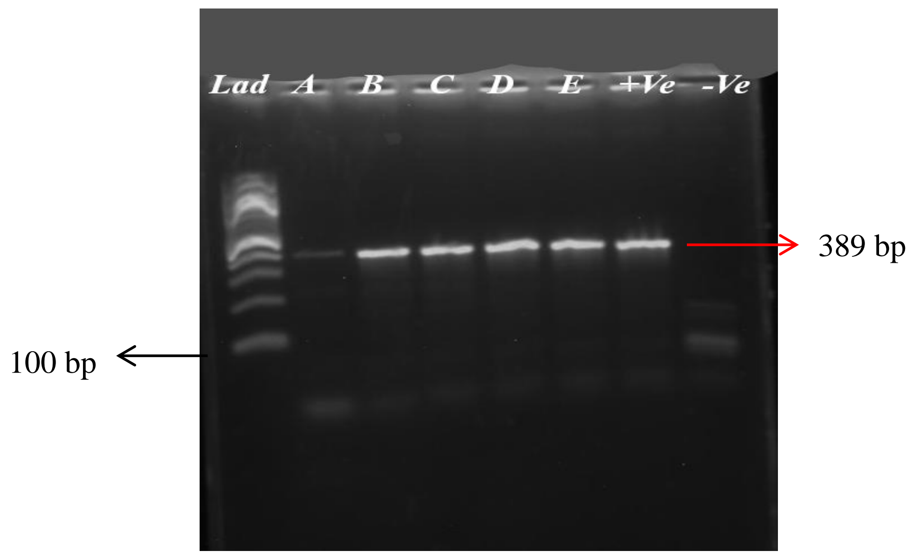


Figure 3. Representative gel of invA PCR

(Lad=Ladder 100bp; A, B, C, D, E= *S. enterica* positive isolates; +Ve=Positive control; and –Ve= Negative control)

4.3. Prevalence of *Salmonella* serotypes by multiplex PCR

Among the 71 *S. enterica* isolate processed by *invA* PCR, 32(45.07%), 42(59.15 %), and 27(38.03 %) were found to be Salm. Enteritidis, Salm. Heidelberg, and Salm. Typhimurium, as seen in Figure 5 below. The representative gel image for multiplex PCR-based serotyping is shown above in Figure 4. and the distribution graph of the three serotypes is illustrated below in Figure 5. Among the 71 isolates, 30 of them have at least two serotypes.

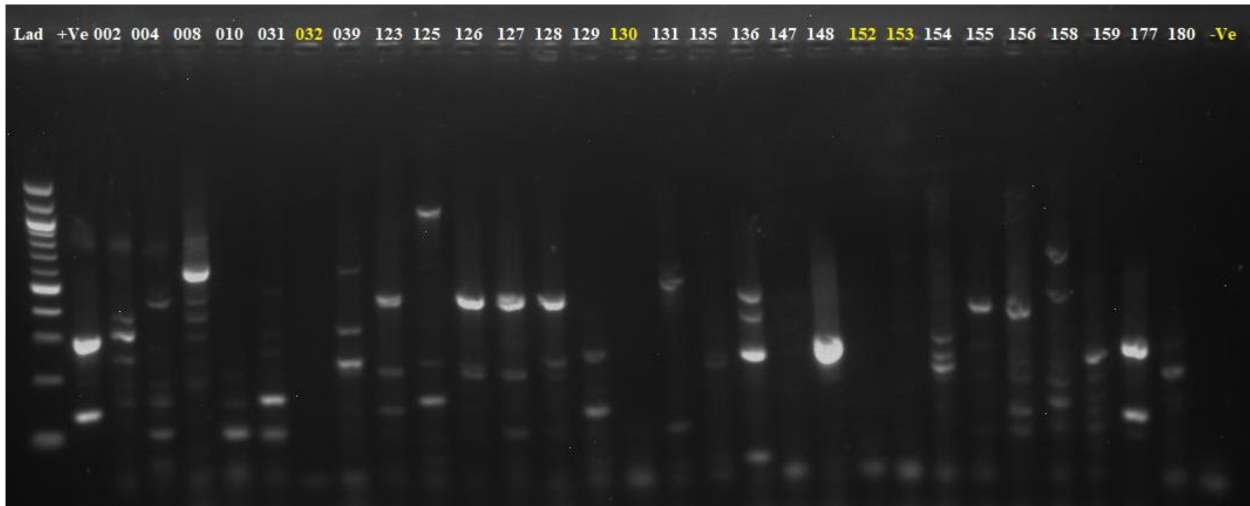
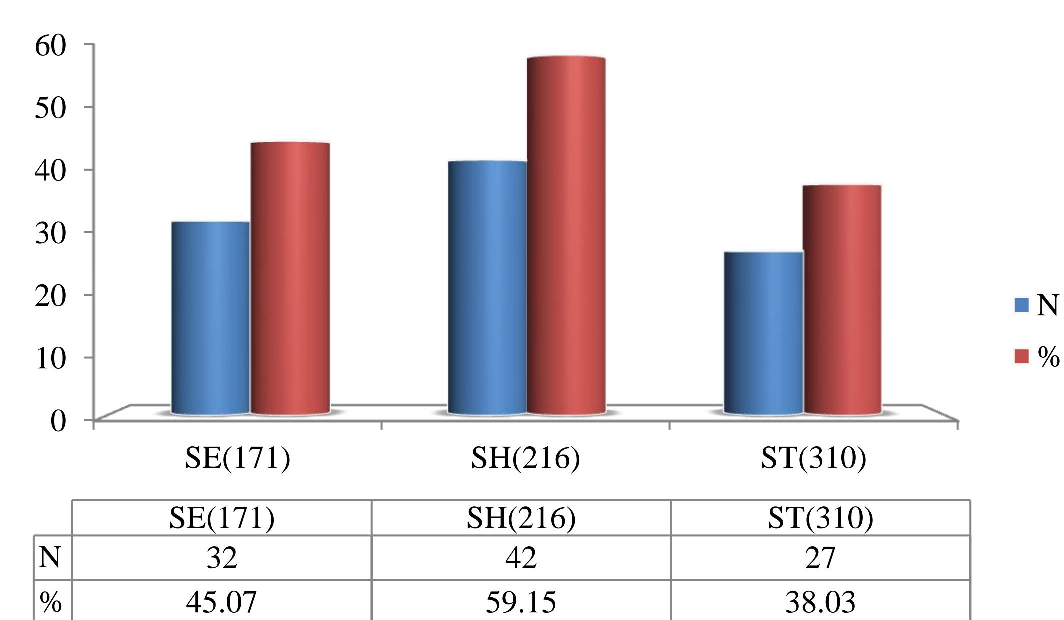


Figure 4. Representative results of multiplex PCR based serotyping *Salmonella* isolates

Comprising Lad= 100 bp Ladder (left the first lane), +ve=positive control, 2nd lane, lane 3-28=isolates, and last lane=negative control



	SE(171)	SH(216)	ST(310)
N	32	42	27
%	45.07	59.15	38.03

Figure 5. The three serotypes distribution assessed in the study

Key: SE=Salm. Enteritidis, SH=Salm. Heidelberg, ST=Salm. Typhimurium

4.3. Features of the Study Respondents

For the study, 304 respondents were engaged (96 milk producers, nine milk collectors, seven milk processors, 96 milk retailers, 48 cottage cheese producers, and 48 cottage cheese retailers).

4.4. Assessment of risk factors

4.4.1. Factors associated with milk production

As shown in Table 7, from 96 interviewed milk farmers/producers, 27 (28.2%) were residing in urban, while 69 (71.8%) were in the peri-urban areas. The interviewees' gender difference comprised approximately 33 (34.4%) men and 63 (65.7%) women. Farms' milk production expertise grouped into less than a year, 1-2 years (3.13%), 3-5 years (4.2%), 5-10 years (20.8%), and over ten years (52.1%).

About 52(54.2%) kept their cattle in a concrete house, and 11(11.46%) of them owned more than ten cows. Many of them (57(59.4%)) used tap water to clean milk-related equipment. Almost all of them (94(97.92%) washed the udder before milking; 51(53.13%) dried the udder and 45(46.87%) did not (Table 7).

Eighty-eight (91.7%) of the respondents filtered the milk, while the rest (8 (8.3%)) did not. Half of the respondents (44 (50%)) used a piece of cloth, and a few of them (7 (7.9%)) used wire mesh as a filtration unit. Almost all of them (85, 88.5%) used a plastic container, while 5 (5.2%) used mazzi plastic, and 6 (6.3%) used an aluminum can to store milk. Besides, 41 (42.7%) of them preserved the milk in the refrigerator and, 55 (57.3%) did not (Table 7).

In this assessment, of all the variables assessed, the educational level, the state of water used to wash udder, the history of mastitis or any other infectious disease, the access to training, and the milk handling containers might be the determinants of the prevalence since they are all significant statistically (Table 8).

Table 7. Assessment of risk factors associated with contamination of raw milk by *S. enterica* during milk production

Variables	Observation	N	n(%)	χ^2	p-value
Gender	Male	33	10(30.30)	2.090	0.148
	Female	63	11(17.46)		
	Total	**96**	**21(21.87)**		
Production system	Urban	27	7(25.93)	0.361	0.548
	Peri-Urban	69	14(20.29)		
	Total	**96**	**21(21.87)**		
Experience	Less than a year	3	0(0.0)	3.276	0.513
	1-2 years	4	2(50.0)		
	2-5 years	20	4(20.0)		
	5-10 years	19	3(15.79)		
	Over 10 years	50	12(24.0)		
	Total	**96**	**21(21.87)**		
Cattle housing	Concrete	52	15(28.85)	*4.527*	*0.210*
	Cattle shed	21	4(19.05)		
	Soil floor	22	2(9.1)		
	Klaar	1	0(0)		
	Total	**96**	**21(21.87)**		
Number of lactating cows	1-2	32	3(9.37)	*6.891*	*0.075*
	3-5	31	7(22.58)		
	6-10	22	6(27.27)		
	Over 10	11	5(45.45)		
	Total	**96**	**21(21.87)**		
Source of water for washing of milk handling equipment	Groundwater	9	2(22.22)	*7.596*	*0.108*
	Pump water	23	9(39.13)		
	Rain water	1	0(0)		
	River water	6	0(0)		
	Tap water	57	10(17.54)		
	Total	**96**	**21(21.87)**		
Do you wash Udder?	Yes	94	20(30.85)	*0.830*	*0.362*
	No	2	1(50)		
	Total	**96**	**21(21.87)**		
Do you dry the udder you washed?	**Yes**	51	15(29.41)	*3.73*	*0.053*
	No	45	6(13.33)		
	Total	**96**	**21(21.87)**		
Do You Filter Milk?	Yes	88	19(21.59)	*0.050*	*0.824*
	No	8	2(25.00)		
	Total	**96**	**21(21.87)**		
Material of Filtration	Piece of cloth	44	13(29.55)	*0.048*	*0.826*
	Plastic filter	37	6(16.22)		
	Wire mesh	7	0(0)		
	Total	**88**	**21(21.87)**		
Do you preserve milk?	Yes	41	6(14.63)	*2.136*	*0.144*
	No	55	15(27.27)		
	Total	**96**	**21(21.87)**		

Key: N=number of respondents, n=number of positive isolates, χ^2= Chi-square

Table 8. Attribution of statistically significant variables on the apparent prevalence of *S. enterica*

Variable	Observation	N	n (A.P.)	95% CI of A. Prevalence	OR	χ^2	P-Value
Educational level	Informal	31	4(12.9)	4.9 - 29.7	1	10.48	0.033
	Primary school	31	5(16.1)	1.6 - 69.5	1.3		
	High school	17	4(23.5)	2.3 - 80.3	2.1		
	Preparatory	7	5(71.4)	11.1 - 98.0	16.9		
	Diploma/ degree	10	3(30.0)	2.6 - 87.2	2.9		
	Total	**96**	**21(21.9)**	**14.7 – 31.2**			
Training	Trained	52	17(32.3)	5.1- 81.4	4.9	8.33	0.0039
	Untrained	44	4(9.1)	3.5-21.8	1		
	Total	**96**	**21(21.9)**	**14.7 – 31.2**			
Water state to wash udder	Cold water	28	11(39.3)	23.3 – 58.0	4.1	7.21	0.0072
	Warm water	66	9(13.6)	1.9 – 48.7	1		
	Total	**94**	**20(21.3)**	**14.7 – 31.2**			
Mastitis or other disease check-up	Yes (1)	27	10(37.0)	10.1 – 75.5	3.1	4.73	0.0297
	No (0)	69	11(15.9)	9.1 – 26.5	1		
	Total	**96**	**21(21.9)**	**14.7 – 31.2**			
Type of milk container	Plastic	85	16(18.8)	11.9 – 28.5	1	6.0	0.0498
	Mazzi plastic	5	1(20.0)	1.5 – 80.5	1.1		
	Aluminum can	6	4(66.7)	16.3 – 95.3	8.6		
	Total	**96**	**21(21.9)**	**14.7 – 31.2**			

Key: N=Total number of respondents, n=number of positive isolates, A.P=Apparent Prevalence

All the risk factors, except for the water temperature with which udder was washed, had a significant association with the prevalence of *S. enterica* in milk (Table 8). The preparatory school completed subjects had 17 times exposure as illiterates. Workers that had training contributed to contamination of milk 5 times compared to those without it. Owners who checked for mastitis were three times exposed to contamination compared to those who did not.

4.4.2. Factors Associated with milk collectors

There were altogether nine milk collection centers, of which 3 from urban settlers. More than half of the participants attended elementary school but illiterates had similar frequency. On the other hand, 17(17.7%), 7(7.3%), and 10(10.4 %) of them had completed high school, preparatory, and holders of diploma/degree respectively. The majority of participants had more than ten years of experience (2 had 2-5 years, and 3 had 5-10 years. Moreover, all of the respondents (i.e., 9), have received training related to the production of milk (Table 9).

Of the nine respondents, 6 (66.7%) of them collected the milk by trekking on foot. Also, the same figure was observed for the query about cooling style existence. Four of them were users of the deep freezer, while one respondent used bulk tankers as means of refrigeration, and also 4 did not use any means of refrigeration (Table 9). The collection house of 8 of them was concrete and only 1 was observed to be soil floor. Even though these variables were assessed in the study, none of them indicated statistical significance (p>0.05).

Table 9. Assessment of milk collection centers with the apparent prevalence of *S.enterica*

Variable	Observation	N	A.P.(%)	χ^2	p-value
Production system	Urban	3	21.87	0313	0.717
	Peri-Urban	6	18.75		
	Total	**9**	**19.79**		
Educational Status	High school	4	20.45	0.022	0.881
	Diploma/degree	5	19.23		
	Total	**9**	**19.79**		
Experience	2-5 years	2	28.57	2.334	0.311
	5-10 years	3	11.11		
	Over 10 years	5	20.83		
	Total	**9**	**19.79**		
Means of transportation	Trekking on foot	6	16.67	0.591	0.442
	Four-wheel drive	3	22.92		
	Total	**9**	**19.79**		
Cooling style	Yes	6	20.59	0.093	0.760
	No	3	17.86		
	Total	**9**	**19.79**		
What type of refrigeration did you use?	Deep freezer	4	25.0	3.504	0.173
	Bulk tanker	1	0.0		
	None	4	21.67		
	Total	**9**	**19.79**		
Collection house	Concrete	8	9.78	1.030	0.310
	Soil floor	1	0.0		
	Total	**9**	**19.79**		

The evaluation of the sex and milk filtration of milk collectors showed statistical relevance ($p<0.05$) as seen in Table 10. In contrast to male respondents, the gender difference was more than 8 times higher among women at case exposure. The deviation between the respondents who filter and who have not been adversely related (i.e., collectors who filtered the milk were 5 times exposed than those who do not).

Table 10. The attribute of gender and milk filtration towards the prevalence of *S. enterica*

Variable	Observation	N	A.P (%)	95% CI of A.P	OR	χ^2	p-value
Sex	Male	8	14.28	7.61 – 23.62	1	10.32	0.001**
	Female	1	58.33	27.67 – 84.83	8.4		
	Total	**9**	**19.79**	**12.36 – 29.17**			
Do you filter milk?	Yes	3	36.11	20.82 – 53.78	5.1	9.418	0.002**
	No	6	10.0	3.76 – 20.51	1		
	Total	**9**	**19.79**	**12.35 – 29.17**			

4.4.3. Factors associated with milk processors

Interview participants from pasteurized milk processors were 7, of which 6 were males (Table 11). All of them are diploma/degree holders and trained (i.e., 7). The production system of 5 of them was from urban areas, while the rest were from peri-urban. Above half of them (55 (57.29%)) maintained the temperature to preserve the milk.

The characteristics of statistically significant variables measured in milk processors were given in Table 11. Those with 2 to 5 years of experience were the most exposed to the pathogen by two and a half fold compared to those with ten years and greater. Piece of cloth has an incidence of exposure of one and a half of the centrifugal pump to sieve the milk. The evaluation of the chemical test showed an additional negative relationship in which the cold, warm water, and the acid-base test was 38-fold, and the Acid-base test was 14-fold than warm water and base test. In the case of exposure, the microbiological test had shown 14 times more than the alcohol test, and it had preceded by one and a half as of lactometer (Table 12).

Table 11. Assessment of milk processors towards the prevalence of *S. enterica*

Variable	Observation	N	n(%)	χ^2	p-value
Production System	Urban	64	11(17.19)	0.356	0.551
	Peri-Urban	32	4(12.5)		
	Total	**96**	**15(15.63)**		
Gender	Female	12	0(0.0)	2.540	0.111
	Male	84	15(17.86)		
	Total	**96**	**15(15.63)**		
Means of Transportation	Public transport	24	2(8.33)	1.291	0.256
	Four-wheel drive	72	13(18.05)		
	Total	**96**	**15(15.63)**		
How often do you maintain the temperature?	Once in a year	52	8(15.38)	0.005	0.944
	Twice in a year	44	7(15.90)		
	Total	**96**	**15(15.63)**		

Table 12. The attribute of statistically significant variables assessed in milk processors

Variable	Observation	N	n (%)	95% CI of A. P	OR	χ^2	p-value
Experience	Less than a year	24	0(0.0)	0.0 – 14.25		7.822	0.020*
	2-5 years	8	3(37.50)	8.52 – 75.51	2.6		
	Over 10 years	64	12(18.75)	10.08 – 30.46	1		
	Total	**96**	**15(15.63)**	**9.02 – 24.45**			
How do you sieve the milk?	Centrifugal pump	60	13(21.67)	12.07 – 34.19	1		
	Piece of cloth	12	2(16.67)	2.09 – 48.41	1.4	9.68	0.008*
	Metal sieve	24	0(0.0)	0.0 – 14.25			
	Total	**96**	**15(15.63)**	**9.02 – 24.45**			
What sort of chemicals did you test?	Water only	24	0(0.0)	0.0 – 14.25		34.83	0.001**
	Warm water and base	48	2(4.17)	0.51 – 14.25	1		
	Acid base	8	3(37.50)	8.52 – 75.51	13.8		
	Cold, warm water and acid-base	16	10(62.50)	35.43 – 84.80	38.33		
	Total	**96**	**15(15.63)**	**9.02 – -24.45**			
What parameters of adulteration did you test?	Lactometer	32	4(12.5)	3.51 – 28.99	1.6	23.40	0.002**
	Alcohol test	24	2(8.33)	1.03 – 26.99	1		
	Organoleptic	24	0(0.0)	0.0 – 14.25			
	Microbiological test	16	9(56.25)	29.88 – 80.25	14.14		
	Total	**96**	**15(15.63)**	**9.02 – 24.45**			

4.4.4. Factors associated with milk retailors

Of the 96 pasteurized milk retailers participating in the study, 55 (57.5 %) were males. The entire development structures were built in urban dwellings (Table 13). Five of them (5.2 %) were illiterate, while 36(37.5 %), 29(30.2 %), 9(9.4 %), and 17(17.7 %) were primary, secondary, preparatory, and diploma/degree holders. Involvement in milk production was 19(19.8%) for less than one year, 28(29.2%) for 1-2 years, 21(21.9%) for 2-5 years, 13(13.5%) for 5-10 years, and 15(15.6%) for 10 years. Almost all of them (92 (95.83 %)) were not trained (Table 13).

The sellers were predominantly (81.25%) selling pasteurized dairy whole-sellers; whereas 17.71% of the retailers but a few (1.04%) bought milk directly from the market. A plastic pouch was used for the packing of products by most retailers (95%, 98.96%) but one (1.04%) used paper board.

Eighty percent of retailers regulated the temperature of pasteurized milk before it is sold and stored it in a different refrigerator, while the rest did not. A large number (90%) of them monitored milk quality but 10% did not.

All of the variables assessed in this value chain had not shown statistical significance with the prevalence of *Salmonella* in milk products (Table 13).

Table 13. Assessment of milk retailers with the apparent prevalence of *S. enterica*

Variable	Observation	N	n (%)	χ^2	p-value
Access to training	Trained	4	0(0.0)	0.432	0.511
	Untrained	92	9(9.78)		
	Total	**96**	**9(9.38)**		
Sex	Female	42	2(4.76)	1.870	0.171
	Male	54	7(12.96)		
	Total	**96**	**9(9.38)**		
Education	Illiterate	5	0(0.0)	4.737	0.315
	Primary school	36	5(13.89)		
	High school	29	4(13.79)		
	Preparatory school	9	0(0.0)		
	Diploma/Degree	17	0(0.0)		
	Total	**96**	**9(9.38)**		
Experience	Less than a year	19	1(5.26)	1.346	0.853
	1-2 years	28	4(14.28)		
	2-5 years	21	2(9.52)		
	5-10 years	12	1(8.33)		
	Over 10 years	15	1(6.67)		
	Total	**96**	**9(9.38)**		
From whom do you get pasteurized milk?	Directly purchased from market	1	0(0)	0.419	0.811
	Whole sellers	78	8(10.26)		
	Factories delivery	17	1(5.88)		
	Total	**96**	**9(9.38)**		
How was the milk package?	Plastic pouch	95	9(9.47)	0.105	0.746
	Paper board package	1	0(0)		
	Total	**96**	**9(9.38)**		
Do you maintain temperature until sale?	Yes	17	1(5.88)	0.297	0.586
	No	79	8(10.13)		
	Total	**96**	**9(9.38)**		
Do you keep the milk in a separate refrigerator?	Yes	18	1(5.56)	0.380	0537
	No	78	8 (10.26)		
	Total	**96**	**9(9.38)**		
Do you check the quality upon receiving it?	Yes	86	8(9.30)	0.005	0.943
	No	10	1(10.0)		
	Total	**96**	**9(9.38)**		

4.4.5. Factors Associated with cottage cheese production

Among 48 farmer/producer respondents, 47(97.9%) were males. The production system settlement was 13(27.1%) urban and 35(72.9%) peri-urban based. Twenty-two (45.8%) of the participants were illiterate, but, 17(35.5%) and 9(18.7%) were attended primary and high school completes, respectively (Table 14).

Table 14. Assessment of cottage cheese with the apparent prevalence of *S. enterica*

Variable	Observation	N	n(p%)	χ^2	p-value
Sex	Female	47	2(4.26)	0.044	0.833
	Male	1	0(0.0)		
	Total	**48**	**2(4.17)**		
Production system	Urban	13	1(7.69)	0.555	0.456
	Peri-Urban	35	1(2.86)		
	Total	**48**	**2(4.17)**		
Training	Trained	4	0(0.0)	0.190	0.663
	Untrained	44	2(4.55)		
	Total	**48**	**2(4.17)**		
Education	Illiterate	23	1(4.35)	0.567	0.753
	Primary school	16	1(6.25)		
	High school	9	0(0.0)		
	Total	**48**	**2(4.17)**		
Experience	Less than a year	5	0(0.0)	2.970	0.563
	1-2 years	9	0(0.0)		
	2-5 years	13	1(7.69)		
	5-10 years	8	1(12.5)		
	Over 10 years	13	0(0.0)		
	Total	**48**	**2(4.17)**		
How do you preserve cottage cheese in a household until sale or consumption?	Placing underground	34	2(5.88)	0.248	0.883
	Placing underwater	1	0(0)		
	Refrigerator	3	0(0)		
	Total	**38**	**2(5.26)**		
What type of packaging material do you use to handle cottage cheese?	Plastic bucket	39	2(5.13)	0.051	0.821
	House paint bucket	3	0(0)		
	Total	**42**	**2(100)**		
How is cottage cheese transported to the farm markets/ retail shops/hotels?	Animal drawn cart	6	1(16.67)	3.540	0.472
	Trekking on foot	16	0(0)		
	Motor bicycle	8	0(0)		
	Three-wheel drive	5	0(0)		
	Public transport	23	1(4.35)		
	Total	**43**	**2(100)**		
How long does it take to transport cottage cheese to the farm market?	Less than 30 min	11	1(9.09)	1.205	0.547
	30 min- 1 hr	18	1(5.55)		
	Over 1 hr	14	0(0)		
	Total	**43**	**2(100)**		
Do you regularly wash cottage cheese handling equipment and utensils before and after use?	Yes	47	2(4.25)	0.044	0.833
	No	1	0(0)		
	Total	**48**	**3(100)**		
What kind of cleaning chemical do you use for washing and cleaning cottage cheese handling equipment and utensils?	Water with soap	32	1(3.13)	0.484	0.785
	Different herbs	14	1(7.14)		
	Coldwater only	2	0(0)		
	Total	**48**	**2(100)**		

The experience in milk production for 7(14.6%) of them was less than a year, 9(18.8%) of 1-2 years, 24(50%) of 2-5 years, 5(10.4%) of 5-10 years, but, 3(6.3%) of them had over ten years of experience. Most of them (44(91.7%) did not receive training (Table 14).

Ninety percent of them stored the cottage cheese in a plastic bucket for preservation. Over half of the respondents (53.45%) used public transport to reach the market (farm market/hotels/restaurants), while 37.21% used to walk. It took about 30 minutes to 1 hour for about half of them to hit the market from home. Almost all (97.92%) of them washed cottage cheese containers daily before and after use, using water and soap (66.67%) while the rest did not (Table 14).

4.4.6. Characteristics and practices of cottage cheese retailers

A total of 48 respondents engaged in the cottage cheese retail market, with high participation of females (39(81.3%) (Table 15). A large number of them (42(87.5%)) were settled in urban residences, while 6(12.5%) were in the peri-urban area. More than half of the actors (30(65.2%)) were diploma/degree holders, but 2(4.2%) of them were illiterate, 4(8.3%) primary school, 9(18.8%) high school, and 3(6.3%) of them from preparatory school. Half of the participants (24(50%)) had 2-5 years of experience but 7(14.6%), 9(18.8%), 5(10.4%), and 3(6.3%) of them had less than a year, 1-2 years, 5-10 years, and over ten years of involvement. Few of them (10(20.8%)) had taken training (Table 15).

Cottage cheese was received or purchased by 45.8% of the participants from producers, 20.8 % from household producers, and the remainder (33.33%) from the factory. Nearly half of them were transported by refrigerated truck from the processor to the retail location, 27.1 % by public transportation, and the remainder by other means. Almost all of them (97.9%) had cottage cheese in the refrigerator before they sell it, but 70.8 % had a different cottage cheese refrigerator (dairy product). Furthermore, 50% of them had used a standby generator in the event of a power outage (Table 15).

Except for the production system (p=0.003) (Table 16), all of the measured risk factors were statistically insignificant, as seen in the table below (Table 15).

Table 15. Assessment of Cottage cheese retailers with the apparent prevalence of *S. enterica*

Variable	Observation	N	n(p%)	χ^2	p-value
Sex	Male	9	0(0.0)	0.738	0.390
	Female	39	3(7.69)		
	Total	**48**	**3(6.25)**		
Education	Illiterate	2	0(0.0)	1.920	0.750
	Primary school	4	0(0.0)		
	High school	9	0(0.0)		
	Preparatory	3	0(0.0)		
	Diploma/Degree	30	3(10.0)		
	Total	**48**	**3(6.25)**		
Training	Trained	25	2(8.0)	0.273	0.602
	Untrained	23	1(4.35)		
	Total	**48**	**3(6.25)**		
Experience	Less than a year	7	0(0.0)	3.200	0.525
	1-2 years	9	0(0.0)		
	2-5 years	24	3(12.5)		
	5-10 years	5	0(0.0)		
	Over 10 years	3	0(0.0)		
	Total	**48**	**3(6.25)**		
From whom do you receive or buy cottage cheese?	Household producer	10	0(0)	2.944	0.229
	Market	16	0(0)		
	Factory	22	3(13.64)		
	Total	**48**	**3(6.25)**		
How is cottage cheese transported from processor to retailing point?	Four-wheel drive	2	0(0.0)	3.478	0.627
	Three-wheel drive	2	0(0.0)		
	Public transport	13	0(0.0)		
	Animal drawn cart	3	0(0.0)		
	Trekking on foot	5	0(0.0)		
	Refrigerated vehicle	23	3(13.04)		
	Total	**48**	**3(6.25)**		
Do you keep cottage cheese in a refrigerator until sell?	Yes	47	3(6.38)	0.068	0.794
	No	1	0(0)		
	Total	**48**	**3(6.25)**		
Do you have a separate refrigerator for cottage cheese (dairy product)?	Yes	34	3(8.82)	1.317	0.251
	No	14	0(0)		
	Total	**48**	**3(6.25)**		
Do you have a backup generator to be used in the case when electric power is out?	Yes	24	1(8.33)	0.356	0.551
	No	24	2(4.17)		
	Total	**48**	**3(6.25)**		

The production system was the only statistically significant variable measured for the value chain of cottage cheese retailers. As the logistic regression shows, the odds ratio of Peri-Urban was 20.5 as high as that of the urban settlements. This shows that the probability of occurrence of *Salmonella enterica* in urban areas was approximately 20 times lower than that of peri-urban areas (Table 16).

Table 16. Attribution of production system towards the prevalence of *S. enterica*

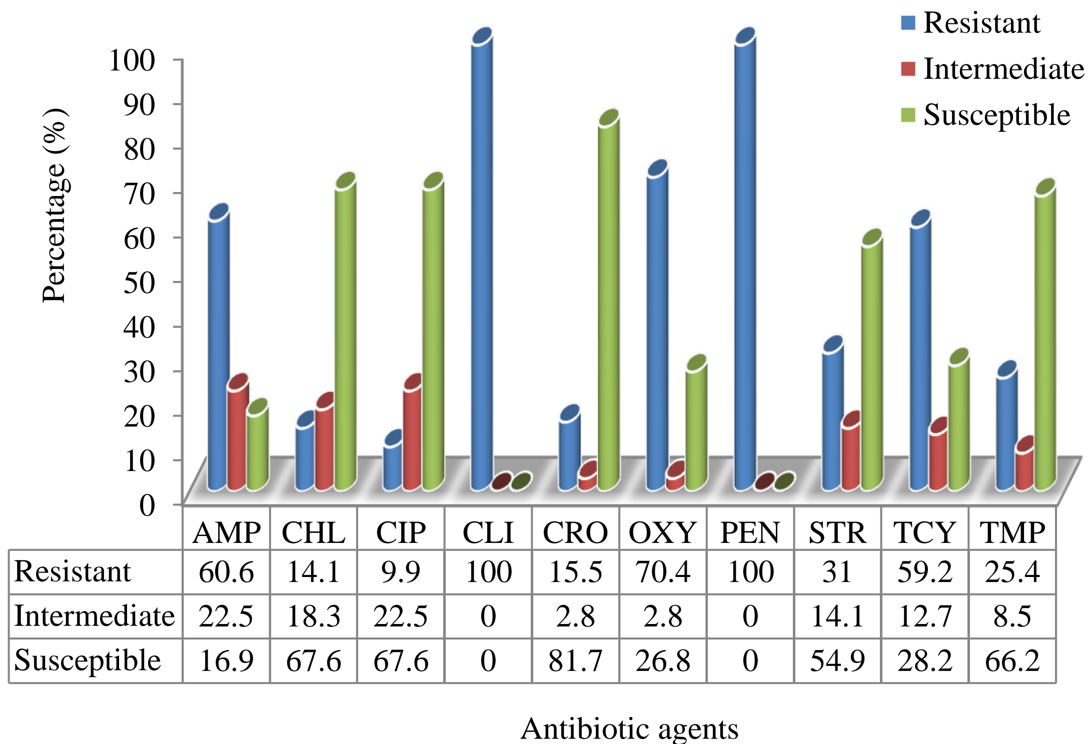
Variable	**Observation**	**N**	**n(%)**	**95% CI of A. P**	**OR**	χ^2	**p-value**
Production system	Urban	42	1(2.38)	0.06 – 12.57	1	8.584	0.003**
	Peri-Urban	6	2(33.33)	4.33 – 77.72	20.5		
	Total	**48**	**3(6.25)**	**1.31 – 17.19**			

4.5. Antimicrobial susceptibility results

The antimicrobial resistance test included all verified *S. enterica* (71) by invA PCR. The isolates then exposed to the ten antimicrobials, and halo zone diameters were measured and classified as resistant, intermediate, or susceptible using CLSI standards.

4.5.1. Mono-drug resistance result

The results of the antimicrobial susceptibility testing of *Salmonella enterica* isolates from milk and cottage cheese to the selected ten different antimicrobial disks are indicated in the graph below (Figure 6).



	AMP	CHL	CIP	CLI	CRO	OXY	PEN	STR	TCY	TMP
Resistant	60.6	14.1	9.9	100	15.5	70.4	100	31	59.2	25.4
Intermediate	22.5	18.3	22.5	0	2.8	2.8	0	14.1	12.7	8.5
Susceptible	16.9	67.6	67.6	0	81.7	26.8	0	54.9	28.2	66.2

Figure 6. Percent of the level of resistance of 71 bacterial isolates to different antimicrobials.

The highest level of resistance observed was for clindamycin and penicillin (100%) followed by oxy-tetracycline (71.83%). Two isolates were resistant to all the antibiotics (Figure 6).

The susceptibility of the pathogen was also observed and assessed in percentages. The potent antimicrobial was Ceftriaxone (81.7%) followed by Chloramphenicol and Ciprofloxacin (67.6 %), while the Trimethoprim was 66.2% (Table 17).

Table 17. The number and percent of the level of resistance of bacterial isolates to different antimicrobials

Antibiotic agent (Code)	Number of isolates				
	Disc concentration	R (%)	I (%)	S (%)	%R 95%C.I.
Ampicillin (AMP)	10 µg	43(60.6)	16(22.5)	12(16.9)	48.3-71.8
Ceftriaxone (CRO)	30 µg	10(14.1)	13(18.3)	48(67.6)	7.3-24.9
Chloramphenicol (CHL)	30 µg	7(9.9)	16(22.5)	48(67.6)	4.4-19.9
Ciprofloxacin (CIP)	5 µg	11(15.5)	2(2.8)	58(81.7)	8.4-26.5
Clindamycin (CLN)	2 µg	71(100)	0(0)	0(0)	93.6-100
Oxy-tetracycline (OT)	30 µg	50(70.4)	2(2.8)	19(26.8)	58.2-80.3
Penicillin (P)	10 units	71(100)	0(0)	0(0)	93.6-100
Streptomycin (S)	10 µg	22(31)	10(14.1)	39(54.9)	20.8-43.2
Tetracycline (TE)	30 µg	42(59.2)	9(12.7)	20(28.2)	46.9-70.5
Trimethoprim (W)	5 µg	18(25.4)	6(8.5)	47(66.2)	16.1-37.4

Key: R=Resistant, I=Intermediate, S=Susceptible C.I. =Confidence Interval

4.5.2. Multi-drug resistance patterns

Almost all of the isolates [69 (97.2%)] were multi-antimicrobial resistant (Table 18). The resistance levels ranged from 9.9 % to 100 %, to all the 10 antibiotics tested with some resistance. Several of the isolates (54%) were resistant to <5 antibiotics but the reaming isolates (46%) were resistant to >6. Two isolates were resistant to all remaining antibiotics. Additionally, all of the isolates were resistant to Penicillin and Clindamycin.

Table 18. Antimicrobial resistance patterns of the *Salmonella* isolates

Number	Antimicrobial resistance pattern (No.)	Number of isolates (%)
Zero	None	0(0%)
Two	CLI, PEN	2(2.89%)
Three	CLI, OXY, PEN / CIP, CLI, PEN / AMP, CLI, PEN	6(8.69%)
Four	CLI, OXY, PEN, TMP / CLI, OXY, PEN, TCY / AMP, CLI, PEN, TCY / AMP, CLI, OXY, PEN / AMP, CIP, CLI, PEN	10(14.49%)
Five	CLI, OXY, PEN, STR, TCY / CIP, CLI, PEN, TCY, TMP / CIP, CLI, PEN, STR, TMP / CRO, CLI, OXY, PEN, TCY / AMP, CLI, PEN, TCY TMP / AMP, CLI, PEN, STR, TCY / AMP, CLI, OXY, PEN, TCY /AMP, CLI, OXY, PEN, STR / AMP, CIP, CLI, PEN, STR / AMP, CRO, CLI, OXY, PEN	17(24.64 %)
Six	AMP, CLI, OXY, PEN, TCY, TMP / AMP, CLI, OXY, PEN, STR, TMP / AMP, CLI, OXY, PEN, STR, TCY / AMP, CRO, CLI, PEN, STR, TMP / AMP, CRO, CLI, OXY, PEN, TCY / AMP, CRO, CIP, CLI, PEN, TMP / AMP, CRO, CIP, CLI, PEN, STR	19(27.54%)
Seven	AMP, CLI, OXY, PEN, STR, TCY, TMP / AMP, CIP, CLI, OXY, PEN, TCY, TMP / AMP, CIP, CLI, OXY, PEN, STR, TCY / AMP, CRO, CLI, OXY, PEN, TCY, TMP	11(15.94%)
Eight	AMP, CIP, CLI, OXY, PEN, STR, TCY, TMP / AMP, CRO, CLI, OXY, PEN, STR, TCY, TMP / AMP, CRO, CIP, CLI, OXY, PEN, STR, TCY	3(4.35%)
Nine	AMP, CRO, CIP, CLI, OXY, PEN, STR, TCY, TMP	4(5.8%)
Ten	AMP, CRO, CIP, CHL, CLI, OXY, PEN, STR, TCY, TMP	2(2.89%)

Other antibiotics that showed resistance included oxy-tetracycline (70.4 %), ampicillin (60.6 %), tetracycline (59.2 %), and streptomycin (31 %). Three antimicrobials (ceftriaxone, ciprofloxacin, and chloramphenicol) were the most effective antimicrobials with the highest % of efficacy (Table 17).

4.5.3. Antimicrobial Resistance of the serotypes

Clindamycin scored the greatest proportion of resistance (100%) across all the three serotypes (Table 19). Penicillin had the second greatest resistance, with 90.6 %, 81.5 %, and 78.6 % to S. Enteritidis, S. Heidelberg, and S. Typhimurium, respectively. Ciprofloxacin had the lowest proportion of resistance (3.7 % in both S. Enteritidis and S. Typhimurium, but 9.5 % in S. Heidelberg). Among the 10 drugs tested for resistivity in the three serotypes, 15.6 % and 18.7 % against Streptomycin and trimethoprim by S. Enteritidis serotype ($p<0.05$). Ceftriaxone also exhibited lesser resistance, to 12.5% and 14.8% against S. Enteritidis and S. Typhimurium, respectively, whereas chloramphenicol showed 6.3% and 14.8% resistance to S. Enteritidis and S. Typhimurium, respectively.

All three serotypes showed resistance to all the tested drugs. Ampicillin and oxytetracycline resistance by S. enteritidis was the second-highest, accounting for 65.6% resistance. It was 66.7% for ampicillin and tetracycline by S. Heidelberg, but 73.8% for oxytetracycline. In the instance of S. Typhimurium, ampicillin and oxytetracycline resulted in 62.9 % and 77.8 % resistance, respectively (Table 19).

Table 19. The attribution of AMR and serotypes

Serotype	Antibiotics	Reaction in N (%)			Total positive	χ^2	P value
		S	I	R			
SE	AMP	5(15.6)	6(18.7)	21(65.6)	32 (45.1)	0.673	0.714
	CRO	28(87.5)	0(0)	4(12.5)	32 (45.1)	2.219	0.33
	CHL	23(71.9)	7(21.9)	2(6.3)	32 (45.1)	3.1	0.212
	CIP	27(84.4)	4(12.5)	1(3.1)	32 (45.1)	4.016	0.134
	CLN	0(0)	0(0)	32(100)	32 (45.1)	0(0)	0(0)
	OT	9(28.1)	2(6.3)	21(65.6)	32 (45.1)	2.668	0.263
	P	1(3.1)	2(6.3)	29(90.6)	32 (45.1)	4.883	0.087
	S	21(65.6)	6(18.7)	5(15.6)	32 (45.1)	7.552	0.023*
	TE	9(28.1)	5(15.6)	18(56.3)	32 (45.1)	0.454	0.797
	W	25(78.1)	1(3.1)	6(18.7)	32 (45.1)	6.525	0.038*
SH	AMP	6(14.3)	8(19.0)	28(66.7)	42(59.1)	1.604	0.448
	CRO	23(54.7)	1(2.4)	8(19.0)	42(59.1)	1.03	0.597
	CHL	26(61.9)	11(26.2)	5(11.9)	42(59.1)	4.329	0.115
	CIP	30(71.4)	8(19.0)	4(9.5)	42(59.1)	0.217	0.897
	CLN	0(0)	0(0)	42(100)	42(59.1)	0(0)	0(0)
	OT	10(23.8)	1(2.4)	31(73.8)	42(59.1)	0.572	0.751
	P	2(4.7)	7(16.7)	33(78.6)	42(59.1)	0.231	0.891
	S	20(47.6)	7(16.7)	15(35.7)	42(59.1)	1.506	0.471
	TE	9(21.4)	5(11.9)	28(66.7)	42(59.1)	1.604	0.448
	W	24(57.1)	1(2.4)	17(40.5)	42(59.1)	2.569	0.277
ST	AMP	4(14.8)	6(22.2)	17(62.9)	27(38.0)	0.156	0.925
	CRO	22(81.5)	1(3.7	4(14.8)	27(38.0)	0.135	0.935
	CHL	18(66.7)	5(18.5)	4(14.8)	27(38.0)	0.023	0.988
	CIP	23(85.2)	3(11.1)	1(3.7)	27(38.0)	3.244	0.198
	CLN	0(0)	0(0)	27(100)	27(38.0)	0(0)	0(0)
	OT	6(22.2)	0(0)	21(77.8)	27(38.0)	1.897	0.387
	P	1(3.7)	4(18.8)	22(81.5)	27(38.0)	0.339	0.844
	S	15(55.6)	3(11.1)	8(29.6)	27(38.0)	0.319	0.853
	TE	7(25.9)	4(18.8)	16(59.3)	27(38.0)	0.034	0.983
	W	15(55.6)	1(3.7)	11(40.7)	27(38.0)	0.938	0.626

*Significant at p value < 0.05, N (%)=Positive serotype No.(Percentage from the total, S= Susceptible, I=Intermediate, R=Resistant

5. DISCUSSION

The prevalence of *S. enterica* in cow's milk and cottage cheese was predicted by the extent of contamination of raw milk in production, collection, processing, and retailing units. The figure 14.79% in cow's milk and cottage cheese in this study is in agreement with 14.2%, 14.3%, 14.6%, and 15% of the prevalence of *Salmonella* spp. in dairy products reported by Singh *et al*. (2018), Beyene *et al*. (2016), Ali *et al.* (2020) and El-baz *et al*. (2017), respectively. The result is also consistent with the 12.1% and 20% recorded by Abunna *et al.* (2017) in udder milk and Tadesse and Dabassa (2012) in raw milk from the Kersa district of Ethiopia.

The prevalence of *Salmonella* spp. found in raw milk in this study was similar to the work of Gwida and Al-Ashmawy (2014) who documented *Salmonella* spp in raw milk with a prevalence of 21% in Egypt and also 16.4% of Mpatswenumugabo *et al*. (2019) in Rwanda. In comparison, Endris *et al.* (2013), Addis *et al*. (2011), and Elafify *et al*. (2019) showed a higher *Salmonella* prevalence of 35.7%, 28.6% in Ethiopia, and 44.44% in Egyptian dairy products. This higher prevalence of *Salmonella* may be attributed to various reasons such as the difference in geographical location as well as husbandry practices of the studying animal population including hygiene, transportation, farm administration, and farm-scale, environmental and seasonal factors (Pires *et al*., 2019).

In Ethiopia, Dadi *et al.* (2020), Hailu *et al.* (2015), Liyuwork *et al*. (2013), Reta *et al*. (2016), Ejo *et al.* (2016a), and Tadesse and Gebremedhin (2015) reported a low prevalence of 0.7%, 2.3%, 3.1%, 3.3%, 6%, and 10.76% in raw milk, respectively. Except for Dadi *et al.* (2020), who reported the prevalence of *S. enterica* (one of the two species), all the research was based at the species level. The decreased prevalence may be attributed to the procedures being focused on traditional culture approaches and biochemical tests rather than a molecular approach with high sensitivity.

In contrast to Liyuwork *et al*. (2013) and Ejo *et al.* (2016), who recorded 2.1% and 0% *Salmonella* in pasteurized milk in Ethiopia, the prevalence of 12.5% *Salmonella* in pasteurized milk was higher. However, according to a report by CDC (1984), there is a 15% prevalence in this value chain, which supports the current study. This high occurrence may be attributed to the samples' insufficient pasteurization and post-pasteurization contamination. Pasteurized milk, too,

has been implicated for spreading these microbes that entered the food by contamination after heating (Marth, 1969; Olsen *et al.*, 2004; Fusco *et al.*, 2020).

The prevalence of 6.25% of *Salmonella* spp. in cottage cheese in the present study was higher than that of Ejo *et al.* (2016a), Liyuwork *et al*. (2013), *Zewdu* (2004), and Taye *et al.* (2013), with a rate of 0%, 1.6%, 2.1%, and 3.1% in Gondar, Addis Ababa, Bishoftu and Addis Ababa, respectively. It is also higher compared to the work of Elbagory *et al.* (2016) in Egypt, which is around 2.5%. Higher rates have been reported, including 20% by El-baz *et al*. (2017) and 8.8% by Martínez *et al.* (2019).

In the current analysis, *Salmonella* serotypes were identified as S. Enteritidis, which is 45.07%, while S. Heidelberg is 59.15% and S. Typhimurium is 38.03%. For S. Typhimurium, the finding was consistent with the analysis performed by Eguale *et al*. (2015) and Beshiru *et al.* (2019) which is 37.3% and 31.1%, respectively, while the 0%, 8%, and 20% prevalence in the analysis conducted by Jibril *et al*. (2020), Ketema *et al.* (2018), and Alemayehu *et al.* (2003), respectively, in the milk sample. It was quite related to the 25.9% and 21.43% prevalence recorded by El-baz *et al*. (2017) and Alemu and Zewde (2012), respectively. In comparison, higher proportions also were present in studies like Jassim and Al-gburi (2020) and Wang *et al.* (2020) with rates of 75% and 63.4%, respectively. The last two reports were consistent with current work since they were molecularly based on identifying virulence genes, whereas the others were done using a slide agglutination test, which is less sensitive.

The prevalence of S. Enteritidis by Eguale *et al.* (2015), Alemayehu *et al.* (2003), and Jibril *et al*. (2020) of 1.49, 12, and 17.81%, respectively, differed with the current investigation since it was higher. Similar works reported by Omar *et al*. (2018) indicating a prevalence of 43.1% that matched with this study. The results of the current analysis were also higher relative to the work of El-baz *et al*. (2017) which is about 33.3%, and Beshiru *et al.* (2019) which is 24.4%.

For S. Heidelberg, the prevalence reports of 2, 12.8, 14.8, and 25.86% by Davidson *et al.* (2018), Odoch *et al.* (2017), Eguale *et al*. (2015), and Omar *et al*. (2018), respectively, were smaller as compared to this work.

The survey findings described the general sanitation and biosafety activities of the dairy value chains in these regions. It was noted that bacterial contamination of milk was substantially and

strongly correlated with containers used during milk transportation ($p<0.5$), the water temperature used to wash udder ($p<0.01$), the academic level of respondents ($p<0.05$), the management of mastitis ($p<0.05$) and exposure to training ($p<0.01$) in the milk producer value chain.

As the result of logistic regression indicates in this study for the aluminum can that farmers used to handle milk, the risk was octupled, like that of plastic users, in contrast to the work by Tigabu *et al.* (2015), who reported 0% prevalence for aluminum can user in the case of *S. aureus*. It also disagrees with the Donkor *et al.* (2007) and Mpatswenumugabo *et al*. (2019) studies, in which a possible risk factor associated with coliform bacteria contamination of milk in Ghana and Rwanda with the use of plastic milk containers (CI: 16.3 – 95.3). The usage of plastic and traditional containers can be a source of bacterial contamination of milk because they enable bacteria to multiply on milk contact surfaces during the interval between milkings, and being difficult to clean, which are porous by nature, using regular cleaning procedures and corrosion properties of the materials as compared to aluminum or stainless steel containers (Bereda *et al*. 2012; Babage *et al*. 2020). This suggests that the habit of cleaning milk equipment is inadequate because the risk is higher in the owners of the high-quality equipment (aluminum cans).

In the report of Mbindyo *et al.* (2020) from Kenya, there is also a somewhat similar figure in the case of mastitis check-up, in which the incidence is leading by more than one and half times for who will perform it than those do not; in this study, it leads by 3.1 times. The findings might be dealing with the reality that farmers with mastitis knowledge should not contaminate their milk and mostly seek their milk for containing the bacteria. Besides, even such producers could inspect for it and get mastitis, they may not dispose of the milk and they could potentially infect other cows in the herd.

In the assessment of exposure to the training of milk farmers, this study opposed the work of Niang *et al.* (2019), whose occurrence of pathogen exposure was doubled among those untrained. However, the latest research dictated five times more exposure for those trained than those not. In addition, the paper by Niang *et al.* (2019) was consistent with this study on the use of cold and warm water to wash utensils by doubling the exposure when this experiment quadrupled it. This is a capacity of heat treatment to destroy the bacteria that may be attached to the surface of the pieces of equipment by forming a biofilm. Illiteracy decreased the risk of

contamination of milk by more than 5 times and 3 times than completion of the preparatory and graduating by diploma/degree, as found in this report. This can be related to the lack of concern of the literates, the sample size attribution, and the close distance of the educated people to each other that could sustain the influence, then the illiterates who live far apart. This is hardly consistent with the work of Chimonyo *et al.* (2018), in which the risk to uneducated people is indicated to be high.

There were a link and high interaction of bacterial contamination of milk with the sex of respondents and milk filtration ($p<0.01$) in the value chain of collectors. Men collectors were subordinating the degree of exposure by 12% more than women collectors. Male collectors may be more observant than female collectors, resulting in this less exposure. In reality, since they were following certain procedures of milk and its products is a cultural matter of women, they are less likely to contaminate the milk, but the results of the evaluation disagreed with this fact. This could also be because women, especially in the countryside where this study was conducted, are engaged in various households such as cleaning of family premises and also that if animals, food preparation, carrying off children (Galhena *et al.* 2013). With all this burden that has to handle, there is no such sufficient clean water and detergent available for personal hygiene. Therefore, they may cross-contaminate milk from other sources. Furthermore, collectors who do not filter the milk, contaminate milk 20% less than those who do. However, contrary to what this study suggests, filtering can increase milk quality (Elwell and Barbano, 2006). This may be attributed to the unevenness of the sample size since milk filtration was supposed to result in less microbial contamination. The other variables assessed as a factor of risk for the prevalence in this value chain were statistically insignificant.

As stated by Knutson *et al.* (2010), conceivably perilous microorganisms in raw milk originate in both the milk when it is exuded and consequent debasement during the collection, processing, distribution, and storage manners. Because of environmental determinants in the factory, *Salmonella* most likely will contaminate the containers or milk contact surfaces during pasteurization, and could contaminate raw milk (Olsen *et al*., 2004).

The experience attribution against the vulnerability of the processors to the pathogen was roughly 38.5 % and 61.5 % of those of more than 10 years of experience and 2 to 5 years of experience, respectively. The usage of a piece of cloth has a 71.4 % exposure and the centrifugal

pump has a 28.6 % exposure to sieve the milk. In addition, when the microbiological test is played more than 14 times greater than the alcohol test for pathogen detection, the alcohol assay was followed by a one and a half lactometer test for adulteration parameters. According to Jüttner *et al.* (2003), this difference among processors may be due to variations in the distribution of potential value chain inputs, their chance, and their relative contributions.

There was no major association with the prevalence of *Salmonella enterica* in raw milk retailers and cottage cheese producer value chains in the study. Since then, no further inquiries have been carried out for the two value chains even if the pathogen presents. This may be due to the sample aspect, heat processing of the cottage cheese, or lack of stratification in the experiment design.

Only the production system has displayed the statistical significance of all the assessed risk factors in the value chain of the cottage cheese retailer. *Salmonella* was 5% likely to present in urban dwellers compared to peri-urban dwellings. This may be associated with urban dwellers' sanitation processes, water condition, veterinary access, availability of education and training, and the quality of livestock residences, and/or the matter of vicinity.

The resistance to antimicrobials by *Salmonella* isolates was observed for widely used antimicrobials. All the isolates were resistant to at least one antimicrobial. The entire isolates tested were 100% resistant to Clindamycin and Penicillin, which is in agreement with the reports of Singh *et al*. (2018), Carvalho *et al.* (2013), and Beshiru *et al.* (2019). Following the former drugs, oxy-tetracycline, ampicillin, and tetracycline, the resistance recorded in the current study was 70.4, 60.6, and 59.2%, respectively, relative to the work of Ali *et al.* (2020) who reported 82% and 70% resistance to oxytetracycline and ampicillin. Reports are similar to recent work, including Awol *et al.* (2019), which is 60%, Ali *et al.* (2020) which is 70%, Beyene *et al.* (2016), which is 58.3% for ampicillin, and 55.6% for tetracycline tolerance by Tosisa *et al*. (2020).

In contrast to this study, Abdi *et al*. (2017) and Awol *et al.* (2019) reported higher resistance to ampicillin, tetracycline, and streptomycin (97.8% each) and chloramphenicol (91.3%) and sequentially 60% to ampicillin and 80% to tetracycline. Wabeto *et al.* (2017) and Assefa and Girma (2019) recorded higher resistance, which is approximately 83.93% and 79.3% for tetracycline. Meanwhile, lower resistance estimates relative to this work were published in Banti (2018) and Eguale *et al*. (2015), respectively, 13.3% and 16.4% for Ampicillin; Beyene *et al.*

(2016) also reported 0 and 16.7% resistance in ciprofloxacin and chloramphenicol, respectively. The study conducted by Jorga *et al*. (2020) showed that 0 and 7.7% lower resistance to chloramphenicol and ciprofloxacin. Two isolates have demonstrated tolerance to all antimicrobials and this study is consistent with the article by Ali *et al.* (2020).

Antimicrobial resistance is the result of the use of antimicrobials in animals and humans and the resulting transmission of resistance genes and bacteria between animals, humans, food products, and the environment (Batt, 2014). The present study has shown that the resistance of screened antimicrobials varied from 0 percent to 81.7%. Ceftriaxone accounted for 81.7%, chloramphenicol, and ciprofloxacin for 67.6%, and trimethoprim for 66.2%. Addis *et al*. (2011), and Ali *et al.* (2020), 83.3 and 84% reported comparative work on ceftriaxone. Assefa and Girma (2019) recorded 100% susceptibility to ceftriaxone and ciprofloxacin and 24.15% susceptibility to chloramphenicol below the current report, while Jorga *et al*. (2020) reported higher susceptibility to chloramphenicol (92.3%).

All the isolates were resistant to one or more of the antimicrobials tested and 97.2% were resistant to multiple antimicrobials. It was higher than those recorded by Ali *et al.* (2020), Banti (2018), and Assefa and Girma (2019), which were 86, 86.7, and 79.3%, respectively. This study is in contrast to Zewdu (2004) who published 25% antimicrobial-resistant *Salmonella* isolates from cottage cheese. Detection of antimicrobial-resistant *Salmonella* can be consistent with their widespread use in both the livestock and public health markets since these antimicrobials are comparatively cheaper and widely available (D'Aoust, 1994).

The results of 100% antimicrobial-resistant *Salmonella* isolates from the sampled dairy products were remarkable. It poses a risk to public health since food poisoning outbreaks will be difficult to handle and this source of MDR *Salmonella* in food supplies is a repository for transferable, resistant genes (Muñoz-Aguayo, *et al.*, 2007). Reasons to restore antimicrobial resistance *Salmonella* isolates are most likely due to the indiscriminate application of antimicrobials (Mehadi *et al.,* 2015), self-medication due to quick access to non-prescription antibiotics in the public health industry, and the use of sub-therapeutic doses of antimicrobials to animals for prophylactic or medicinal purposes (Acha and Szyfres, 2005). This may also be due to the use of antimicrobials to promote the growth and prevention of disease in food animals.

6. CONCLUSION AND RECOMMENDATION

The present study results revealed a high prevalence of S. *enterica* in cow milk and cottage cheese. T*he* contamination was widespread in dairy value chains especially, in the collectors followed by producers, processors, and retailers. It also showed the resistance of Salmonella to most antimicrobials, of which 97.2% were MDR, and two of the isolates were completely resistant to all of the antimicrobials tested. It was evidence of a low level of public awareness about contamination of milk with Salmonella and the associated risk in the study area. Three serotypes (of health relevance) revealed in the study areas, in higher percentages, were requiring a high degree of effort to cope with them.

Based on the above conclusion the following recommendations are forwarded:

- ❖ Training programs on best practices for milk handling must be provided for handlers, as well as raising public awareness.
- ❖ There is a need to teach consumers, food handlers, and everyone else who comes into contact with food about the value of sanitation, cooling systems, and so on.
- ❖ Training on the monitoring and surveillance program of antimicrobial use in animals is recommended.
- ❖ In the veterinary and public health domains, encouraging the responsible and judicious utilization of antimicrobial drugs.
- ❖ Improvement in the milk delivery methods and sanitation from production to retail markets, such as the use of refrigerated transport vehicles.
- ❖ Application of the HACCP concept as a method to assess the exact causes of contamination at different key points from farm to table is important in developing effective policies to significantly minimize the incidence and related risk.
- ❖ An amendment prohibiting the storage of food until it is clean of *Salmonella* or excretion (shedding) is repealed.
- ❖ To reduce contamination of milk and milk products with pathogens of public health significance, a relevant action program and knowledge building on best practices in milk handling should be implemented.
- ❖ More studies should be performed to classify the pathogen and identify its serotypes.
- ❖ Milk manufacturers, collectors, processors, and marketers should be trained experts who understand the concepts of food quality and food safety.

7. REFERENCES

Abd El-Ghany WA (2020) Salmonellosis: A foodborne zoonotic and public health disease in Egypt. *Journal of Infection in Developing Countries, 14*(07), 674-678.

Abdi RD, Mengstie F, Beyi AF, Beyene T, Waktole H, Mammo B, Ayana D, Abunna F. (2017). Determination of the sources and antimicrobial resistance patterns of Salmonella isolated from the poultry industry in Southern Ethiopia. *BMC Infectious Diseases*, *17*(1), 1-12.

Abdissa, R., Haile, W., Fite, A. T., Beyi, A. F., Agga, G. E., Edao, B. M., … Goddeeris, B. M. (2017). Prevalence of Escherichia coli O157: H7 in beef cattle at slaughter and beef carcasses at retail shops in Ethiopia. *BMC Infectious Diseases*, *17*(1), 1–6.

Abunna, F., Ashenafi, D., Beyene, T., Ayana, D., Mamo, B., and Duguma, R. (2017). Isolation, identification and antimicrobial susceptibility profiles of *Salmonella* isolates from dairy farms in and around Modjo town, Ethiopia. *Ethiopian Veterinary Journal*, *21*(2), 92-108.

Acha, P. N., and Szyfres, B. (2005). Zoonosis and common communicable diseases in man *79*(3), 423–423.

Achtman M, Wain J, Weill FX, Nair S, Zhou Z, Sangal V, Krauland MG, Hale JL, Harbottle H, Uesbeck A, Dougan G, Harrison LH, Brisse S (2012); *S. Enterica* MLST Study Group. Multilocus sequence typing as a replacement for serotyping in *Salmonella enterica*. *PLoS Pathog*. *8*(6):e1002776.

Addis Z, Kebede N, Worku Z, Gezahegn H, Yirsaw A, Kassa T. (2011). Prevalence and antimicrobial resistance of *Salmonella* isolated from lactating cows and in contact humans in dairy farms of Addis Ababa. *BMC Infectious Diseases*, *11*(1), 1-7.

Adugna, M., Asresie, A., & Adigrat, E. (2015). A review on the microbiological quality of Ethiopian raw bovine milk. *Food Sci Qual Manage*, *35*, 17-34.

Afolami, O. I., and Onifade, A. K. (2018). Antibiotic-Resistant *Salmonella* spp: Mechanism of Drug Resistance, Gene Variations and Clinical Implications. *Asian Journal of Research in Medical and Pharmaceutical Sciences*, *4*(4), 1–6.

Ahmed, Mohamed, Ehui, Simeon and Yemesrach, Assefa. (2004). Dairy Development in

Ethiopia. International Food Policy Research Institute (IFPRI), EPTD discussion papers. *123*, 1-50.

Al-Bayati, A. M. K., and Al-Khafaji, N. S. K. (2020). Biofilm and antibiotic resistance profile among Salmonella typhi isolated from patients with typhoid fever. *Drug Invention Today*, *13*(6), 966–969.

Alemayehu, D., Molla, B., and Muckle, A. (2003). Prevalence and Antimicrobial Resistance Pattern of Salmonella Isolate from Apparently Healthy Slaughtered Cattle in Ethiopia. *Tropical Animal Health and Production, 35*(4), 309–319.

Alemneh, T., Getabalew, M., and Akeberegn, D. (2019). Dairy Production in Ethiopia - Existing Scenario and Constraints. *Biomedical Journal of Scientific & Technical Research*, *16*(5), 12304–12309.

Alemu, S., and Zewde, B. M. (2012). Prevalence and antimicrobial resistance profiles of *Salmonella enterica* serovars isolated from slaughtered cattle in Bahir Dar, Ethiopia. *Tropical Animal Health and Production*, *44*(3), 595–600.

Ali, D. A., Tadesse, B., and Ebabu, A. (2020). Prevalence and Antibiotic Resistance Pattern of *Salmonella* Isolated from Caecal contents of Exotic Chicken in Debre Zeit and Modjo, Ethiopia. *International Journal of Microbiology, 2020.*

Almeida, F., Seribelli, A. A., da Silva, P., Medeiros, M. I. C., dos Prazeres Rodrigues, D., Moreira, C. G., and Falcão, J. P. (2017). Multilocus sequence typing of *Salmonella* Typhimurium reveals the presence of the highly invasive ST313 in Brazil. *Infection, Genetics, and Evolution*, *51*, 41–44.

Alnakip, M., Quintela-Baluja, M., Böhme, K., Fernández-No, I., Caamaño-Antelo, S., Calo-Mata, P., and Barros-Velázquez, J. (2014). The Immunology of Mammary Gland of Dairy Ruminants between Healthy and Inflammatory Conditions. *Journal of Veterinary Medicine*, *2014*, 1–31.

Amagliani, G., Brandi, G., and Schiavano, G. F. (2012). Incidence and role of *Salmonella* in seafood safety. *Food Research International*, *45*(2), 780–788.

Amentie, T., Eshetu, M., Mekasha, Y., and Kebede, A. (2016). Milk postharvest handling practices across the supply chain in Eastern Ethiopia. *Journal of Advanced Veterinary and Animal Research*, *3*(2), 112–126.

Andino, A., and Hanning, I. (2015). *Salmonella enterica*: survival, colonization, and virulence differences among serovars. *The Scientific World Journal*, *2015*.

Andrews, W. H., Jacobson, A., and Hammack, T. (2018). Chapter 5: Salmonella. Bacteriological Analytical Manual (BAM).

Anvarinejad, M., Pouladfar, G. R., Pourabbas, B., Shahidi, M. A., Rafaatpour, N., Dehyadegari, M. A., and Mardaneh, J. (2016). Detection of salmonella spp. With the BACTEC 9240 automated blood culture system in 2008-2014 in Southern Iran (Shiraz): biogrouping, MIC, and antimicrobial susceptibility profiles of isolates. *Jundishapur Journal of Microbiology*, *9*(4), 0-0.

Assefa, A., & Girma, M. (2019). Prevalence and antimicrobial susceptibility patterns of *Salmonella* and *Shigella* isolate among children aged below five years with diarrhea attending Robe General Hospital and Goba Referral Hospital, South East Ethiopia. *Tropical diseases travel medicine and vaccines*, *5*(1), 1-11.

Awofisayo-Okuyelu, A., McCarthy, N., Mgbakor, I., and Hall, I. (2018). Incubation period of typhoidal salmonellosis: a systematic review and meta-analysis of outbreaks and experimental studies occurring over the last century. *BMC Infectious Diseases*, *18*(1), 1-13.

Awol, N., Nigusse, D., and Ali, M. (2019). Prevalence and antimicrobial susceptibility profile of *Salmonella* and Shigella among food handlers working in food establishment at Hawassa city, Southern Ethiopia. *BMC Research Notes*, 12(1), 1–7.

Ayalew, H., Birhanu A., and Asrade B. (2013). Review on food safety system: Ethiopian perspective. *African Journal of Food Science*, *7*(12), 431–440.

Babege, K., Eshetu, M. and Kassa, F. (2020). Hygienic Production Practices and Microbial Quality of Cow Milk in Cheha District of Gurage Zone, Southern Ethiopia. *Open Journal of Animal Science*s, *10*(03), 592-607.

Baker, S., and Dougan, G. (2007). The genome of *Salmonella enterica* serovar Typhi. *Clinical Infectious Diseases*, *45*(Supplement. 1), 29–33.

Banti, H. B. (2018). Isolation, Identification and Antimicrobial Susceptibility Profile of *Salmonella* Isolate from Abattoir and Selected Dairy Farms of Addis Ababa City, Ethiopia. *Global Veterinaria 20*(6), 285–292.

Batt, C. A. (2014). Escherichia coli: *Encyclopedia of Food Microbiology,* 688–694.

Beaubrun, J., Ewing, L., Dudley, K., Benhamed, F., Wang, H., and Hanes, D. E. (2017). Evaluation of a multiplex PCR method to serotype *Salmonella* in animal feeds pre-enrichment broth cultures. *MethodsX*, *4*, 335–345.

Bereda, A., Yesuf Kurtu, M., and Yilma, Z. (2014). Handling, Processing and Utilization of Milk and Milk Products in Ethiopia: A Review. *World Journal of Dairy & Food Science*, *9*(2), 105–112.

Bereda, A., Yilma, Z., and Nurfeta, A. (2012). Hygienic and microbial quality of raw whole cow's milk produced in Ezha district of the Gurage zone, Southern Ethiopia. *Journal of Agriculture Research*, *1*(1), 459–465.

Beshiru, A., Igbinosa, I. H., and Igbinosa, E. O. (2019). Prevalence of antimicrobial resistance and virulence gene elements of *Salmonella* serovars from ready-to-eat (RTE) shrimps. *Frontiers in Microbiology*, *10*, 1–11.

Betancourt, W. (2019). Part Three. Specific Excreted Pathogens: Environmental and Epidemiology Aspects Cryptosporidium spp. *Global Water Pathogens Project*, *3*(1), 1–50.

Beyene, T., Yibeltie, H., Chebo, B., Abunna, F., Beyi, A. F., Mammo, B., … Duguma, R. (2016). Identification and Antimicrobial Susceptibility Profile of *Salmonella* Isolated from Selected Dairy Farms, Abattoir, and Humans at Asella Town. *Journal of Veterinary Science & Technology, 7*(3), 320.

Boko CK, Marc Kpodekon T, Duprez J-N, Imberechts H, Taminiau B, Bertrand S, Jacques Mainil G (2013). Identification and typing of *Salmonella enterica* serotypes isolated from guinea fowl (Numida meleagris) farms in Benin during four laying seasons (2007 to 2010).

Avian Pathology, 42(1), 1-8.

Bonny AC, Karou TG, Atobla. K, Bohoua LG, Niamkey LS (2011). Carriage of *salmonella* in the raw chicken gizzard sold in Abidjan, Ivory Coast]. *Applied Biosciences, 47*, 3230- 3234

Brenner FW, Villar RG, Angulo FJ, Tauxe R, Swaminathan B (2000). *Salmonella* nomenclature. *Journal of Clinical Microbiology*, *38*(7), 2465- 2467

Brisabois A (2001). Intérêt et limites des techniques de caractérisation des *Salmonella* [Interest and limitations of *salmonella* characterization techniques]. *Epidemiology on Animal Health, 39*, 31-42.

Bayer, A. W., M. M. Kirby, J. C. Sherris, M.D., M. Turck, M.D., (1996). Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *American Journal of Clinical Pathology*, *45*(4), 493-496.

Callan, K., & F. Westblade, L. (2020). CLSI AST News Update. *The Importance of Being Specific: Staphylococcus Species-Specific Breakpoints for Methicillin (Oxacillin) Resistance*, *5*(1), 9–11.

ÇAM, D. (2020). Lateral Flow Assay for *Salmonella* Detection and Potential Reagents. *New Insight into Brucella Infection and Foodborne Diseases*.

Carip C, Jacky B, Dorsainvil E, Salvert MH, Tandeau A (2008). Microbiologie hygiène: base microbiologique de la diététique. LondresParis-New York.

Carvalho, F. C. T., Sousa, O. V, Carvalho, E. M. R., Hofer, E., and Vieira, R. H. S. F. (2013). Antibiotic Resistance of Salmonella spp . Isolated from Shrimp Farming Freshwater Environment in Northeast Region of Brazil. *Journal of Pathogens, 2013*, 1-5.

Chimonyo, M., Paraffin, A.S., and Zindove, T. J. (2018). Perceptions of Factors Affecting Milk Quality and Safety among Large- and Small-Scale Dairy Farmers in Zimbabwe. J*ournal of Food Quality, 3,* 1-7.

Chiu, C.-H., Wu, T.-L., Su, L.-H., Chu, C., Chia, J.-H., Kuo, A.-J., … Lin, T.-Y. (2002). The Emergence in Taiwan of Fluoroquinolone Resistance in *Salmonella enterica* Serotype

Choleraesuis. *New England Journal of Medicine*, *346*(6), 413–419.

CLSI. (2013). Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard—Second Edition. *National Committee of Clinical and Laboratory Standards, 19*(11), 6-11.

Cooke, V. M., Miles, R. J., Price, R. G., and Richardson, A. C. (1999). A novel chromogenic ester agar medium for detection of *salmonellae*. *Applied and Environmental Microbiology*, *65*(2), 807–812.

Cox, J. M. (1993). Lysine-mannitol-glycerol agar, a medium for the isolation of *Salmonella* spp., including S. Typhi and atypical strains. *Applied and Environmental Microbiology*, *59*(8), 2602–2606.

CSA. (2018). Agricultural Sample Survey. Report on Livestock and Livestock Characteristics (Private Peasant Holdings). The *Federal Democratic Republic of Ethiopia*, *II*(Statistical Bulletin 587), 104.

D'Aoust, J. Y. (1994). *Salmonella* and the international food trade. *International Journal of Food Microbiology*, *24*(1–2), 11–31.

Dabassa, A. and Bacha, K., 2012. The prevalence and antibiogram of *Salmonella* and Shigella isolated from the abattoir, Jimma Town, South West Ethiopia. *Journal of Pharmaceutical and Biological Research*, *3*,143-148.

Dadi S, Lakew M, Seid M, and Koran T, O. A. (2020). Isolation of *Salmonella* and *E. coli* O157:H7 and its Antimicrobial Resistance Pattern from Bulk Tank Raw Milk in Sebeta Town, Ethiopia. *HSOA Journal of Animal Research and Veterinary Science*, *4*(21), 1–7.

Daniel, W. W. (1991). Biostatistics: A Foundation for Analysis in the Health Sciences, 5th Edition. In *Biometrics* (Vol. 47).

Daquigan, N., Grim, C. J., White, J. R., Hanes, D. E., and Jarvis, K. G. (2016). Early recovery of *salmonella* from food using a 6-hour non-selective pre-enrichment and reformulation of tetrathionate broth. *Frontiers in Microbiology*, *7*, 1–12.

Darby, J., and Sheorey, H. (2008). Searching for *Salmonella*. *Australian Family Physician*, *37*(10), 806–810.

Davidson, K. E., Byrne, B. A., Pires, A. F. A., and Magdesian, K. G. (2018). Antimicrobial resistance trends in fecal *Salmonella* isolates from northern California dairy cattle admitted to a veterinary teaching hospital*, PLoS One. 13*(6), 2002–2016.

Davies, R. H., and Wales, A. D. (2013). *Salmonella* contamination of cereal ingredients for animal feeds. *Veterinary Microbiology*, *166*(3–4), 543–549.

Davis, B. J. K., Li, C. X., and Nachman, K. E. (2014). A Literature Review of the Risks and Benefits of Consuming Raw and Pasteurized Cow's Milk. *Johns Hopkins Center for a Livable Future*, 7,1–36.

DeWaal, C. S., & Robert, N. (2005). *Global & local: food safety around the world*. Center for Science in the Public Interest.

Donkor, E. S., Aning, K. G., Omore, A., Nurah, G. K., Osafo, E. L. K., & Staal, S. (2007). Risk factors in the hygienic quality of milk in Ghana. *The Open Food Science Journal*, *1*(1), 6-9.

Doyle, M. P., Erickson, M. C., Alali, W., Cannon, J., Deng, X., Ortega, Y., … Zhao, T. (2015). The Food Industry's Current and Future Role in Preventing Microbial Foodborne Illness Within the United States. *Clinical Infectious Diseases*, *61*(2), 252–259.

EAC. (2008). EAST AFRICAN COMMUNITY. Microbiology of food and animal feeding stuffs — Part 6: Horizontal method for the detection of *Salmonella* spp. EAST. *Eac*, *6,* 1-55.

EFSA. (2012). Scientific Opinion on Review of the European Union Summary Report on trends and sources of zoonoses, zoonotic agents, and food-borne outbreaks in 2009 and 2010 specifically for the data related to bovine tuberculosis, Echinococcus, Q fever, brucellosis, and non-food borne diseases. *EFSA Journal, 10*(6), 2765.

Eguale, T., Gebreyes, W. A., Asrat, D., Alemayehu, H., Gunn, J. S., and Engidawork, E. (2015). Non-typhoidal *Salmonella* serotypes, antimicrobial resistance, and co-infection with parasites among patients with diarrhea and other gastrointestinal complaints in Addis Ababa, Ethiopia. *BMC Infectious Diseases*, *15*(1), 1-9.

Ejo, M., Garedew, L., Alebachew, Z., and Worku, W. (2016). Prevalence and Antimicrobial Resistance of *Salmonella* Isolated from Animal-Origin Food Items in Gondar, Ethiopia. *BioMed Research International*, *1*, 8.

El-baz, A. H., El-sherbini, M., Abdelkhalek, A., and Al-ashmawy, M. A. (2017). Prevalence and molecular characterization of *Salmonella* serovars in milk and cheese in Mansoura city, Egypt. *Journal of Advanced Veterinary and Animal Research, 4*(1), 45–51.

Elafify, M., Darwish, W. S., Al-Ashmawy, M., Elsherbini, M., Koseki, S., Kawamura, S., and Abdelkhalek, A. (2019). Prevalence of *Salmonella* spp. in Egyptian dairy products: molecular, antimicrobial profiles and a reduction trial using d-tryptophan. *Journal Fur Verbraucherschutz Und Lebensmittelsicherheit*, *14*(4), 399–407.

Elbagory, A. (2016). Nutrition and Food Technology : Open Access Impact of Probiotic Strains on Growth of Some Food Poisoning Bacteria from Milk and Soft Cheese. *Nutrition and Food Technology, 1*(2).

Elwell, M. W., & Barbano, D. M. (2006). Use of Microfiltration to Improve Fluid Milk Quality. *Journal of Dairy Science, 89,* 20–30.

Endris, M., Taddesse, F., Geloye, M., Degefa, T., and Jibat, T. (2013). Sero and media culture prevalence of Salmonellosis in local and exotic chicken, Debre Zeit, Ethiopia. *African Journal of Microbiology Research*, *7*(12), 1041–1044.

Eng, S. K., Pusparajah, P., Ab Mutalib, N. S., Ser, H. L., Chan, K. G., & Lee, L. H. (2015). *Salmonella*: A review on pathogenesis, epidemiology, and antibiotic resistance. *Frontiers in Life Science*, *8*(3), 284–293. https://doi.org/10.1080/21553769.2015.1051243

Eng, S. K., Pusparajah, P., Ab Mutalib, N. S., Ser, H. L., Chan, K. G., and Lee, L. H. (2015b). *Salmonella*: A review on pathogenesis, epidemiology, and antibiotic resistance. *Frontiers in Life Science*, *8*(3), 284–293. https://doi.org/10.1080/21553769.2015.1051243

Eriksson, J., Löfström, C., Aspán, A., Gunnarsson, A., Karlsson, I., Borch, E., Rådström, P. (2005). Comparison of genotyping methods by application to *Salmonella* Livingstone strains associated with an outbreak of human salmonellosis. *International Journal of Food*

Microbiology, *104*(1), 93–103.

Fang, H., Xu, J., Ding, D., Jackson, S. A., Patel, I. R., Frye, J. G., Tong, W. (2010). An FDA bioinformatics tool for microbial genomics research on molecular characterization of bacterial foodborne pathogens using microarrays. *BMC Bioinformatics*, *11,* 6.

FAO. (2005). National food safety systems in Africa–A situation analysis. In FAO/WHO Regional Conference on Food Safety for Africa (pp. 47-57).

FAO/WHO. (2010). *FAO/WHO framework for developing national food safety emergency response plans.*

FAO. (2013). *Milk and Dairy Products in Human Nutrition. Food and Agriculture Organization of the United Nations*. 67(2).

FAO. (2017). Food Safety Risk Management. *Evidence-Informed Policies and Decisions Considering Multiple FActors*, *4*(2017).

FAO. (2019). Food and Agriculture Organization of the United Nations. *The Future of Food Contents*. 1–26.

FAO, F. and A. O. of the U. N., and WHO, W. H. O. (2007). Principles and Guidelines for the Conduct of Microbiological Risk Assessment. *FAO: Agriculture and Consumer Protection*, *63*, 68.

Ferrari, R. G., Panzenhagen, P. H. N., and Conte-Junior, C. A. (2017). Phenotypic and genotypic eligible methods for Salmonella Typhimurium source tracking. *Frontiers in Microbiology*, *8*, 1–21.

Ferretti, R., Mannazzu, I., Cocolin, L., Comi, G., and Clementi, F. (2001). Twelve-hour PCR-based method for detection of *Salmonella* spp. in food. *Applied and Environmental Microbiology*, *67*(2), 977–978.

Finn S, Condell O, McClure P, Amézquita A and Fanning S (2013). Mechanisms of survival, responses, and sources of Salmonella in low-moisture environments. *Frontiers Microbiology*, *4,* 331.

Foley, S. L., White, D. G., McDermott, P. F., Walker, R. D., Rhodes, B., Fedorka-Cray, P. J., … Zhao, S. (2006). Comparison of subtyping methods for differentiating *Salmonella enterica* serovar Typhimurium isolates obtained from food animal sources. *Journal of Clinical Microbiology*, *44*(10), 3569–3577.

Fraser, R. W., Williams, N. T., Powell, L. F., and Cook, A. J. C. (2010). Reducing Campylobacter and *Salmonella* infection: Two studies of the economic cost and attitude to the adoption of on-farm biosecurity measures. *Zoonoses and Public Health*, *57*(7–8), 109–115. https://doi.org/10.1111/j.1863-2378.2009.01295.x

Frye, J. G., & Jackson, C. R. (2013). Genetic mechanisms of antimicrobial resistance identified in *Salmonella enterica*, *Escherichia coli*, and *Enterococcus* spp. isolated from U.S. food animals. *Frontiers in microbiology*, *4*, 135.

Fusco, V., Chieffi, D., Fanelli, F., Logrieco, A. F., Cho, G. S., Kabisch, J., … Franz, C. M. A. P. (2020). Microbial quality and safety of milk and milk products in the 21st century. *Comprehensive Reviews in Food Science and Food Safety*, *19*(4), 2013–2049.

Gal-Mor, O., Boyle, E. C., and Grassl, G. A. (2014). Same species, different diseases: How and why typhoidal and non-typhoidal *Salmonella enterica* serovars differ. *Frontiers in Microbiology*, *5*, 1–10.

Getabalew, M., Dairy, H., and Alemneh, T. (2020). Review paper Review on Milk and Milk product Handling Practices, Utilization and Microbial quality in Ethiopia. *International Journal of Dairy Science and Technology, 4*(1), 218-224.

Getto, L., Zeserson, E., and Breyer, M. (2011). Vomiting, Diarrhea, Constipation, and Gastroenteritis. *Emergency Medicine Clinics of North America*, *29*(2), 211–237.

Gibani, M. M., Britto, C., and Pollard, A. J. (2018). Typhoid and paratyphoid fever: A call to action. *Current Opinion in Infectious Diseases*, *31*(5), 440–448.

Girma., S. (2012). Assessment of awareness on food-borne zoonoses and their relation with veterinary public health services in and around Addis Ababa, Ethiopia. *Journal of Public Health and Epidemiology*, *4*(2), 48-51.

Gizaw, S., Abera, M., Muluye, M., Aliy, M., Alemayehu, K., & Tegegne, A. (2017). Validating the classification of smallholder dairy farming systems based on herd genetic structure and access to breeding services. *Agricultural Sciences*, *8*(07), 545.

Gonzalez-Escobedo, G., Marshall, J. M., and Gunn, J. S. (2011). Chronic and acute infection of the gall bladder by Salmonella Typhi: Understanding the carrier state. *Nature Reviews Microbiology*, *9*(1), 9–14.

Gossner, C. M., Le Hello, S., De Jong, B., Rolfhamre, P., Faensen, D., Weill, F. X., and Giesecke, J. (2016). Around the world in 1,475 *Salmonella* geo-serotypes. *Emerging Infectious Diseases*, *22*(7), 1298–1302.

Guiney, D. G. (2005). The role of host cell death in *Salmonella* infections. *Current Topics in Microbiology and Immunology*, *289*, 131–150.

Guiney, Donald G., and Fierer, J. (2011). The role of the *spv* genes in *Salmonella* pathogenesis. *Frontiers in Microbiology*, *2*, 1–10.

Gut, A. M., Vasiljevic, T., Yeager, T., & Donkor, O. N. (2018). Salmonella infection–prevention and treatment by antibiotics and probiotic yeasts: a review. *Microbiology*, *164*(11), 1327-1344.

Gwida, M. M., & Al-Ashmawy, M. A. (2014). Culture versus PCR for Salmonella Species Identification in Some Dairy Products and Dairy Handlers with Special Concern to Its Zoonotic Importance. *Veterinary medicine international*, *2014*, 502370.

Hadrya, F., Elouardi, A., Benali, D., Hami, H., Soulaymani, A., and Senouci, S. (2012). Bacterial quality of informally marketed raw milk in Kenitra city, Morocco. *Pakistan Journal of Nutrition*, *11*(8), 662–669.

Hailu, D., Gelaw, A., Molla, W., Garedew, L., Cole, L., and Johnson, R. (2015). Prevalence and antibiotic resistance patterns of *Salmonella* isolates from lactating cows and in-contact humans in dairy farms, Northwest Ethiopia. *Journal of Environmental and Occupational Science*, *4*(4), 171–178.

Hall-Stoodley, L., Nistico, L., Sambanthamoorthy, K., Dice, B., Nguyen, D., Mershon, W. J.,

Post, J. C. (2008). Characterization of biofilm matrix, degradation by DNase treatment, and evidence of capsule downregulation in *Streptococcus pneumoniae* clinical isolates. *BMC Microbiology*, *8*, 1–16.

Hansen-Wester, I., Stecher, B., and Hensel, M. (2002). Type III secretion of *Salmonella enterica* serovar Typhimurium translocated effectors and SseFG. *Infection and Immunity*, *70*(3), 1403–1409.

Henley, S. C., Stein, S. E., and Quinlan, J. J. (2012). Identification of unique food handling practices that could represent food safety risks for minority consumers. *Journal of Food Protection*, *75*(11), 2050–2054.

Herikstad, H., Motarjemi, Y., and Tauxe, R. V. (2002). *Salmonella* surveillance: A global survey of public health serotyping. *Epidemiology and Infection*, *129*(1), 1–8.

Herrero, M., Grace, D., Njuki, J., Johnson, N., Enahoro, D., Silvestri, S., & Rufino, M. C. (2013). The roles of livestock in developing countries. *Animal*, *7*(1), 3-18.

Humphries, R. M., and Linscott, A. J. (2015). Laboratory diagnosis of bacterial gastroenteritis. *Clinical Microbiology Reviews*, *28*(1), 3–31.

ISO, (2017). The new ISO 6579-1: A real horizontal standard for detection of Salmonella, at last! *Food Microbiology, 71*, 2-7.

Jajere, S. M. (2019). A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and adaptation, and antimicrobial resistance including multidrug resistance. *Veterinary World*, *12*(4), 504–521.

Jassim, A. A., and Al-gburi, N. M. (2020). Virulence Genes And Antimicrobial Resistance of *Salmonella* Virulence Genes And Antimicrobial Resistance Of *Salmonella* Isolated From Milk In Wasit Province, Iraq. *Plant Archives* *20*(1), 2033-2039.

Jensen, A. N., Dalsgaard, A., Stockmarr, A., Nielsen, E. M., and Baggesen, D. L. (2006). Survival and transmission of *Salmonella enterica* serovar Typhimurium in an outdoor organic pig farming environment. *Applied and Environmental Microbiology*, *72*(3), 1833–1842.

Jibril, A. H., Okeke, I. N., Dalsgaard, A., Kudirkiene, E., Akinlabi, O. C., Bello, M. B., and Olsen, J. E. (2020). Prevalence and risk factors of *Salmonella* in commercial poultry farms in Nigeria. *PLoS ONE*, *15*(9), 1–17.

Jorga, E. S., Kudama, K., Dandecha, M., Megersa, L., Borena, M., and Gebremdhin, E. Z. (2020). Prevalence, organ distribution, and antimicrobial susceptibility profile of *Salmonella* isolated from chickens purchased from markets in selected districts of West Shoa, Ethiopia. *Ethiopian Veterinary Journal 24*(2), 73–89.

Joseph, A., and Carlos, G. (2012). *Salmonella* detection methods for food and food ingredients. *Salmonella-A Dangerous Foodborne Pathogen. Rijeka, Croatia: InTec*, 373-392.

Jüttner, U., Peck, H., and Christopher, M. (2003). Supply chain risk management: outlining an agenda for future research. *International Journal of Logistics Research and Applications*, *6*(4), 197–210.

Karalis, T. (2000). Cultural Methods for the Detection and Enumeration of Salmonella in Foods. http://unsworks.unsw.edu.au/fapi/datastream/unsworks:35904/SOURCE01?view=true

Karshima. N.S., Pam. V. A., Bata S. I., Dung P. A. and Paman N. D. (2013). Isolation of *Salmonella* Species from Milk and Locally Processed Milk Products Traded for Human Consumption and Isolation of *Salmonella* Species from Milk and Locally Processed Milk Products Traded For Human Consumption and Associated Risk Factors in Kanam. *J Anim Prod A*, *3*(3), 69–74.

Kauffmann, F. (1972). Serological diagnosis of *Salmonella*-species, Kauffmann-White-schema. *Bacterial Serotyping Guide for Salmonella*, 122-124.

Keba, A., Rolon, M. L., Tamene, A., Dessie, K., Vipham, J., Kovac, J., and Zewdu, A. (2020). Review of the prevalence of foodborne pathogens in milk and dairy products in Ethiopia. *International Dairy Journal*, *109*(2), 104762.

Ketema, L., Ketema, Z., Kiflu, B., Alemayehu, H., Terefe, Y., Ibrahim, M., … Laranjo, M. (2018). Prevalence and Antimicrobial Susceptibility Profile of *Salmonella* Serovars Isolated

from Slaughtered Cattle in Addis Ababa, Ethiopia. *BioMed Research International*, *2018*.

Ki-Bok Yoon, Byung-Joon Song, Mi-Yeong Shin, Hyun-Cheol Lim, Yeon-Hee Yoon, Doo-Young Jeon, Hoon Ha, Soo-In Yang, Jung-Beom Kim (2017). Antibiotic Resistance Patterns and Serotypes of *Salmonella* spp. Isolated at Jeollanam-do in Korea. *Osong Public Health Research Perspective*, *8*(3):211-219.

Kibret, M., and Abera, B. (2012). The sanitary conditions of food service establishments and food safety knowledge and practices of food handlers in Bahir dar town. *Ethiopian Journal of Health Sciences*, *22*(1), 27–35.

Kitinoja, L. (2013). Use of cold chains for reducing food losses in developing countries. *Postharvest Education Foundation White Paper*, *6*(13), 1–16.

Knutson, R. D., Currier, R. W., Ribera, L. A., & Goeringer, L. P. (2010). *Asymmetry in raw milk safety perceptions and information: Implications for risk in fresh produce marketing and policy* (No. 700-2016-48005).

Koczula, K. M., and Gallotta, A. (2016). Lateral flow assays. *Essays in Biochemistry*, *60*(1), 111–120.

Lamboro, T., Ketema, T., & Bacha, K. (2016). Prevalence and Antimicrobial Resistance in *Salmonella* and *Shigella* Species Isolated from Outpatients, Jimma University Specialized Hospital, Southwest Ethiopia. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *2016*, 1–8.

LeJeune, J. T., and Rajala-Schultz, P. J. (2009). Unpasteurized milk: A continued public health threat. *Clinical Infectious Diseases*, *48*(1), 93–100.

Liu, W. bing, Liu, B., Zhu, Xin-na, Yu, S. jing, and Shi, X. ming. (2011). Diversity of *Salmonella* isolates using serotyping and multilocus sequence typing. *Food Microbiology*, *28*(6), 1182–1189.

Liyuwork, T., Biruhalem, T., Sefinew, A., Haile, A., Zufan, S., and Haileleul, N. (2013). Prevalence and antimicrobial resistance profile of *Salmonella* isolated from dairy products in Addis Ababa, Ethiopia. *African Journal of Microbiology Research*, *7*(43), 5046–5050.

Lohiniva, A. L., Saeed, M., El-Sayeed, N., and Talaat, M. (2008). Clean hands: prevention of typhoid fever in rural communities in Egypt. *International quarterly of community health education*, *28*(3), 215-227.

Mainar-Jaime, R. C., Andrés, S., Vico, J. P., San Román, B., Garrido, V., and Grilló, M. J. (2013). Sensitivity of the ISO 6579:2002/Amd 1:2007 standard method for detection of *Salmonella* spp. on mesenteric lymph nodes from slaughter pigs. *Journal of Clinical Microbiology*, *51*(1), 89–94.

Majowicz, S. E., Musto, J., Scallan, E., Angulo, F. J., Kirk, M., O'Brien, S. J., … Hoekstra, R. M. (2010). The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clinical Infectious Diseases*, *50*(6), 882–889.

Mandefero, D., and Yeshibelay, G. (2018). Assessment of community knowledge, attitude, and practice on milk-borne zoonoses disease in Debre- Birhan town, north Shewa, Ethiopia. *Journal of Public Health and Epidemiology*, *10*(4), 123–131.

Marchello, C. S., Carr, S. D., and Crump, J. A. (2020). A systematic review on antimicrobial resistance among Salmonella Typhi worldwide. *American Journal of Tropical Medicine and Hygiene*, *103*(6), 2518–2527.

Marth, E. H. (1969). *Salmonellae* and Salmonellosis Associated with Milk and Milk Products. A Review. *Journal of Dairy Science*, *52*(3), 283–315.

Mattick, K. L., Jorgensen, F., Legan, J. D., Lappin-Scott, H. M., and Humphrey, T. J. (2000). Habituation of *Salmonella* spp. at reduced water activity and its effect on heat tolerance. *Applied and Environmental Microbiology*, *66*(11), 4921–4925.

Mbindyo, C. M., Gitao, G. C., and Mulei, C. M. (2020). Prevalence, Etiology, and Risk Factors of Mastitis in Dairy Cattle in Embu and Kajiado Counties, Kenya. *Veterinary Medicine International, 20*(12), 2.

Mehadi, M., Chowdhury, H., Islam, M. T., Rahman, M., and Mehedy, E. (2015). Indiscriminate Uses of Antibiotics as a Threat to Public Health Demand Implementation of Effective Drug Practices and Enhancement of Public Awareness in Bangladesh. *European Journal of*

Scientific Research, *133*(2), 187-195.

Mehlhorn, H. (2015). Food-Borne Disease Burden Epidemiology Reference Group. *Encyclopedia of Parasitology*, 1–1.

Mojica, F. J. M., Díez-Villaseñor, C., Soria, E., and Juez, G. (2000). The biological significance of a family of regularly spaced repeats in the genomes of Archaea, Bacteria, and mitochondria. *Molecular Microbiology*, *36*(1), 244–246.

Monack, D. M., Mueller, A., and Falkow, S. (2004). Persistent bacterial infections: The interface of the pathogen and the host immune system. *Nature Reviews Microbiology*, *2*(9), 747–765.

Mooijman, K. A. (2018). The new ISO 6579-1: A real horizontal standard for detection of *Salmonella*, at last! *Food Microbiology*, *71*, 2–7.

Mosalagae, D., Pfukenyi, D. M., and Matope, G. (2011). Milk producers' awareness of milk-borne zoonoses in selected smallholder and commercial dairy farms of Zimbabwe. *Tropical Animal Health and Production*, *43*(3), 733–739.

Mossie, H. (2019). Review on Traditional Handling, Processing, and Marketing of Milk and its Derivative in Ethiopia. *Journal of Dairy and Veterinary Science*, *13*(5), 555874.

Mpatswenumugabo, J. P. M., Bebora, L. C., Gitao, G. C., Mobegi, V. A., Iraguha, B., and Shumbusho, B. (2019). Assessment of bacterial contamination and milk handling practices along the raw milk market chain in the north-western region of Rwanda. *African Journal of Microbiology Research 13*(29), 640–648.

Mthembu, T. P., Zishiri, O. T., & El Zowalaty, M. E. (2019). Molecular detection of multidrug-resistant Salmonella isolated from livestock production systems in South Africa. *Infection and drug resistance*, *12*, 3537.

Munita, J. M., and Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiology spectrum*, *4*(2), 4-2.

Muñoz-Aguayo, J., Lang, K. S., LaPara, T. M., González, G., and Singer, R. S. (2007). Evaluating the effects of chlortetracycline on the proliferation of antibiotic-resistant bacteria

in a simulated river water ecosystem. *Applied and Environmental Microbiology*, *73*(17), 5421–5425.

Nada, S., Ilija, D., Igor, T., Jelena, M., and Ruzica, G. (2012). Implication of food safety measures on the microbiological quality of raw and pasteurized milk. *Food Control*, *25*(2), 728–731.

Negash, F., Tadesse, E., Aseffa, E., Yimamu, C., and Hundessa, F. (2012). Production, handling, processing, utilization, and marketing of milk in the Mid Rift Valley of Ethiopia. *Livestock Research for Rural Development*, *24*(9).

Niang, Y., Soe, N., Wai, S., Lin, T. N., Thu, W. P., Lat, L., Myaing, T. (2019). Bacterial content and associated risk factors influencing the quality of bulk tank milk collected from dairy cattle farms in Mandalay Region. *Food Science and Nutrition, 7*(3), 1063–1071.

Ngogo, F.A., Joachim, A., Abade, A.M., *et al.* (2020). Factors associated with *Salmonella* infection in patients with gastrointestinal complaints seeking health care at Regional Hospital in Southern Highland of Tanzania. *BMC Infectious Diseases,* 20**,** 135.

Odoch, T., Wasteson, Y., Abée-lund, T. L., Muwonge, A., Kankya, C., Nyakarahuka, L., Skjerve, E. (2017). Prevalence, antimicrobial susceptibility, and risk factors associated with non-typhoidal *Salmonella* on Ugandan layer hen farms. *BMC Veterinary Research, 13*(1), 1–10.

Oliveira, S. D., Rodenbusch, C. R., Cé, M. C., Rocha, S. L. S., and Canal, C. W. (2003). Evaluation of selective and non-selective enrichment PCR procedures for *Salmonella* detection. *Letters in Applied Microbiology*, *36*(4), 217–221. https://doi.org/10.1046/j.1472-765X.2003.01294.x

Oliver Al., E. T. (2005). Foodborne Pathogens in Milk and the Dairy Farm Environment: Food Safety and Public. *Foodborne pathogens and disease* *2*(2), 115–129.

Oliver, S. P., Boor, K. J., Murphy, S. C., and Murinda, S. E. (2009). Food safety hazards associated with consumption of raw milk. *Foodborne Pathogens and Disease*, *6*(7), 793–806.

Omar, D., Al-Ashmawy, M., Ramadan, H., & El-Sherbiny, M. (2018). Occurrence and PCR identification of Salmonella spp. from milk and dairy products in Mansoura, Egypt. *International Food Research Journal*, *25*(1), 446-452.

Omulo, S., Thumbi, S. M., Njenga, M. K., & Call, D. R. (2015). A review of 40 years of enteric antimicrobial resistance research in Eastern Africa: what can be done better?. *Antimicrobial resistance and infection control*, *4*(1), 1-13.

Pal, M., Mulu, S., Tekle, M., Pinto, S. V, and Prajapati, J. P. (2016). Bacterial Contamination of Dairy Products. *Beverage and Food World*, *43*(9), 40–43.

Paniel, N., & Noguer, T. (2019). Detection of *Salmonella* in food matrices, from conventional methods to recent aptamer-sensing technologies. *Foods*, *8*(9), 371.

Park, S. H., & Ricke, S. C. (2015). Development of multiplex PCR assay for simultaneous detection of *Salmonella* genus, *Salmonella* subspecies I, Salm. Enteritidis, Salm. Heidelberg and Salm. Typhimurium. *Journal of Applied Microbiology*, *118*(1), 152–160.

Park, Sang Hyun, Ryu, S., and Kang, D. H. (2012). Development of an improved selective and differential medium for isolation of *Salmonella* spp. *Journal of Clinical Microbiology*, *50*(10), 3222–3226.

Pegues, D. A., Ohl, M. E., and Miller, S. I. (2006). Nontyphoidal Salmonellosis. *Tropical Infectious Diseases*, *1*, 241–254.

Pérez-Carrera, A. L., Arellano, F. E., and Fernández-Cirelli, A. (2016). Concentration of trace elements in raw milk from cows in the southeast of Córdoba province, Argentina. *Dairy Science and Technology*, *96*(5), 591–602.

Petersen, R. F., Litrup, E., Larsson, J. T., Torpdahl, M., Sørensen, G., Müller, L., and Nielsen, E. M. (2011). Molecular characterization of Salmonella Typhimurium highly successful outbreak strains. *Foodborne Pathogens and Disease*, *8*(6), 655–661.

Pires, A., Patterson, L., Kukielka, E. A., Aminabadi, P., Navarro-Gonzalez, N., & Jay-Russell, M. T. (2019). Prevalence and risk factors associated with *Campylobacter* spp. and *Salmonella enterica* in livestock raised on diversified small-scale farms in

California. *Epidemiology and infection*, *147*, 321.

Rambach, A. (1990). New plate medium for facilitated differentiation of *Salmonella* spp. from Proteus spp. and other enteric bacteria. *Applied and Environmental Microbiology*, *56*(1), 301–303.

Ranieri, M. L., Shi, C., Moreno Switt, A. I., Den Bakker, H. C., and Wiedmann, M. (2013). Comparison of typing methods with a new procedure based on sequence characterization for *Salmonella* serovar prediction. *Journal of Clinical Microbiology*, *51*(6), 1786–1797.

Rasschaert, G. (2007). *Molecular epidemiology of Salmonella and Campylobacter contamination of poultry during transport and slaughter* (Doctoral dissertation, Ghent University).

Ratiner, Y. A., Salmenlinna, S., Eklund, M., Keskimäki, M., and Siitonen, A. (2003). Serology and genetics of the flagellar antigen of *Escherichia coli* O157:H7a,7c. *Journal of Clinical Microbiology*, *41*(3), 1033–1040.

Reischl, U., and Wittwer, C. (2001). Rapid Cycle Real-Time PCR: Methods and Applications. *Springer-Verlag Berlin Heidelberg 2001*, pp. 1–8.

Report, M. W. (1984). *Salmonellosis from Inadequately Pasteurized Milk — Kentucky Outbreaks of Respiratory Illness among Employees in Large Office Buildings — Tennessee, District of Columbia*. *33*(36), 505–516.

Reta, M. A., Bereda, T. W., & Alemu, A. N. (2016). Bacterial contamination of raw cow's milk consumed at Jigjiga City of Somali Regional State, Eastern Ethiopia. *International Journal of Food Contamination*, *3*(1), 1-9.

Reygaert W. C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology*, *4*(3), 482–501.

Ruiz, J. (2003). Mechanisms of resistance to quinolones: target alterations, decreased accumulation, and DNA gyrase protection. *Journal of Antimicrobial Chemotherapy*, *51*(5), 1109-1117.

Saba, D., Ercan, A., Şenkaya, I., Gebitekin, C., and Özkan, H. (2001). The impact of water,

sanitation, and hygiene on key health and social outcomes. *Ulusal Travma ve Acil Cerrahi Dergisi*, *7*(3), 201–203.

Sanchez, J., Dohoo, I. R., Christensen, J., and Rajic, A. (2007). Factors influencing the prevalence of *Salmonella* spp . in swine farms : A meta-analysis approach. *Preventive Veterinary Medicine*, *81*, 148–177.

Shanmugasamy, M., Velayutham, T., and Rajeswar, J. (2011). Inv a gene-specific PCR for detection of *Salmonella* from broilers. *Veterinary World*, *4*(12), 562–564.

Sheehy, T., and Sharma, S. (2013). Trends in energy and nutrient supply in Trinidad and Tobago from 1961 to 2007 using FAO food balance sheets. *Public Health Nutrition*, *16*(9), 1693–1702.

Shen, H., Chen, H., Ou, Y., Huang, T., Chen, S., Zhou, L., ... & Ma, W. (2020). Prevalence, serotypes, and antimicrobial resistance of Salmonella isolated from patients with diarrhea in Shenzhen, China. *BMC microbiology*, *20*(1), 1-10.

Shunda, D., Habtamu, T., and Endale, B. (2013). Assessment of bacteriological quality of raw cow milk at different critical points in Mekelle, Ethiopia. *International Journal of Livestock Research*, *3*(4), 42–48.

Singh, P., Singh, R. V, Gupta, B., Tripathi, S. S., Tomar, K. S., Jain, S., and Sahni, Y. P. (2018). Prevalence study of *Salmonella* spp . in milk and milk products. *Asian Journal of Dairy & Food Research*, *37*(1), 7–12.

Siriken B. (2013). *Salmonella* Patojenite Adaları [*Salmonella* pathogenicity islands]. *Microbiol Bul, 47*(1),181-188.

Smith, A. M., Tau, N., Sooka, A., and Keddy, K. H. (2015). Microbiological characterization of *Salmonella enterica* serotype Paratyphi, South Africa, 2003–2014. *Journal of Medical Microbiology*, *64*(11), 1450–1453.

Solomon, T., & Ketema, T. (2011). Microbiological safety of street vended ayib in Jimma town, southwest Ethiopia. *Ethiopian Journal of Education and Sciences*, *7*(1), 81-91.

Stanaway, J. D., Parisi, A., Sarkar, K., Blacker, B. F., Reiner, R. C., Hay, S. I., … Crump, J. A. (2019). The global burden of non-typhoidal *salmonella* invasive disease: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet Infectious Diseases*, *19*(12), 1312–1324.

Tadesse, A., and Alem, M. (2006). LECTURE NOTES Degree and Diploma Programs For Environmental Health Students Medical Bacteriology.

Tadesse, G. (2015). A meta-analysis of the proportion of animal *Salmonella* isolates resistant to drugs used against human salmonellosis in Ethiopia. *BMC Infectious Diseases*, *15*, 84.

Tadesse, G., and Gebremedhin, E. Z. (2015). Prevalence of *Salmonella* in raw animal products in Ethiopia: A meta-analysis. *BMC Research Notes*, *8*(1), 1–8.

Tadesse, G., & Yilma, Z. (2018). Dairy Trade in Ethiopia: Current scenario and way forwardreview. *Journal of Dairy and Veterinary Sciences*, *8*(1), 1-13.

Tadesse, T., and Dabassa, A. (2012). Prevalence and antimicrobial resistance of *Salmonella* isolated from raw milk samples collected from Kersa District, Jimma Zone, Southwest Ethiopia. *Journal of Medical Sciences*, *12*, 224–228.

Takaya, A., Suzuki, M., Matsui, H., Tomoyasu, T., Sashinami, H., Nakane, A., & Yamamoto, T. (2003). Lon, a stress-induced ATP-dependent protease, is critically important for systemic *Salmonella enterica* serovar Typhimurium infection of mice. *Infection and Immunity*, *71*(2), 690–696.

Taye, B., Alemu, S., Alemayehu, H., and Sisay, Z. (2013). Prevalence and antimicrobial resistance profile of *Salmonella* isolates from dairy products in Addis Ababa, Ethiopia. *African Journal of Microbiology Research*. 7(43), 5046-5050.

Teferi SC. (2020). A Review on Food Hygiene Knowledge, Practice and Food Safety in Ethiopia. *Journal of Biomedical Research and Environmental Research, 6*(1), 4–10.

Tegegne, A., Gebremedhin, B., Hoekstra, D., Belay, B., and Mekasha, Y. (2013). *Smallholder dairy production and marketing systems in Ethiopia: IPMS experiences and opportunities for market-oriented development. IPMS (Improving Productivity and Market Success) of*

Ethiopian Farmers Project Working Paper 31. Nairobi: ILRI. (31), 78.

Tegegne, B., and Tesfaye, S. (2017). Bacteriological milk quality: Possible hygienic factors and the role of *Staphylococcus aureus* in raw bovine milk in and around Gondar, Ethiopia. *International Journal of Food Contamination*, *4*(1), 1–9.

Tigabu, E., Asrat, D., Kassa, T., Sinmegn, T., Molla, B., and Gebreyes, W. (2015). Assessment of Risk Factors in Milk Contamination with Staphylococcus aureus in Urban and Peri-Urban Small-Holder Dairy Farming in Central Ethiopia. *Zoonoses and Public Health*, *62*(8), 637–643.

Todd, E. (2020). Food-borne disease prevention and risk assessment. *International Journal of Environmental Research and Public Health*, *17*(14), 1–13.

Tomaska, L. (2004). The Present State of Foodborne Disease in OECD Countries [Book Review]. *Australian Journal of Emergency Management, 19*(3), 94.

Tosisa, W., Mihret, A., Ararsa, A., Eguale, T., & Abebe, T. (2020). Prevalence and antimicrobial susceptibility of Salmonella and Shigella species isolated from diarrheic children in Ambo town. *BMC pediatrics*, *20*(1), 1-8.

Trawinski, H., Wendt, S., Lippmann, N., Heinitz, S., von Braun, A., & Lübbert, C. (2020). Typhoid and paratyphoid fever. *Zeitschrift fur Gastroenterologie*, *58*(2), 160-170.

Uçar, A., Yilmaz, M. V., & Cakiroglu, F. P. (2016). Food safety–problems and solutions. *Significance, prevention, and control of food-related diseases*, 3-15.

van Belkum, A., Tassios, P. T., Dijkshoorn, L., Haeggman, S., Cookson, B., Fry, N. K., … Struelens, M. (2007). Guidelines for the validation and application of typing methods for use in bacterial epidemiology. *Clinical Microbiology and Infection*, *13*(3), 1–46.

Wabeto, W., Abraham, Y., & Anjulo, A. A. (2017). Detection and identification of antimicrobial-resistant *Salmonella* in raw beef at Wolaita Sodo municipal abattoir, Southern Ethiopia. *Journal of Health, Population and Nutrition, 36*(52), 1–7.

Wallace, H Andrews, and Thomas, H. (2009). BAM: *Salmonella*. *Bacteriological Analytical*

Manual, (December 2007), 21.

Wang, M., Zhang, M., Lu, Y., Kang, X., Meng, C., and Zhou, L. (2015). Analyses of prevalence and molecular typing of *Salmonella* in the goose production chain. *Poultry Science*, *99*(4), 2136–2145.

Wattiau, P., Boland, C., and Bertrand, S. (2011). Methodologies for *Salmonella enterica* subsp. Enterica Subtyping: Gold Standards and Alternatives. *Applied and Environmental Microbiology*, *77*(22), 7877–7885.

Woods, D. F., Reen, F. J., Gilroy, D., Buckley, J., Frye, J. G., and Boyd, E. F. (2008). Rapid multiplex PCR and real-time TaqMan PCR assays for detection of *Salmonella enterica* and the highly virulent serovars Choleraesuis and Paratyphi C. *Journal of Clinical Microbiology*, *46*(12), 4018–4022.

Wang, J., Xue, K., Yi, P., Zhu, X., Peng, Q., Wang, Z., ... & Aleri, J. W. (2020). An abattoir-based study on the prevalence of Salmonella fecal carriage and ESBL related antimicrobial resistance from culled adult dairy cows in Wuhan, China. *Pathogens*, *9*(10), 853.

Yalew ST (2020). *Salmonella* and Its Status in Ethiopia. Au*stin Journal of Veterinary Science & Animal Husbandry, 7*(1), 1069.

Yap, K. P., Gan, H. M., Teh, C. S. J., Chai, L. C., and Thong, K. L. (2014). Comparative genomics of closely related serovar Typhi strains reveals genome dynamics and the acquisition of novel pathogenic elements. *BMC Genomics*, *15*(1), 1–20.

Yilma, Z. (2012). Microbial properties of Ethiopian marketed milk and milk products and associated critical points of contamination: An epidemiological perspective. *Epidemiology insights*, *15*, 298-322.

Yilma, Z., Guernebleich, E., Sebsibe, A., & Fombad, R. (2011). A review of the Ethiopian dairy sector. *Addis Ababa, Ethiopia: FAO Sub Regional Office for Eastern Africa (FAO/SFE)*.

Yilma, Z., Haile, A., & Dessie, T. (2007). Payment systems influence quality: Ethiopian milk and milk products current situation and future move. *Pastoral Livestock Systems: Opportunities and Challenges as a Livelihood Strategy*, 81.

Yilma, Z., Loiseau, G., and Faye, B. (2007). Manufacturing efficiencies and microbial properties of butter and Ayib-Ethiopian cottage cheese. *Livestock Research for Rural Development*, *19*(7).

Yilma, Z., Hailu, Y., Wolkaro, T., and Eshetu, M. (2017). The Ethiopian Dairy Value Chain with a Particular Focus on Cattle and Camel Milk: Current Scenarios and Investment Opportunities. *East African Journal of Sciences*, *11*(2), 81–92.

Zadernowska, A., and Chajęcka, W. (2012). Detection of Salmonella spp. presence in food. *Salmonella-A Dangerous Foodborne Pathogen*, *21*.

Zeinhom, M. M. A., and Abdel-Latef, G. K. (2014). Public health risk of some milk-borne pathogens. *Beni-Suef University Journal of Basic and Applied Sciences*, *3*(3), 209–215.

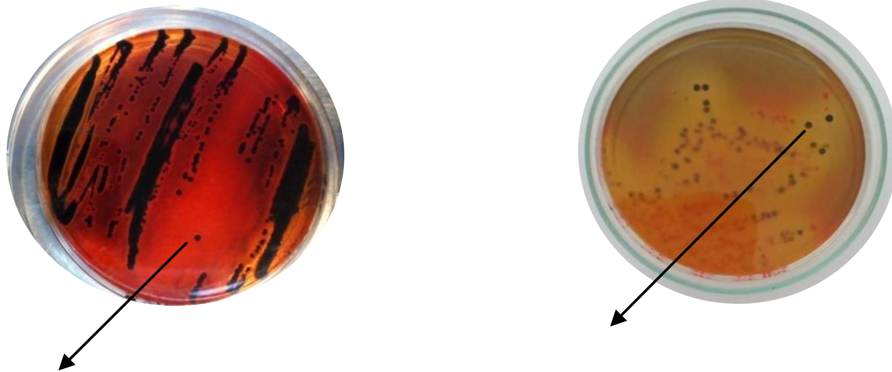
Zenk, S. N., Schulz, A. J., Israel, B. A., James, S. A., Bao, S., and Wilson, M. L. (2005). Neighborhood racial composition, neighborhood poverty, and the spatial accessibility of supermarkets in metropolitan Detroit. *American Journal of Public Health*, *95*(4), 660–667.

Zewdu, E. (2004). Prevalence, distribution and antimicrobial resistance profile of Salmonella isolated from food items and personnel in Addis Ababa, Ethiopia. *Unpublished MSc thesis, Addis Ababa University*.

Zou, W., Tang, H., Zhao, W., Meehan, J., Foley, S. L., Lin, W. J., … Chen, J. J. (2013). Data mining tools for *Salmonella* characterization: Application to gel-based fingerprinting analysis. *BMC Bioinformatics*, *14*(14), 1-9.

ANNEXES

Annex 1: Representative colony morphology of Salmonella on selective agars



A. A pink colony with black center

B. A Blue-green colony with a black center

Figure 7. *Salmonella* species colony morphology on XLD agar (A) and HE agar (B)

Annex 2: Pure colony for DNA extraction

Figure 8. Pure colonies of *Salmonella* spps on BHI agar

Annex 3: Representative photo showing the disc diffusion using different 9 antimicrobials

Figure 9. Disc Diffusion Test for *salmonella* positive isolates

Annex 4: Sample Record Sheet

S.No	Sample code	Site	Sample Type	Value Chain	Receiving Date	Testing date	Remarks

Annex 5: Culture and PCR Record sheet

S.No	Sample code	XLD		HE		*invA* PCR	Serotype PCR
		RVS	MKTTn	RVS	MKTTn		
	OD00X	+	-	-	+	+	+

Annex 6: Zone diameter and disk diffusion breakpoints for *Enterobacteriaceae*

Antibiotic agent (Code)	Disc concentration	S	I	R
Ampicillin (AMP)	10 µg	≥17	15 – 16	≤14
Ceftriaxone (CRO)	30 µg	≥23	20 – 22	≤19
Chloramphenicol (CHL)	30 µg	≥18	13 – 17	≤12
Ciprofloxacin (CIP)	5 µg	≥31	21 – 30	≤20
Clindamycin (CLN)	2 µg	≥21	15 – 20	≤14
Oxy-tetracycline (OT)	30 µg	≥15	12 – 14	≤11
Penicillin (P)	10 units	≥17	15 – 16	≤14
Streptomycin (S)	10 µg	≥15	12 – 14	≤11
Tetracycline (TE)	30 µg	≥15	12 – 14	≤11
Trimethoprim (W)	5 µg	≥16	11 – 15	≤10

Annex 7: **Questionnaire and consent form**

Dear respondent, good morning/good afternoon. Thank you for your interest in talking with me today. I am Abdi Bedassa (Addis Ababa University), who is a member of a research team conducting a study on isolation and molecular characterization of *Salmonella enterica* from milk and cottage cheeses collected from some selected Towns of the Oromia Region, Ethiopia.

The purpose of my visit today is to collect information from you on the aforementioned issue. This study will be conducted in selected towns (Fiche/Selale, Wolmera, Deber-Zeit (Bishoftu), and Asela) of Oromia Regional State of Ethiopia.

If you are willing to participate in the study, I will ask you few questions for 20-30 minutes. Your name will be confidential and the data will never be used in disclosing your information. You do not have to answer any question that you are not comfortable with, and you may end this task any time you want to.

However, your honest answers to these questions will help us in a better understanding of the safety of milk and dairy products and will eventually help in designing and implementing appropriate interventions to alleviate related problems. Hence, I greatly appreciate your participation in the study.

Are you willing to participate in the study?
A.Yes B. No

Questionnaire to be filled by Cottage cheese Retailers

Section A: Preliminary Information

1. Name of Interviewer ---------------------- date ----------- Time------ Retailer's code no. -------

2. Geography and location

- **Town/District/ Zone ------------- Woreda/ Kebele -----------Status:** A. Urban B. Peri-Urban(Peri-urban: areas that are located near to cities or large urban areas but retain rural characteristics such as substantial reliance on agriculture)
- **current location** Latitude (x.y °) -------------------- Longitude (x.y °)--------------------- Altitude (m) --------------------------

Section B: Personal details of the respondent representing the retail shop

1. Name of retail shop _____________ Respondent name ______________ (Confidential)

2. Gender of the respondent A. Male B. Female

4. Position of the respondent in the shop: A. Owner B. Employee C. Relative **Other ____**

5. Educational status of the respondent

A. No formal education B. 1-8 B. 9-10 C. Preparatory school D. Diploma/Degree

Section C: Reception, transportation, packaging condition, and cottage cheese quality test

1) From whom do you receive or buy cottage cheese?

A. Farm market B. Household producers C. Factories or processors D. Other, specify

2) How is cottage cheese transported from processor to retail point?

A. Trekking on foot B. Three-wheel drive "Bajaj" C. Animal drawn cart D. Refrigerated vehicles/ cold chain E. Own transport vehicle F. Public transport G. Other, specify

3) Observational: What is the packaging material in which cottage cheese is received by Retailers from farm markets or processors?

A. Plastic bucket B. Plastic bottles C. Polyethylene plastic container "festal" **D.Other, specify**

Section D: Storage conditions of cottage cheese

- Do you keep cottage cheese in a refrigerator until sell? A. Yes B. No
- Do you have a separate refrigerator for cottage cheese (dairy product)? A. Yes B. No

3. What is the temperature of the refrigerator? _____ check using thermometer r in °C

4. Do you have a backup generator to be used in the case when electric power is out? A. Yes B. No

Section E: Additional comments from the respondent about milk retail job

Any comment the respondent gives here about what kind of support the retail shop needs or any other things related to cottage cheese retail job

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Thank you for your time!!!

Questionnaire to be filled by Questionnaire for Cottage cheese producers

Are you willing to participate in the study?

A.Yes B. No

Section A: Preliminary Information

1. Name of Interviewer --------------------- date ----------- Time------ Respondent code no.--------

2. Geography and location

- **Town/District/ Zone ------------- Woreda/ Kebele -----------Status:** A. Urban B. Peri-Urban(Peri-urban: areas that are located near to cities or large urban areas but retain rural characteristics such as substantial reliance on agriculture)
- **current location** Latitude (x.y °) ------------------- Longitude (x.y °)--------------------- Altitude (m) -------------------------

3. Processing and Storage of cottage cheese

1. **Do you make cottage cheese at home? A.** Yes B. No
2. **Observational: How do you preserve cottage cheese in a household until sale or consumption?**
   1. Placing underground B. Placing under water C. Refrigerator D. **Other, Specify**

4. Observational: Is there a refrigerator to store /preserve cottage cheese at the household level until sale or consumption?

A. Yes B. No

Section B: Hygienic condition of cottage cheese handling equipment and packaging material: A. Yes B. No

6. Do you regularly wash cottage cheese handling equipment and utensils before and after use? A. Yes B. No

7. What kind of cleaning chemical do you use for washing and cleaning cottage cheese handling equipment and utensils? A. Soap and detergents **B.** Coldwater only **Other, Specify --**

8. Observational: What type of packaging material do you use to handle cottage cheese?

A. Plastic cans B. Polyethylene bags "festal"

9. Transporting, sells and marketing cottage cheese

1. **How is cottage cheese transported to the farm markets/ retail shops/hotels?**
   1. Trekking on foot B. Three-wheel drive "Bajaj" C. Animal drawn cart
   - i. Refrigerated vehicles/ cold chain E. Own transport vehicle F. Public transport
2. **How long does it take to transport cottage cheese to farm markets/retail shops/hotels?**

   A. Less than 30 min B. ½ hr. – 1 hr. C. Over 1 hr.

10. Consumption of Cottage Cheese at Household Level

3. **Do you or anyone from your family consume cottage cheese at home?** **A.** Yes B. No
4. **Do/did you ever face any illness associated with consumption of cottage cheese?** **A.** Yes B. No
5. **What type of illness is/was that?** **A.** Diarrhea B. Abdominal pain C.Typhoid D. Vomiting

11. Additional comments from the respondent about milk production job

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____

Put any comment the respondent gives here about what kind of support the farmer/producer need or any other things related to cottage cheese production job

Thank you for your time!!!

Questionnaire to be filled by Milk producers/ farmers

Are you willing to participate in the study?
A.Yes B. No

Section A: Preliminary Information

1. Name of Interviewer ---------------------- date ----------- Time------ Respondent code no.--------

2. Geography and location

- **Town/District/ Zone ------------- Woreda/ Kebele -----------Status:** A. Urban B. Peri-Urban(Peri-urban: areas that are located near to cities or large urban areas but retain rural characteristics such as substantial reliance on agriculture)
- **current location** Latitude (x.y °) ------------------- Longitude (x.y °)-------------------- Altitude (m) -------------------------

Section B: Milk handling knowledge gap assessment of the respondent

4. **How long have you been engaged in milk production?** In the case of farm/ union/ cooperatives: How long it's been the farm engaged in milk production? A. Less than one year B. 1-2 years C. 2-5 years

- 5-10 years E. Over 10 years

4. Farm conditions (infrastructure, hygiene, and animal Health)

5. **Observational: What does the milking house look like?** **A.** Kraal B. Cattle shed

   C. Concrete floor barn D. Soil floor barn E. **Other, specify**

6. **Observational: Is there animal manure (remnant of dung/feces) of the animal present in the milking area?**

   1. Yes B. No

7. **Observational: Are there live pests (rodents or insects) or any sign of it (feces, hair, etc.) present in the milking area?** **A.** Yes B. No

8. **What is your major source of water for washing milk and cottage cheese handling equipment?**

   A. Groundwater B. Pump water C. River water D. Rainwater E. Tap water

2. **Milking Conditions (gender role, personal hygiene, milking equipment, and milking time)**

5.1: Gender role in milking and milking time

1) Who does the milking? **A.** Mostly male adult task (Husband) **B.** Mostly female adult task (Wife)

C. Mostly male child task (Son) D. Mostly female child task (Daughter) E. Employees

F. Members of the union/cooperative G. Relatives

5.2: Personal hygiene and sanitation

4. **Observational: Is the milker wearing a head cover or a hairnet during milk handling?** (Ask the respondent if you did not get the chance to observe) A. Yes B. No

5. **Observational: Does the milker wash their hands before milking and milk handling?** (Ask the respondent if you did not get the chance to observe) A. Yes B. No

6. **Observational: Does the milker wash their hands after touching their face, clothing, and cows?** (Ask the respondent if you did not get the chance to observe) A. Yes B. No

7. **Do you cut your nails regularly? (at least twice a week)** (Ask the respondent if you did not get the chance to observe) A. Yes B. No

5.3: Milking Equipment

1. What type of milk handling equipment or container do you use?

A. Mazzi plastic container B. Plastic containersC. Aluminum cans D. Others_________

2. **Do you wash the milking equipment and utensils before and after milking?**

A. Yes B. No

3. **Observational: How is the milking equipment stored after milking?**

A. Upright and open B. Upright but covered

C. Upside down in contact with the ground D. Upside down on a shelf

6. Post Milking Conditions (Storage, Cooling, Transportation, Marketing, and Consumption)

6.1: Storage and Cooling of milk

1. Observational: Do you have a refrigerator as a cooling mechanism to store or preserve milk at the household level until sale or consumption? A. Yes (if yes Ask to show you) B. No

2. **Do you use other preserving mechanisms other than cooling in a refrigerator to store or preserve milk at household until sale or consumption?** A. Yes B. No

- **What do you use as a preserving mechanism other than cooling in a refrigerator to store or preserve milk at household until sale or consumption?**
  4. Using artificial chemicals (such as formalin farmer call it milks medicine)
  5. Using natural herbs or chemicals
  6. Concentrating it to powder
  7. Fermentation or processing to other milk products (Yogurt, cheese, or butter) E. **Other**

6.2. Transportation and marketing of milk

1. Do you sell milk to others? A. Yes B. No

2. What do they use as a means of transportation for delivering milk to the consumer/ other?

A. Motor bicycles B. Three-wheel drive "Bajaj" C. Animal drawn cart

6.3. Consumption of milk at the household level

1. Do you or anyone from your family consume raw milk at home? A. Yes B. No

2. Do you have awareness about bacteria that might get you sick if you consume raw milk?

A. Yes B. No

3. Have you or anyone from your family ever faced any illness associated with raw milk consumption? A. Yes B. No

4. Do you boil milk before consumption? A. Yes B. No

5. Have you or anyone from your family ever faced any illness associated with boiled milk consumption? A. Yes B. No

Additional comments from the respondent about milk production job.

Put any comment the respondent gives here about what kind of support the farmer need or any other things related to milk production job

Thank You for your time!!!

Questionnaire to be filled by Milk collectors

Are you willing to participate in the study? A.Yes B. No

Section A: Preliminary Information

1. Name of Interviewer ----------------- date ----------- Time------ Collection center code. -------

2. Geography and location

- **Town/District/ Zone ------------- Woreda/ Kebele -----------Status:** A. Urban B. Peri-Urban(Peri-urban: areas that are located near to cities or large urban areas but retain rural characteristics such as substantial reliance on agriculture)
- **current location** Latitude (x.y °) ------------------- Longitude (x.y °)-------------------- Altitude (m) -------------------------

Section B: Personal details of the respondent representing the retail shop

1. Gender of the respondent A. Male B. Female

2. Position of the respondent in the shop: A. Owner B. Employee C. Relative **Other ____**

3. Educational status of the respondent

A. No formal education B. 1-8 B. 9-10 C. Preparatory school D. Diploma/Degree

Section C: Transportation of milk from farm to collection center (milk collection and transportation system, milk cooling and storage)

1. What do they use as a means of transportation for delivering milk to the center?

A. Trekking on foot B. Three-wheel drive "Bajaj" C. Animal drawn cart D. Refrigerated vehicles/ cold chain E. Own transport vehicle F. Public transport G. Other, specify

2. Observational: What does the Personnel hygiene of milk transporters (drivers) look like?

(Ask the respondent if you did not get the chance to observe)

A. Poor (Very dirty) B. Medium (Dirty) C. Good (Clean)

3. Do you filter milk upon reception? **A.** Yes B. No

4) What do you use for filtration? **A.** Piece of cloth **B.** Plastic filter

Section C-2: Milk cooling and storage conditions at the center

1) Do you have a cooling system to preserve your milk? A. Yes B.No

5) What do you use as a cooling system to preserve milk?

A. Refrigerator set at 4°C B. Deep freezer/s C. Bulk tankers

Section D: Collection center conditions (infrastructure, milk handling equipment, and personal hygiene)

» Section D-1: Infrastructure and milk handling equipment

1) Observational: What does the storage and/or collection house look like?

A. Soil floor storage room B. Concrete floor storage room

2) Observational: What is the hygiene condition of the collection center look like?

A. Poor (soil and dirt floor, irregular cleaning, no ventilation, inadequate lighting,...)

B. Medium (partially concrete floor, irregular cleaning, insufficient ventilation,…)

C. Good (concrete/cement floor, regular cleaning, sufficient ventilation, adequate lighting,..)

3) Observational: Is the floor in the milk collection and/or storage area wet with accumulated dirty water (washing/cleaning water)? **A.** Yes B.No

4) What is the major source of water for washing milk handling equipment?

A. Groundwater B. Pump water C . River water D. Rainwater

5) What type of milk handling equipment or container do you use?

A. Plastic containers B. Aluminum cans C. Mazzi cans

Section D-2: Personal Hygiene

1) Observational: Is the milk handler wearing a headcover or a hairnet during milk handling? (Ask the respondent if you did not get the chance to observe) A. Yes B. No

2) Observational: Does the milk handler wear any gloves to handle milk? (Ask the respondent if you did not get the chance to observe) A. Yes B. No

Section E: Marketing and transportation to the consumers

1) Do you transport milk to the buyers by using your transportation? A. Yes B. No

2) What do you use as a means of transportation for delivering milk to buyers?

A. Insulated bulk tanks/cold chain B. Hoarse carts C.Three wheel drive "Bajaj" D. Four-wheel drive without insulated bulk tankers E. Trekking on foot F. Public transport

Thank you for your time!!!

Questionnaire to be filled by Milk processors

Are you willing to participate in the study? A.Yes B. No

Section A: Preliminary Information

1. Name of Interviewer --------------- date ------ Time------- Processing factory code no. -------

2. Geography and location

- **Town/District/ Zone ------------- Woreda/ Kebele -----------Status:** A. Urban B. Peri-Urban(Peri-urban: areas that are located near to cities or large urban areas but retain rural characteristics such as substantial reliance on agriculture)
- **current location** Latitude (x.y °) ------------------- Longitude (x.y °)--------------------- Altitude (m) --------------------------

Section B: Personal details of the respondent representing the retail shop

1. Name of Milk processor______________ **Respondent name** _______________(Confidential)

2.Gender of the respondent A. Male B. Female

3. Position of the respondent in the shop: A. Owner B. Employee C. Relative **Other ____**

4. Educational status of the respondent

- A. No formal education B. 1-8 B. 9-10 C. Preparatory school D. Diploma/Degree

Section C: Milk collection and transportation conditions to the processing plant

2. What do you use as a means of transportation for delivering milk to the processing plant? **A.** Motor bicycles B. Three-wheel drive "Bajaj" C. Four-wheel drive D. Trekking on foot E. Public transport

Section D: Milk reception and pre-pasteurization conditions at the processing plant (milk quality test at reception point, hygienic condition of the processing facility and personnel)

» Section D-1: Milk quality test at the reception point

1. Do you check the quality of milk during the reception? A. Yes B. No

2. How often do you check or test the quality of milk? A. Regularly B. Sometimes

3. Do you filter milk before processing it? A. Yes B. No

Section D-2: Hygienic condition of the processing facility, milk handling equipment, and personnel)

1. Observational: What is the hygiene condition of the processing plant look like?
A. Poor (soil and dirt floor, irregular cleaning, no ventilation, inadequate lighting,...)
B. Good (concrete/cement floor, regular cleaning, sufficient ventilation, adequate lighting,..)

2. Observational: What is the source of water for washing milk handling equipment?
A. Tap water B. Groundwater C. Pump water D. River water

Section E: Pasteurization processing technology and hygienic conditions of the pasteurization process

1. What is the holding time and temperature combination you used for pasteurization?
A. LTLT (low-temperature long time) (63 °C for 30 minutes)
B. HTST (High-temperature short time) (72 °C for 15 seconds)
C. UHT (Ultra-high temperature) (135°C for 2-4°C)
D. Flash Pasteurization

2. Do you calibrate your system to assure that appropriate temperature and time are maintained in the process line during pasteurization? A. Yes B. No

Section F: Post pasteurization conditions (storage, packaging, and storage conditions)

1. Do you have cold storage to store the pasteurized milk before dispatch? A. Yes B. No

2. What is the temperature of the cold storage? A. 4 °C B. -8 °C

3. Observational: What is the packaging material in which pasteurized milk is packed?

A. Paper board packages B. Plastic pouches C. Plastic bottles C. Plastic pouches

4. What do you label on the packaging material?

A. Expiration date B. Batch number C. Brand name

Additional comments from the respondent about the milk processing job

Put any comment the respondent gives here about what kind of support the processor need or any other things related to the milk processing job

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Thank you for your time!!!

Questionnaire to be filled by Milk Retailers

Are you willing to participate in the study? A.Yes B. No

Section A: Preliminary Information

1. Name of Interviewer --------------- date ------ Time------- **Retail shop code No. -----------**

2. Geography and location

- **Town/District/ Zone ------------- Woreda/ Kebele -----------Status:** A. Urban B. Peri-Urban(Peri-urban: areas that are located near to cities or large urban areas but retain rural characteristics such as substantial reliance on agriculture)
- **current location** Latitude (x.y °) ------------------- Longitude (x.y °)-------------------- Altitude (m) --------------------------

Section B: Personal details of the respondent representing the retail shop

1. Respondent name ______________ (Confidential)

2. Gender of the respondent A. Male B. Female

3. Position of the respondent in the shop: A. Owner B. Employee C. Relative **Other ____**

4. Educational status of the respondent

- A. No formal education B. 1-8 B. 9-10 C. Preparatory school D. Diploma/Degree

Section c: packaging material and quality test of pasteurized milk

1. What do they use as a means of transportation for delivering milk to the retail shop?

B. Motor bicycles B. Three-wheel drive "Bajaj" C. Animal drawn cart

2. Observational: What is the packaging material in which pasteurized milk is received from processors? A. Paper board packages B. Plastic bottles C. Plastic pouches

Section D: Storage conditions at the retail level

1. Observational: What do you use to maintain the temperature of the pasteurized milk until sell?

A. Refrigerator B. Deep freezer/s C. Bulk tankers D. Coldwater

2. Observational: Is your refrigerator temperature set at 4 -8°C to store pasteurized milk until sell? (please check the temperature of the refrigerator by using a thermometer if they allow you to do that) A. Yes B. No

3. Observational: Do you have a backup generator to be used in the case when electric power is out? A.Yes B. No

Section E: Storage conditions at the retail level

1. Did you ever face rejection of your pasteurized milk by buyers? A. Yes B. No

2. How often do you face? A. Rarely B. Mostly C. Sometimes

3. Do you know their reason for rejection? A. Yes B. No

4. What is their reason for rejection? A. Expiration of milk B. Color

Additional comments from the respondent about milk production job

Put any comment the respondent gives here about what kind of support the farmer need or any other things related to milk production job

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Thank you for your time!!!