

## PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY PROFILE AND PUBLIC HEALTH SIGNIFICANCE OF E.COLI IN MILK SUPPLY CHAIN OF BAMBASI DISTRICT, WESTERN ETHIOPIA

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**ABSTRACT: Back ground :** The most prevalent contamination in raw and pasteurized milk is *Escherichia coli*. It's a good sign of fecal contamination in water and foods like milk and dairy. **Objectives:** The objectives were to isolate and identify *E.coli* from raw cow milk supply chain, to assess the public health significance associated with risk factors and to determine antimicrobial susceptibility patterns of *Escherichia coli* isolates. **Methods:** Cross-sectional study was conducted on Isolation, Identification and Antimicrobial susceptibility profile of *E.coli* and its Public health significance in milk supply chain of Banbasi District, western Ethiopia from October 2024 to April 2025 in dairy cows. A total of 384 samples were collected from dairy cow and processed bacteriologically and the isolate were tested with a number of biochemical tests for confirmation and identification of *E. coli*. **4. Results:** The study revealed that 18.22% of the collected raw milk was contaminated with *E. coli*. Higher (35%) *E.coli* contamination was reported in milkers' hand swab followed by (18.75%) milk samples, (14.67%) udder swab and 13.33% container (bucket) swab. Besides, all *E. coli* isolates exhibited bright pink color with lactose fermentation on MacConkey agar plates, metallic sheen on Eosin Methylene Blue agar plate and gram-negative, pink-colored, small rod-shaped organisms arranged in single with pairs or short chains on Gram's staining. In this study, there was significant ( $p < 0.05$ ) association between the udder washing practices, parity, lactation stage, pregnancy status, milking hygiene, lesion and blind teat, body condition, age, drainage, and breed of the cow with the isolates of *E.coli*. In other way, previous udder infection, floor type, and herd size risk factors was not significant (P.0.05). Majority (85%) of drug resistance prevalence was reported in penicillin G, followed by (60%) vancomycin; 50% chloramphenicol; and 42.5% streptomycin. Whereas higher (85%) of drug sensitivity or susceptibility was recorded in Gentamycin, followed by 50% kanamycin, 45% streptomycin, 40% vancomycin and 32.5% chloramphenicol. In this study 30% MDR were recorded in three (27.5%) and four classes (2.5%) of antibiotic discs. The presence and consumption of raw milk may constitute a public health hazard and reduced milk quality due to *E. coli*. **5. Conclusion and Recommendations:** Thus health professionals should create awareness about milk handling practice, storage and milking process to Dairy farmer and milk collectors. And hence, regular resistance follow-up, using antimicrobials sensitivity tests helps to select effective antibiotics and to reduce the problems of drug resistance developments towards commonly used antimicrobials so as to reduce the problem encountered.

[Arega muleta, Asmamaw Aki, Bihonegn wodajnew. **PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY PROFILE AND PUBLIC HEALTH SIGNIFICANCE OF E.COLI IN MILK SUPPLY CHAIN OF BAMBASI DISTRICT, WESTERN ETHIOPIA.** *N Y Sci J* 2026;19(5):1-27]. ISSN 1554-0200 (print); ISSN 2375-723X (online). <http://www.sciencepub.net/newyork>. 01. Doi: [10.7537/marsnys190526.01](https://doi.org/10.7537/marsnys190526.01)

**Key words:** Antimicrobial; Bovine; Bambasi; Dairy cows; *E.coli*; MDR; milk

### 1. INTRODUCTION

#### 1.1. Background

Milk and dairy products are important sources of vital nutrients for human beings (Berhe *et al.*, 2020). Raw milk has become a perfect medium for the growth of several types of microorganisms, which could lead to the deterioration of the milk due to its high water content, pH close to neutral, and diversity of nutrients. The presence and proliferation of bacteria induce changes in milk quality, decreasing its shelf life, and harming the economy as well as public health (Mesfine *et al.*, 2015). *Escherichia coli*,

a harmful bacterium found in milk, has become a major public health concern, particularly among individuals who still consume raw milk (Lye *et al.*, 2013). Furthermore, raw milk is a major source of bacteria that can be dangerous to humans. Consuming contaminated raw milk and raw milk products has been linked to foodborne infection outbreaks in Indonesia (Effendi *et al.*, 2018). Cow's milk has long been regarded as a highly nutritious and important human meal; millions of people eat it every day in a range of products (Faisal and Ahmed, 2018). When milk is secreted in the udder, it is free of infection, but it becomes infected

with bacteria before being milked out. However, the bacteria present in milk at this time are insignificant and incapable of causing disease, except for mastitis. The majority of harmful milk infections occur during the milking process, due to improper storage, unhealthy handling techniques, and other activities performed before processing (Perveen, 2021). Consumption of raw milk can result in zoonotic because milk is frequently contaminated with cattle feces during milking and serves as a good microorganism growth medium (Tiimub *et al.*, 2020). Because of its role as a transmission channel, milk can induce a condition known as milk borne disease. Humans and microorganisms both benefit from cow's milk. Bacteria that contaminate milk quickly proliferate to a great number, resulting in a significant number of instances of infection with milk intermediaries (cows); in addition, humans have limited resistance (Perwira *et al.*, 2019). Humans require milk and dairy products as a source of essential nutrients (Berhe *et al.*, 2020). It is a good source of glucose, proteins (all 10 amino acids), vital fatty acids (immunoglobulin), and other micronutrients (Friday *et al.*, 2021; Limbu *et al.*, 2020). Because of its high nutrient content, raw milk is thought to be an excellent medium for microorganism development (Elmonir *et al.*, 2017; Fathi *et al.*, 2019).

Milk and its derivatives, on the other hand, if not treated hygienically, serve as nutritious food sources for humans, but they also serve as excellent media for the multiplication of numerous microbes (Abebe *et al.*, 2018).

*Escherichia coli* are a broad and diversified bacterial genus; it is the type of *Escherichia* that contains predominantly motile Gram-negative bacilli belonging to the Enterobacteriaceae family. It is the human colonic flora's most common facultative anaerobe. *E. coli* colonize the infant's gastrointestinal tract within hours of birth, and *E. coli* and the host benefit from each other for decades (Asime *et al.*, 2020). This organism is typically found in the lower intestine of warm-blooded organisms (Igbinosa and Chiadika, 2021). Raw milk is considered a high risk food, as it is highly nutritious and serves as an ideal medium for bacterial growth. Several factors are responsible for milk contamination such as poor hygienic milking conditions, contaminated equipment, milking utensils, and milk handlers with poor personal hygiene (Alam *et al.*, 2017), and (Igbinosa and Chiadika, 2021)

The most prevalent contamination in raw and pasteurized milk is *Escherichia coli*. It's a good sign of fecal contamination in water and foods like milk

and dairy. Because of the possibility of Enteropathogenic and/or toxigenic strains, their presence in food may pose a public health risk. Milk contamination has been caused by udders with subclinical mastitis and a damp environment. Some *Escherichia coli* strains are known to be pathogenic bacteria, producing serious intestinal and extra intestinal illnesses in humans (Ahmida, 2020). Raw milk reaches consumers with a higher coliform content (Adzitey *et al.*, 2018).

Microbial contamination might generally occur from three main sources: within the udder, exterior to the udder and from the surface of milk handling and storage equipment, but the surrounding air, feed, soil, faces and grass are also possible sources of contamination (Mosu, 2013). Unsafe sources, contaminated raw food items, improper food storage, poor personal hygiene during food preparation, inadequate cooling and reheating of food items, and a prolonged time lapse between preparing and consuming food items have been identified as contributing factors for outbreaks of food borne diseases (Oliver *et al.*, 2005). Outbreaks of VTEC infections involving serogroup O157 have been reported from different countries of the world including United States, Canada, Asia, Australia, Europe, and Africa through various sources of infection and different case fatality (Dufffy, 2006).

Human health is exposed to pathogenic microbes, often identified in milk and milk products. *E. coli*, *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Brucella abortus*, *Mycobacterium* spp., *Campylobacter* spp., *Leptospira* spp., *Clostridium* spp., *Pseudomonas aeruginosa*, *Pseudomonas* (Alam *et al.*, 2017; Pal *et al.*, 2016), Limiting contamination levels, cooling quickly after milking, and maintaining cold storage temperatures are all important steps in preventing the growth of contaminating bacteria in milk. Cleaning, sanitizing, and drying the teats and udders of cows before milking, as well as utilizing sanitized milking equipment, are the most effective ways to limit germs. To control psychotropic bacteria, remove any remaining solid milk from milk containers (Garedew *et al.*, 2012).

## 1.2 Statement of the Problem

Food-borne pathogens are the leading causes of food-borne human illness and death in the world (Agueria *et al.*, 2018). The severity of food-borne illness is higher among developing countries, including Ethiopia (Abdissa *et al.*, 2017; Bey *et al.*, 2017). 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). 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. Milk is nutritious food but, when contaminated; it can support the growth of spoilage and pathogenic microorganisms (Solomon and Ketema, 2011)

Risk of pathogenic *E. coli* infection related to consumption of raw milk is high, indicating that there is risk of *E. coli* infection (Lye *et al.*, 2013). *Escherichia coli* O157:H7 is associated with outbreaks and sporadic cases of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) and other enteric infections all over the world especially in children under 5 years of age (Fernandez and Padola, 2012). It is also responsible for 20 % of foodborne outbreaks globally (WHO, 2017). In comparison with other foodborne bacterial pathogens the severe consequence of the disease and their low infectious dose which is being fewer than 40 cells (Strachan *et al.*, 2005) and might be as few as 10 cells (Ateba and Bezuidenhout, 2008), makes *E. coli* O157:H7 an important emerging public health problem particularly for under-five children. There is an irrational drug use for farming and therapeutic purpose in animals and humans in developing countries (Akbar *et al.*, 2014). The organism also shading through feces from animal reservoirs and has the ability to transfer antimicrobial resistance traits from animal to human (Newell *et al.*, 2010).

However, recently there is an increasing trend of reporting occurrence level of the organism in dairy products (Tassew *et al.*, 2010; Haile Selassie *et al.*, 2013; Taye, 2013; Abebe *et al.*, 2014. and Desta *et al.*, 2016). Despite the fact that numerous studies in various parts of Ethiopia isolated food-borne pathogens of public health significance from raw milk, people still consume raw milk claiming flavor, availability, price and perceived higher nutritional value benefits (Amenu *et al.*, 2019; Ayele *et al.*, 2017; Keba *et al.*, 2020).

In Ethiopia, milk from dairy farms is sold and distributed without being pasteurized or subjected to quality control. According to various reports in Ethiopia, between 71 and 97% of total milk output is consumed through an informal market without quality controls (Tsehay, 2002). Because raw milk is easily contaminated during milking and handling, it is an essential vehicle for the transfer of milk-borne

diseases to people (Addo *et al.*, 2011). This study is expected to fill a knowledge gap regarding hygienic practices and risk factors associated with *E. coli* from raw cow milk, as well as outline measures that milk producers and regulators must take to ensure the safety and quality of milk in dairy farms.

### 1.3 Significance of the Study

Dairy farming was an important activity for the livelihood of the farming community in the study area. The farming community's life is primarily dependent on livestock and agricultural practices, with agricultural practices and their products taking precedence over other milk production in this research area. Nowadays, due to the increased human population in urban areas, the demand for cow milk and products is increasing rapidly. This suggests that the use of cow milk is increasing. Although those large numbers of people have a satisfying need for milk and milk products, the production of high-quality milk is important because production without quality is worthless and health is compromised. Raw milk contamination with *E. coli* is a major problem, mostly in poor hygienic milk practices. Therefore, the expected multiple benefits of this study were: Data on antibiotic resistance were used to characterize these opportunistic pathogens that may further limit the risks associated with the consumption of contaminated raw milk and its products.

Shortage of information on milking hygiene practices, and dairy worker awareness of *E. coli* results in public health risks and economic losses for dairy producers, which may expose the consumer to contracting *E. coli* and other pathogenic microbes from the milk. Promoting the hygienic quality of raw cow milk, therefore, involves maintaining strict compliance with sanitary measures on dairy farms, all stages of milk handling activities, and the equipment's and dairy workers' personal cleanliness.

However, a limited study on this important product is currently being conducted to protect public health by isolating and identifying *E. coli* from raw cow milk and potential sources of contamination along milk supply chain in Bambasi, in milking channels as well as milking equipment and handlers. Furthermore, the study will help the researchers as a baseline for conducting another study, and to recommend for the Bambasi woreda agricultural and livestock health office to improve their efforts in dairy farms workers in milk handling practice, and environmental hygiene.

### 1.4. Objectives of the Study

#### 1.4.1. General Objective

- The goal of this study is to isolate and identify pathogenic *Escherichia coli* from raw cow milk as well as determine likely sources of contamination along the dairy farms of milk supply chain (production channels).

#### 1.4.2. Specific Objectives

- To isolate and identify *Escherichia coli* from cow milk production channels.
- Evaluate the associated risk factors for *E.coli* prevalence
- To assess the source of milk contamination and its public health significance
- To determine antimicrobial susceptibility patterns of *Escherichia coli* isolates.

## 2. MATERIALS AND METHODS

### 2.1. Study Area

Bambasi district is one of the districts found in Benishangul Gumuz Regional State under Assosa

zone. The district is located at 616 km on the main road from Addis Ababa to the regional capital, Assosa town and 45km in the North East direction of Assosa town. The geographical location of the study area is depicted using figure 1. The study will be conducted from November to April, 2024/2025 in Bambasi districts of Asossa zone of Benishangul Gumuz Regional State. The study will be conducted in Bambasi Dairy farm milk supply chain (production channels). Bambasi district has 38 kebeles stretches over an area of 2210.16 k.m.square with human population of 62693. The region is found in the north west of the country between latitude of 9 and 11° N and longitude of 34 and 35° E and its altitude range is 1500-1900 meter above sea level. Annual rain fall is between 1350-1400 mm with uni modal type of rain fall that occurs between April and October. Annual temperature ranges between 21<sup>o</sup>c - 35<sup>o</sup> c. The livelihood of the society largely depends on mixed livestock and crop production having livestock population of 36,735 Cattle, 10732 Goat, 3739 Sheep, 4467 Equines, 41438 Poultry and 23423 beehives (CSA,2015).

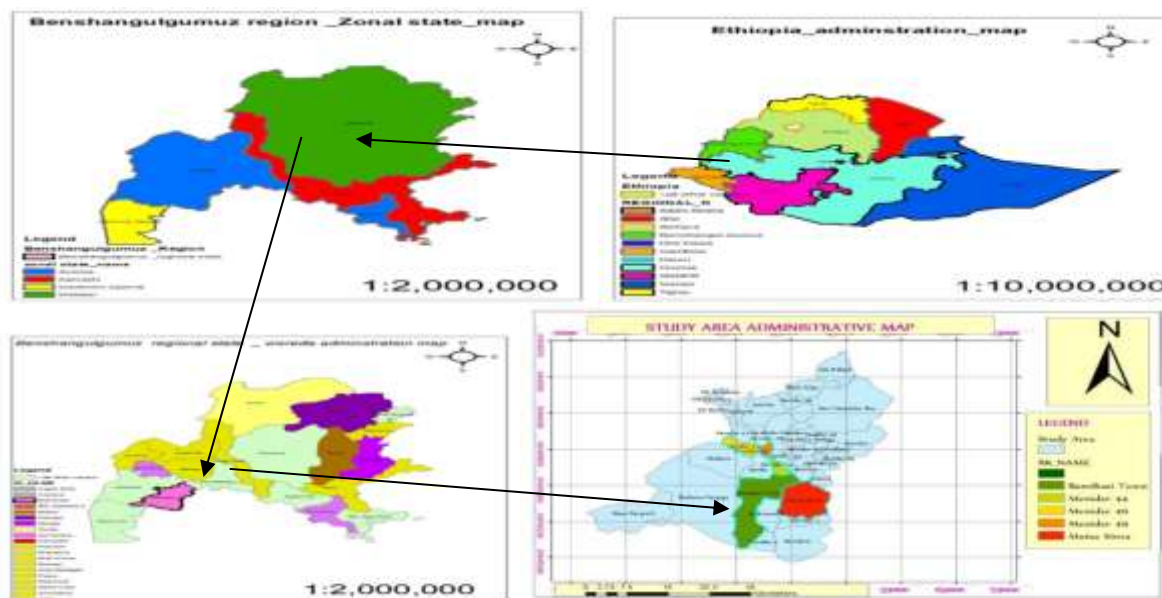


Figure 1: Administrative Map of Bambasi district

### 2.2. Study population

The study was conducted on apparently health lactating dairy cows, from Bambasi district. 109 samples from producers or farm owners from udder teat, 80 samples from collector/ vendor=bulk milk), and 135 samples from end users of milk container swab and 60= Hand swab from milk handlers was collected.

### 2.3. Research Design and period

A cross-sectional study was conducted from December 2024 to April 2025 to isolate and identify *E. coli* from raw cow milk supply, production channels from dairy farms. The milk supply in the production channels was selected purposefully based on the willingness of the farm owners. A total of 384 cow milk samples were sampled from udder (teat), bulk (collected) milk and material (milk handler, equipment, and swab from milk handler). Questionnaires and checklists were made and translated into the local language and was presented to milk handlers working at the dairy farms to define the hygienic condition of the farms and safe milk handling practices. A visual farm observation was conducted before an interview with the dairy workers.

### 2.4 Sampling Size Determination

Raw cow milk obtained straight from the udder, bulk milk, and swabs from farms that were specifically selected, and the sorts of samples were used for this investigation. Based on the availability of one or more lactating cows and the dairy workers' willingness to participate in the study, the dairy farms were specifically selected. In order to determine the total number of lactating cows at dairy farms.

Of 384 samples, 165 swab samples were collected from the milker's hands (milk handlers), milking buckets, and drying towels, milking environment, and milk utensils, depending on the number of workers, frequency of visits, and materials used on the farm. 109 udder milk of cow milk and 110 bulk milk samples were subjected for Bacteriological examination (Table 2).

**Table 1:** Sample size and sample type across production channels /milk supply

Sample type	No. of samples along the production channels			Total
	Producers	Collectors/vender	End users	
Swab(milk material) samples	45	45	45	135
Bulk milk swab	26	27	27	80
Udder milk samples	36	36	37	109
Hand swab	20	20	20	60
Total	127	128	129	384

Therefore, the total sample size for *E.coli* isolation and identification was assigned according to statistical formula of (Thrustfield, 2005). A 5% absolute precision at 95% confidence interval was used during determining the sample size. Since there is no previous work in the study area for *E.coli* prevalence of raw cow milk, the expected prevalence of this analysis was taken as 50%. Therefore, the total sample size for this study was calculated as follows:

$$n = \frac{(1.96)^2 \times P(1-P)}{d^2}$$

Where: n = the total sample size

P = expected prevalence (50%)

d= desired absolute precision (0.05) at 95% CI

$$n = \frac{(1.96) \times (1.96) \times (0.5) \times (1-0.5)}{(0.05) \times (0.05)} = 384 \text{ milk samples}$$

Therefore to isolate *E.coli* a total of (n= 384) milk samples was taken from three parts (udder milk =109, bulk milk=109, hand swab=60 and milk material swabs =135) from milk handlers, milking environment and milk utensils.

### 2.5. Sampling Technique and Sample Collection procedure

#### 2.5.1. Sampling Technique

Purposive sampling was used to get a sample from the lactating dairy cows and bulk milk, as well as material swab samples from the milk handlers, milking environment, and milk utensils. Before sampling, the udders and teats was carefully cleansed, dried, and gently wiped with cotton swabs moistened with 70% ethyl alcohol. The first 3–4 streams of milk will be discarded and about 10 ml of milk will be collected from the udder aseptically and put in a

sterile screw-top universal bottle and placed in a cool box with ice packs and transported in an ice box (+4 °C for further processing and microbiological investigation. Then, the milk and swab samples were labeled and documented. Sampling ID, sampling date, and sample type are all assigned to all collected samples. The sample was transferred using an icebox to the Assosa Regional Veterinary Laboratory, microbiology laboratory. The samples were kept in the refrigerator for 24 hours after arrival at +4 °C until they were processed for isolation (Quinn *et al.*, 2004).

### 2.5.2. Milk samples collection procedure

In total, 384 cow milk samples were collected from the teats, milk tanks and material swabs at the production channels. Among the raw milk samples, 109, 80, 135, and 60 cow milk samples will be collected from udder teat, bulk milk, and swab materials and hand swab along production channels. That is, producers, collectors and end users respectively. Once a week, raw milk samples from producers will be collected in the morning and evening from each sampling point. During the sampling of raw milk directly from udder teats, will be cleaned and dried before sampling; each teat end will be scrubbed gently with cotton swabs moistened with 70% ethyl alcohol. The first 3–4 streams of milk will be discarded, and approximately 5 ml of milk will be collected into sterile sampling bottles from an individual cow's udder. During collection, approximately 25 ml of raw milk consisting of 5 samples will be collected aseptically from the udder, and a volume of 40 ml of bulk milk consisting of 5 bulk milk samples will be collected from milk storage tanks. 10 ml of milk will be taken from each tank and placed into sterile glass bottles. Again, immediately after collection, samples will be labeled and put into an icebox, and then the samples will be transported to the Microbiology Laboratory, under chilled conditions using an icebox. A microbiological examination will be done within 24hr of collection.

### 2.5.3. Swab Sampling Collection Procedure

A total of 304 ( 135 container swab; udder swab, and 60= hand swab) milk swab samples will be collected, from purposively selected dairy farm once a week in the morning and evening during milking time and from vendor or bulk milk collectors. The swab samples will be taken using sterile swabs and kept in sample bottles containing sterile physiological saline solution to prevent desiccation. Aseptically collected swab samples, will be settled in a cool place in an ice box and transported to the laboratory within 24 hours of sampling. Upon arrival at the laboratory, processing of swab samples was commenced for further examinations.

### 2.5.4. Questionnaire Survey on milk handling practices

A structured questionnaire with closed-ended questions will be used to collect data on lactating cows and potential risk factors thought to impact the possible source of *Escherichia coli* contamination in dairy farms. Data on intrinsic factors such as age categorized as young (2- 4), adult (>5-7), and old (>8), body condition categorized as poor, medium, and good (visual and fat deposited) lactation stage categorized as early (3 months), mid (3-6 months), and late (6 (≥6 months), parity level categorized as few (1-2 calves), mid (3-4 calves), and many (≥4 calves) were collected for this purpose. And extrinsic factors such as milking utensils categorized as stainless steel or plastic, teat washing categorized as "yes" or "no., towel use also categorized as "yes" or "no., milking techniques categorized as "hand or machine" were gathered by questioning 30 farm workers.

Data on potential risk factors were collected from the interviews of dairy workers and observations. In addition, observational checklists will be used to rate the hygiene of milk and milk product utensils, milk handlers (Yes or No), and dairy farm hygiene (good or poor). Throughout the study period the three dairy farms' floor condition was visited to characterize as good (rough concrete made floor, having drainage and had cleaned) or poor (soiled floor, muddy or having stored animal dung, haven't drainage, not cleaned even once per day). The survey will be written in English and then translated into the local language (Annex 1 and Annex 2)

## 2.6. Laboratory Analysis

### 2.6.1. Isolation and identification of *E. coli*

Isolation and identification of *Escherichia coli* from swabs, bulk, and teat milk samples will be done using routine culture and biochemical analysis. Approximately 1 ml of milk will be suspended in 1:9 ml of buffered peptone water. Samples will be water spout-formed and incubated overnight at 41 °C. After BPW, 50µl of product will be streaked onto MacConkey agar for primary isolation of *E. coli* and incubated aerobically at 37 °C for 24 hours. Plates will be observed for the growth of *E. coli* (pink colony; lactose fermenter). Sorbitol MacConkey agar was used as selective media for pathogenic *E. coli* identification.

The isolated strains were subjected to a series of different biochemical tests using the procedure of (ISO, 2003) to confirm *E. coli*. Catalase test, oxidase test, indole production test, Methyl red test, Voges-Proskauer test, and Simmons's citrate test on tryptone broth, MR-VP medium, (Appendix: III). The sub-cultured and purified colonies were tested for hydrogen sulphide and indole production using Triple Sugar Iron agar (TSI) slant (Oxoid) and Indole production test.

The isolates giving a result of yellow slant and butt with gas but no hydrogen sulfide (Y/Y/ H<sub>2</sub>S) production on TSI slant agar after incubation of the media at 37°C for 24 hours will be kept with tubes capped loosely to maintain aerobic conditions. Indole test will be carried out using one pure colony inoculated into 4 ml of tryptone soya broth (Oxoid) with a straight inoculation wire. Incubation was done for overnight at 37°C. Then one drop of Indole (Kovac's) reagent will be added to the tryptone soya broth culture to test for indole production (formation of red ring indicating positive reaction) as stated.

The isolation of *E. coli* O157 was carried out as earlier described (E. De-Boer, A.E. Heuvelink, 2000; A. Ivbade *et al.*, 2014) with some modifications. *E. coli* isolates earlier characterized by biochemical assays (one representative colony from each sample) was plated on Sorbitol MacConkey Agar (SMAC) with BCIG and selective supplements (Cefixime-Tellurite, SR0172, Oxoid®, UK) was used for the isolation. Cefixime-Tellurite Selective Supplement was used to knock off other gram-negative organisms present. Non-sorbitol fermenter colonies with a straw colour or pale yellow on SMAC-BCIG plates was considered presumptive *E. coli* O157 isolates (A. Ivbade *et al.*, 2014). This was later sub-cultured (twice) to obtain pure colonies. The serogroup O157 of *E. coli* was determined using a latex agglutination test kit (*E. coli* O157 latex test, Oxoid®, UK) as described by the manufacturer. The positive isolates were stored in 15% glycerol at -20 °C until further processed.

**Table 2:** the growth characters and biochemical processes of *E. coli*

Biochemical reactions.	Expected Result Class Table (+/-)	From	Description of Result Observed	Interpretation
Indole test	+		Red colour ring at the top of the broth	+
Methyl red test	+		Red reagent liquid in test tube	+
Voges-Proskauer	-		Voges-Proskauer -	-
simmon citrate	-		Absence of colour change	-
Triple sugar Iron.	· -		Yellow slant, yellow butt in the test tube	-

## 2.7. Antimicrobial Susceptibility Testing

The agar disc diffusion method, as published by the Clinical and Laboratory Standards Institute, was used to determine antimicrobial susceptibility patterns (CLSI, 2015). A digital caliper was used to determine the zone of inhibition. Spectinomycin (10 µg), Vancomycin (30µg) Norfloxacin (50µg), Kanamycin (30µg), Chloramphenicol (50 µg), Tetracycline (10 g), Erythromycin (15 g), and Penicillin (10g) are used to test the antibiotic susceptibility of *Escherichia coli* isolates. In a nutshell, the bacteria was suspended in a 0.85 percent sterile normal saline solution in a 0.5 McFarland standardized suspension. A sterile cotton swab was dipped in the standardized bacteria suspension and then streaked uniformly across the Mueller-Hinton agar (Oxoid Ltd., Basingstoke, Hampshire, England) surface. The paper discs impregnated with a set concentration of antibiotics are then placed on the agar surface and inverted for 24 hours at 37 °C. The bacterial growth and diffusion of the antibiotics are going to produce obvious zones of inhibition after 24 hours of incubation and are measured in millimeters using a caliper and characterized as susceptible, intermediate, and resistant (CLSI, 2015). The media was checked for free of contamination by incubating 5% of the batch at 37 °C for 18-24 hrs, and ice box was used during sample collection, and transportation and the performance of the media was checked using *E. coli* (ATCC, 25922).

**Table 3:** Antimicrobial susceptibility test interpretive criteria for Enterobacteriaceae

Antibiotics Disc	Disc code	Potency	Zone of diameters		
			Susceptibility (mm)	Intermediate (mm)	Resistance (mm)
Spectomycin	S	10 µg	≥15	12–14	≤11
Norfloxacin	NOR	10 µg	≥22	13-16	≤15
Erythromycin	E	15 µg	≥17	12-16	≤14
Vancomycin	VA	30 µg	>12	10-11	≤9
Kanamycin	KF	30 µg	≥18	13–17	≤13
Tetracycline	T	10 µg	≥15	12–14	≤11
Chloramphenicol C	C	50 µg	≥18	13–17	≤12
Penicillin G	Pen	10 µg	≥29	-	≤28
S10	S10	10 µg	≥15	12-14	≤11
CN10	CN10	10	>15	12-14	<11

## 2.7. Data Processing and Analysis

Data collected from laboratory findings and the questionnaire surveys were entered in to Ms-Excel spread sheet program (Microsoft Corporation, USA), the data were cleaned, checked for completeness and finally imported into STATA Version 17 for analysis. The association between the factors and *E.coli* was assessed using Chi square ( $\chi^2$ ) test. The effect of different risk factors upon the prevalence of the organisms was explained by both multi-variable binary logistic regressions. The finding of the questionnaire survey data was summarized using descriptive Statistics. Odd ratio and 95% CI was computed, and results were considered significant at ( $P < 0.05$ ).

## 3.RESULTS

### 3.1. Occurrence of *E.Coli* in the Milk Production Channel

In the present study, (80) milk samples, (109) udder swab, (60) milkers' hand swab and (135) milk containers swab, of sample types were identified by culturing and biochemical test methods. From the overall samples (N=384), 70/384 (18.22%) of the *E. coli* isolates were able to produce bright pink colored colonies on MacConkey agar; characteristic metallic sheen colonies on the EMB agar and pink colored, small rod-shaped Gram-negative bacilli on Gram's staining. The results of catalase, MR and indole test of the *E. coli* isolates were positive but the V-P test and simmon's citrate utilization were negative. The pattern of sugar fermentation reaction by the isolated *E. coli* with three sugars (triple sugar iron agar) was observed and produced acid and gas. The isolates were able to ferment glucose, lactose, and sucrose completely. Acid production was indicated by the color change from reddish to yellow and the gas production was noted by the appearance of gas bubbles in the test tubes.

#### 3.1.1. The prevalence and associated risk factors of *E.coli* isolated from dairy cow raw milk

Prevalence of *E.coli* infection related to the potential risk factors were determined as the proportion of affected cows of the total examined. As indicated in (Table 5), the questionnaire survey and observation data result shows association of sample type, age factors, breed factors, parity, body conditions, milking hygiene, blind teat, teat lesion, management factors, pregnancy status, body conditions, udder washing, and drainage, are amongst the potential risk factors, which are associated with mastitis disease in dairy cows farmstead.

**Table 4:** Evaluations of potential risk factors associated with the prevalence of E.coli using Uni-variable Logistic Regression

E coli	Crude OR	St.Er.	t-value	p-value	[95% Conf	Interval]	Sig
<b>Sample type:</b> udder swab	Ref	.	.	.	.	.	.
Hand swab	3.019	1.153	2.89	.004	1.428	6.383	***
Container Swab	.885	.328	-0.33	.741	.428	1.83	
Bulk milk sample	1.438	.56	0.93	.351	.67	3.083	
<b>Age:</b> b<3 years	Ref	.	.	.	.	.	.
4-7 years	1.623	.504	1.56	.119	.883	2.983	
<b>BCS:</b> good	1	.	.	.	.	.	.
Poor	.419	.115	-3.16	.002	.244	.718	***
<b>Breed :</b> Cross	Ref	.	.	.	.	.	.
Zebu	.27	.102	-3.47	.001	.13	.568	***
<b>Parity:</b> 1-3 calving	Ref	.	.	.	.	.	.
4-6 calving	.12	.047	-5.39	.0	.056	.26	***
<b>Lactation Stage:</b> early (3)	Ref	.	.	.	.	.	.
Mid(4-6)	1.571	.622	1.14	.254	.723	3.414	
Late(7-9)	1.397	.662	0.71	.481	.552	3.536	
Dry(>)	2.65	.91	2.86	.004	1.36	5.2	***
<b>Herd Size:</b> small	Ref	.	.	.	.	.	.
Medium	.647	.173	-1.63	.104	.383	1.094	
Large	.23	.241	-1.40	.161	.029	1.795	
<b>Pregnant status:</b> Non-pregnant	Ref.	.	.	.	.	.	.
Pregnant	.06	.061	-2.76	.006	.008	.442	***
<b>Milking hygiene:</b> Good	1	.	.	.	.	.	.
Poor	.248	.069	-5.04	.0	.144	.426	***
<b>Barn floor space:</b> muddy	Ref.	.	.	.	.	.	.
Cemented	1.262	.343	0.86	.391	.741	2.15	
<b>Udder shape:</b> Pendiculous	Ref.	.	.	.	.	.	.
High up	.06	.019	-9.02	.0	.032	.11	***
<b>Teat lesion :</b> No	Ref.	.	.	.	.	.	.
Yes	2.687	1.125	2.36	.018	1.182	6.104	**
<b>Udder Washing :</b> No	1	.	.	.	.	.	.
Yes	.229	.064	-5.28	.0	.133	.396	***
<b>Previous udder Rx:</b> NO	Ref.	.	.	.	.	.	.
Yes	.546	.228	-1.45	.147	.241	1.238	
<b>Drainage:</b> No	Ref.	.	.	.	.	.	.
Yes	.577	.159	-2.00	.045	.336	.989	**
Number of obs		384	Prob > chi2			0.042	

\*\*\*  $p < .01$  = highly significant, \*\*  $p < .05$  = significant, OR = odds ratio

As depicted in Table 7, some potential risk factors revealed statistical significant association with the prevalence of E.coli. In the current study area, from different sample types milker's swab showed statistically significant association with respect to E.coli occurrence and was 3.019 times more likely to harbour E.coli bacteria than udder swab (OR = 3.019, [CI=1.428, 6.383], P-value =.004).

**Table 5:** Evaluation of Potential risk factors of E.coli using multivariable logistic regression

Risk factors	Categories	No. examined	No (%) positives	Adjus OR	Confidence Interval		P-value
					Lower CI	Upper CI	
Age(years )	≤3 (year)	23	0 (0%)	4.06	1.667	9.887	0.002
	4-7 years	252	55 (21.82%)				
	> 7 years	108	15 (13.88%)				
Breed	Cross	117	29(24.78%)	.092	.031	.275	0.000
	Zebu	266	41(15.41%)				
Parity	1-3	210	62(29.52%)	.029	.009	.093	0.000
	4-6	164	8(4.87%)				
	≥6	9	0(0%)				
Lactation Stage (m)	Early (≤3)	125	15(12%)	2.02	1.474	2.767	0.000
	Mid (4-6)	85	15(17.64%)				
	Late (7-9)	50	8(16%)				
	Dry (>9)	124	32(25.80%)				
Pregnancy Status	Pregnant	60	0(0%)	.005	0	.066	0.000
	Non-Pregnant	324	70(21.60%)				
Previous udder infection	Infected	353	62(17.56%)	.131	.04	.431	0.001
	Non-infected	31	8(25.80%)				
Teat lesion	No	356	61(17.13%)	6.357	1.712	12.878	0.000
	Yes	28	9(32.14%)				
Udder shape	Pendulous	77	46(59.74)	.049	.025	.125	0.000
	High up	307	24(7.82%)				
Udder washing	Yes	240	24(10)	.529	.242	1.011	.074
	No	143	46(32.16)				

As viewed from the table 8 , there has been statistical significant association between E.coli prevalence and potential risk factors; of which Age(years), Breed, Parity, Lactation stage in month, pregnancy status, previous udder infection, teat lesion, udder shape, udder washing had statistical significant contribution for the occurrence of the organisms in the dairy cow milk channel.

### 3.3 Antimicrobial Susceptibility Test

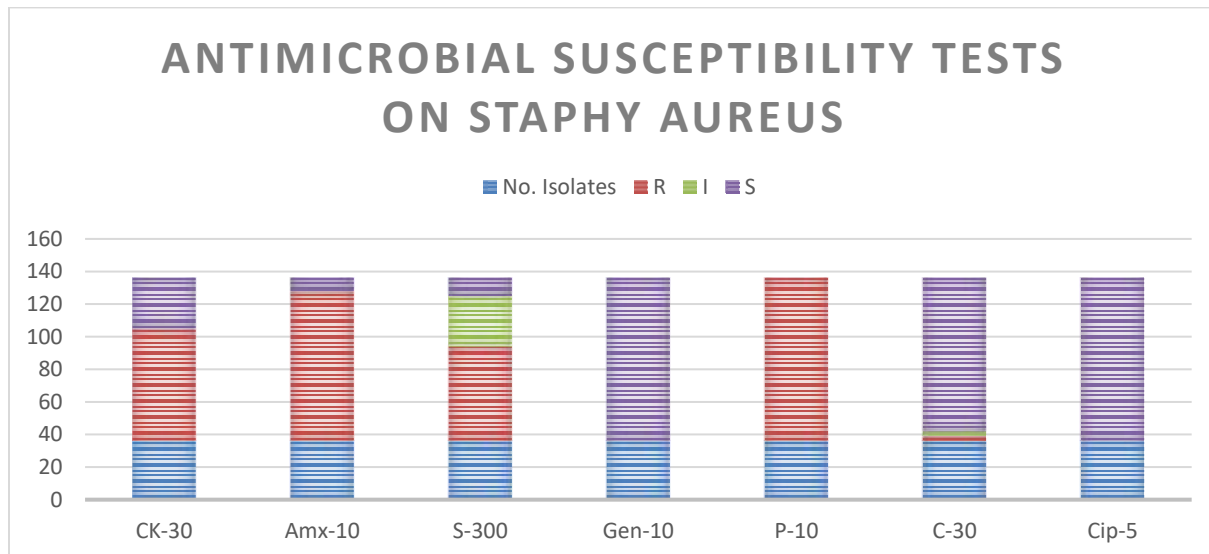
Out of 70 *E.coli*, 40 isolates were subjected to antimicrobial susceptibility tests. Penicillin (85%) followed by kanamycin (60%); chloramphenicol (50%) and streptomycin (42.5%) were drugs to which a large proportion of *E.coli* isolates' resistance. As it is indicated in Table 9, most isolates (60% and 85%) were resistance to these two

drugs respectively. All 40 testes species of E.coli were highly susceptible to Gentamycin (85%) followed by kanamycin (50%); streptomycin (45%) ; vancomycin (40%) and Chloramphenicol (32.5%).

**Table 6:** Resistance and susceptible of E. coli isolates to different antimicrobials (n = 40).

Antimicrobial agents	Disc content (µg)	No. of Isolates	Resistance	Intermediate	Susceptible
			No (%)	No (%)	No (%)
Streptomycin	S10	40	17(42.5)	5(12.5)	18(45)
Gentamycin	CN10	40	6(15)	0(0)	34(85)
Kanamycin	CK	40	7(17.5)	13(32.5)	20(50)
Vancomycin	Van 30	40	24(60)	0(0)	16(40)
Penicillin G	P-10	40	34(85)	0(0)	6(15)
Chloramphenicol	C-30	40	20(50)	7(17.5)	13(32.5)

**Key:** %=percent, S=susceptible; I=intermediate; R=resistance



**Figure 2:** Antimicrobial drugs resistance and susceptibility profile of E. COLI isolated from milk;

Key: CN10, Pen-10; ; C30, CK30, VAN30, S10

### 3.3.1 Multi drug resistance of E.coli

In this study, 13 isolates of E.coli showed resistance to two classes of antimicrobial drugs. From this isolates, (8) eight for vancomycin and penicillin, two(2) for chloramphenicol and penicillin and three for chloramphenicol and streptomycin. Of the 25 isolates of E.coli, 6 isolates were resistance to three classes of antimicrobial (vancomycin, penicillin and streptomycin); two isolates of each for (chloramphenicol, penicillin and streptomycin) and one for vancomycin, penicillin and kanamycin). The maximum multiple drug resistance registered for one isolates were resistance to four classes of antimicrobials as indicated in Table 10.

**Table 7:** Multi drug resistance/MDR/ pattern among *E.coli* isolates

No. AMR	AMR patterns	No. isolates	No.of isolates(%)
Two	CHL, PEN	2	2 (5)
	VAN, PEN	8	8 (20)
	CHL, S	3	3(7.5)
THREE	CHL, PG, S	4	4(10)
	S, VA30, PEN	6	6(15)
	VAN, PEN, CK	1	1(2.5)
FOUR	S, CHL, VAN, PEN	1	1(2.5)

Key; no= number; %=percent; S= Streptomycin; PG=penicillin, VAN= vancomycin; CHL= chloramphenicol; CK= kanamycin

### 3.4 Assessment of the Public Health Significance of *E.Coli*

The issues of public health significance arising from pathogenic *E.coli* and possible sources of milk, general hygiene, hand wash, use of towel, disinfectant, udder hygiene, time of clean, milkers' hand and milk container contamination with *E.coli* were assessed using semi- structured questionnaire survey from Banbasi livestock owners. Besides this, public health significant risk factors associated with *E.coli* infection in lactating cows were assessed by questionnaire on livestock owners milk consumption and acquiring illness due to consuming habit of milk, clinical signs due to *E.coli* infection, illness for milk born disease, awareness on milk born disease and pathogenic *E.coli* food poisoning frequency of milk consumption were assessed.

As Table11 described, dominant livestock owners (61.36%) were participated and indicated as *E.coli* was risk in lactating cows as compared to female domain. Higher (59.09%) participants of 30- 50 years age categories were interviewed followed by 922.72%) participants of greater than 50 years age level and (18.2%) of 15-30 years respondents respond as *E.coli* in milk was risk to consumers. Majority (90.90%) of participants was married and 9.09% were single. Higher (70.45%), followed by 20.45%, 6.81%, and 2.27% of the read and write, illiterate, primary and secondary education level were interviewed respectively about pathogenic *E.coli* as it was public health risk in the lactating cows production channels. In the Banbasi woreda 61.36% of respondents' occupation categories were livestock owners, followed by 34.09% of milk sellers; and 4.44 % of consumers. Regarding the herd size of the community, 52.27%; 43.2% and 4.54% of respondents' herd size were medium, small and large.

With regards to housing of lactating cows, higher 75% of dairy cows housing system was group (shared) while 25% were separated pen.72.72% of lactating cows in the Banbasi district have no bedding materials while 27.3% had bedding materials. Majority (65.90%) of the respondents noticed that their housing system was muddy floor, followed by 22.72% (bad concrete); and 11.36% (good concrete). The dominant (54.54%) of respondents indicated that, time of cleaning of the dairy house were daily; followed by 40.90% of weekly and 4.54% monthly in the areas. 47.72% of respondents indicated that cleaning of milking cows udder were before and after milking; followed by 25% only before milking; 20.45% only after milking; and 6.81% no cleaning. 25% of the lactating cows hygienic conditions were poor; and 75% were good hygienic conditions in the areas.

With respect to milk consumption habit, 47.72%, 27.3%, 15.90% and 9.09% form of milk consumption were boiled, Ayib, raw and ergo respectively. 79.54% of participants had milk consumption habit while 20.45% of respondent interviewers had no habit of consuming practices of milk. 77.3% of respondents did not use refrigerator for milk while 22.72% of participants use refrigerator for milk storage. 90.90% of participant dairy farmers use of soap, detergent to clean milk container whereas 9.09% of lactating cow owners did not use detergents for cleaning milk containers. The consumption of raw milk is relatively higher among read and write categories (70.45%)of respondents than uneducated dairy farmers (20.45%). Only (31.81%) of the dairy farmers were aware of the occurrence of food borne diseases due to raw milk consumption and (25%) of them have aware of *E.coli* food poisoning associated with consumption of raw milk and milk products. 52.27 % dairy farmers consuming milk and milk products, have the chance of acquiring illness whereas those dairy farmers consuming milk have the 47.72% of chance not to acquiring illness.

Higher (54.54%) of diarrhea, and vomiting; followed by 20.45% abdominal pain, cramp; (15.90%) vomiting; and 9.09% diarrhea signs of illness were shown in those consuming raw milk and milk products. In the Banbasi district, the participants indicated that, 75% lactating cows' milk production/ milk yield was low; followed by 22.72% medium and 2.27% high milk yield.

54.54%, 40.90%, and 4.54% of time of cleaning practice of dairy house were daily, weekly, and monthly respectively, however, 64 % of respondents udder hygiene were slightly dirty, 36% and 0 % were moderately dirty and very dirty respectively. 43.2% of respondents were hand washing before milking, 6.81, 47.72, 2.27 and 0 were after milking, before and after milking, in between milking process and not all, respectively.

**Table 8:** the socio-demographic characteristics of the Livestock owners at farm level associated with the public health Significance of E.coli infection (n=44)

Factors	Categories	Freq.	Percentage (N=44)
Sex	Male	17	38.63
	Female	27	61.36
Age	15-30 years	8	18.2
	30-50 years	26	59.09
	>50 years	10	22.72
Marital	Single	4	9.09
	Married	40	90.90
Education level	Illiterate	9	20.45
	Read and write	31	70.45
	Primary	3	6.81
	Secondary	1	2.27
Occupation	Livestock owner	27	61.36
	Milk seller	15	34.09
	Consumers	2	4.44
Herd size	Small	19	43.2
	Medium	23	52.27
	Large	2	4.54
Housing	Separated pen	11	25
	Group ( shared)	33	75
Bedding material	Yes	12	27.27
	No	32	72.72
Floor type	Good concrete	5	11.36
	Bad concrete	10	22.72
	Muddy floor	29	65.90
Time of of cleaning dairy house	Daily	24	54.54

	Weekly	18	40.90
	Monthly	2	4.54
Time length of cleaning milking cows Udder	No cleaning	3	6.81
	Only before milking	11	25
	Only after milking	9	20.45
	Before and after milking	21	47.72
General hygiene	Yes	11	25
	No	33	75
Milk yield	Low	33	75
	Medium	10	22.72
	High	1	2.27
Gross milk quality	Normal	29	65.90
	Watery	1	2.27
	Clots/flakes	2	4.54
	Blood tinged/pus	12	26.66
Hand wash	Before milking	19	43.18
	After milking	3	6.81
	Before and after milking	21	47.72
	Between milking process	1	2.27
	Not at all	0	0
Use of dry towel	Yes	4	9.09
	No	40	90.90
Do you know the different type of mastitis	Yes	11	25
	No	33	75
Previous exposure of mastitis problem in the farm	Yes	11	25
	No	33	75
When do you milk the cows with mastitis	First	3	6.81
	Last	40	90.09
	Any time	1	2.27
Do you treat mastitis case as they occur	Yes	31	70.45
	No	13	29.54
Do you know the name of drugs used for treatments	Yes	10	22.72
	No	34	77.27
Practices of culling chronically	Yes	20	45.45

infected cows	No	24	54.54
Practice of dry cow therapy	Yes	31	70.45
	No	13	29.54
Equipment's used for milking	Aluminum cans	9	20.45
	Plastic can	31	70.45
	Clay pot	0	0
	Other traditional	4	9.09
Use of soap, detergent to clean milk container	Yes	40	90.90
	No	4	9.09
Awareness on milk borne illness	Yes 14	14	31.81
	No	30	68.18
Awareness on E.coli food poisoning	Yes	11	25
	No	33	75
Acquiring illness	Yes	23	52.27
	No	21	47.72
Signs of illness showed	Diarrhea	4	9.09
	Vomiting	7	15.90
	Diarrhea , vomiting	24	54.54
	Abdominal pain, cramp	9	20.45
Form of milk consumption	Raw	7	15.90
	Ergo	4	9.09
	Ayib	12	27.27
	Boiled	21	47.72
Milk consumption habit	Yes	35	79.54
	No	9	20.45
	Yes	10	22.72
Use of refrigeration	No	34	77.27

Higher (55%) of the respondent consumers drink boiled milk followed by 22.5% consume yoghurt/ergo; 15% consume cheese/Ayib and 7.5% raw milk.

57.5% of them had no aware of milk borne disease associated with drinking raw milk and 42.5% of the respondents had knowledge about staphylococcal food poisoning.

Out of 40 consuming milk in different forms, 35 % were acquiring illness; and 65% no acquiring illness. Of the consumers 77.5% of them used plastic containers while the rest 22.5% used metallic containers to transport milk to their homes. 30% of them stored milk in a refrigerator while 70% of them kept milk at room temperature (**Table 12**).

**Table 9:** Assessment of the Public Health significance of E.coli at Consumer level on (n=40)

Issues raised for Milk consumers	Categories	freq.	Response rate N=40, %)
Form of milk consumption	Boiled milk	22	55 %
	Yoghurt/Ergo	9	22.5%
	Cheese/Ayib	6	15%
	Raw milk	3	7.5%
Awareness about milk born disease	Yes	17	42.5%
	No	23	57.5%
Awareness about E.coli food born disease	Yes	3	7.5%
	No	37	92.5%
Acquiring illness after consuming milk and milk product	Yes	14	35%
	No	26	65%
Where do you purchase milk	Farm	22	55%
	Milk selling center	12	30%
	Hotel/ cafe	6	15%
Type of container do you use to collect milk	Plastic	31	77.5%
	Metallic	9	22.5%
Duration of milk stay at home prior consumption	<1hr	8	20%
	1-2hr	11	27.5%
	>2hr	22	55
Temperature of milk storage	<4 <sup>0</sup> c / refrigerator	28	70%
	Room temperature	12	30%
Knowledge on signs of illness	Diarrhea	17	42.5
	Vomiting	6	15
	Diarrhea and vomiting	7	17.5
	Stomach cramp	10	25

#### 4. DISCUSSION

In the present study, out of (N=384) lactating cows' raw milk samples collected and processed by Bacteriologically and Biochemical test methods, 70/384 (18.22%) E.coli prevalence was identified, which was statistically significant ( $P < 0.003$ ). Higher E.coli (35%) contamination rate was recorded in milkers' hand swab followed by milk sample (18.75%), udder swab (14.67%) and container swab (13.33%). All the E. coli isolates were able to produce bright pink colored colonies on MacConkey agar ; characteristic metallic sheen colonies on the EMB agar and pink colored, small rod-shaped Gram-negative bacilli on Gram's staining. The results of catalase, MR and indole test of the E. coli isolates were positive but the V-P test and simmon's citrate utilization were negative which are in agreement with the reports of (Zinnah *et.al.*, 2007).

The pattern of sugar fermentation reaction by the isolated E. coli with three sugars (triple sugar iron agar) was observed and produced acid and gas. The isolates were able to ferment glucose, lactose, and sucrose completely. Acid production was indicated by the color change from reddish to yellow and the gas production was noted by the appearance of gas bubbles in the test tubes. This result was in agreement with the findings (Asmelash, 2015; Bedassa, 2018; Zinnash, 2007; Giwida and Gohary, 2013). This result was partially in agreement with the findings of (Beutin *et al.*, 1993 and Sandhu *et al.*, 1996). They reported that although E. coli ferments all three basic sugars but it partially ferments sucrose and glucose. Variation of the results may be due to genetic factors and the nature of the inhabitant of the organisms.

With regards to E. coli associated risk factors, 18.75% of the raw milk samples were contaminated with E. coli. In this findings, the prevalence of E.coli infection was significantly influenced by age categories ( $P < 0.05$ ). Significant *E.coli* infection associated with age categories which was higher in (21.82%) in 4-7 years age followed by (13.88%) of greater than 7 years age and in 3 years 0(0%) which was significant ( $P < 0.01$ ). Similar result was reported by Shimelis (2014) in Selale /Fitcha.). Adult followed by old cows in this study were more

susceptible to *E.coli* infection than young cows. The increasing occurrence of infection with increasing age were agreed with the findings by Kerro and Tareke, (2003) who found that, the risk of infection with the advancing age of the cow. This might be due to the increased opportunity of infection with time and the prolonged duration of infection, especially in a herd without mastitis control program (Radostits *et al.*, 2007).

Significant (24.78%) *E.coli* infection was recorded in cross breeds followed by 15.41% in local zebu breeds ( $p<0.05$ ). Comparable findings were reported by with Bitew *et al.* (2010) who reported in Bahir Dar, between Cross and Fogera breed, Lakew *et al.* (2009) in cross and local Arsi breed. Increased milk yield from genetic selection may be accompanied in genetic susceptibility to lactating cows infections (Schutz, 1994). Besides this, the low occurrence of mastitis in local breeds in addition to genetic factors could also be one indication for higher occurrence of mastitis prevalence in areas where exotic breeds and their hybrids well adapted. Therefore, the lower prevalence in local zebu breeds in this study could be associated with difference in genetically controlled physical barrier like streak canal sphincter muscles, keratin in the teat canal or shape of teat end where pointed teat ends are prone to lesion (Seykora and Mcdaniel, 1985). In addition to physical barriers, the difference in occurrence of mastitis in these breeds could arise from differences in cellular immunity (Erskine, 2001).

The observed higher occurrence of *E.coli* during dry lactation 25.80% as compared to early 12% ; mid 17.64% and late lactation stage 16% was significant ( $p<0.05$ ). The finding of higher infection in cows in dry lactation stage followed by medium, late and early lactation stages in the study concurs with previous reports. In cows most new infections occur during the the dry period and in the first two months of lactation (Radostits *et al.*, 2007). This may be due to an absence of dry period therapy and birth related influences. During a dry period, due to low bactericidal and bacteriostatic qualities of milk, the pathogens can easily penetrate into the teat canal and multiply (Aylate *et al.*, 2013). Radostits *et al.* (2000) suggested that, the mammary gland is more susceptible to new infection during the early and late dry period, which may be due to the absence of udder washing and teat dipping, which in turn may have increased the presence of potential pathogens on the skin of the teat. Moreover, during a dry period due to the low bactericidal and bacteriostatic qualities of milk, the pathogens can easily penetrate into the teat canal and multiply; this can be carried over into the post parturient period and ultimately develop into infection.

Multivariable binary logistic regression analysis revealed that the prevalence of *E.coli* isolates were significantly different among parity groups. Early birth of cows with 1-3 parity has 29.52% (higher) of infection followed by cow with 4-6 parity or frequency of birth which was ( 4.87%) of infection. In this study infection occurrence was associated with parity of the animal and found statistically significant ( $p<0.05$ ). This might be due to the increased opportunity and contamination of *E.coli* infection and the prolonged duration of infection (Markos *et al.*,2023). The higher early occurrence of infection with parity in the current study is comparable with the previous reports of Mulugeta and Wassie, (2013) in Wolaita Sodo town, Mekibib *et al.*, (2010) in Holota town and Haftu *et al.*, (2012) in northern Ethiopia. The association might be due to the increased opportunity of infection with time and the prolonged duration of infection, especially in a herd without mastitis control program (Radostits *et al.*, 2007). The increased occurrence of infection with parity was reported by Mekibib *et al.* (2010) in Holeta town and Haftu *et al.* (2012) in northern Ethiopia.

In this study, *E.coli* occurrence in muddy floor system was 19.55% prevalent while 16.35% was seen in concrete floor which has non- significant ( $p>0.05$ ). In agreement with Abera *et al.* (2013) in Adama town and Fekadu *et al.* (2005) in southern Ethiopia, Lakew *et al.* (2009) and Sori *et al.* (2005). The findings of a high prevalence of infection in farms with muddy (soil) floors (48.36%) when compared with concrete floor types (35.22%) shows the occurrence of mastitis is significantly associated with the housing (bedding) type or condition of the farm. This is due to association with poor sanitation and cows which were maintained in dirty and muddy common barns with bedding materials that favor the proliferation and transmission of mastitis pathogens. The main sources of infection are udder of infected cows transferred via milker's hand, towels and environment (Radostitis *et al.*, 2007).

This study revealed that dairy cows house with poor drainage was 22.06 times more likely to be harbor *E.coli* than well drainage housing systems. The association can be attributed to poor sanitation practices and the housing of cows in dirty and muddy common barns with bedding materials that promote the survival and transmission of pathogens (Bizunesh *et al.*, 2022).

Occurrence of *E.coli* infection was significantly associated with milking hygienic practice ( $p=0.000$ ). Cows at farms with poor milking hygiene standard are severely affected (31.94%) than those with good milking hygiene practices

(10%). This findings were comparable with the previous findings of (Mulugeta and Wassie, 2013; Lakew *et al.*, 2009; Sori *et al.*, 2005). This might be due to absence of udder washing, milking of cows with common milkers' and using of common udder cloths, which could be vectors of spread especially for contagious mastitis (Radostitis *et al.*, 2007).

The consumption of raw milk and its different forms of product is common in Ethiopia, which is not safe from consumers' health point of view as it may lead to transmission of various diseases. It may be contaminated at the site of production and during processing, the cow itself, unclean milk containers and the milk handlers. The hygienic condition or quality of milk has serious implication on public health safety. The questionnaire results mainly gave broad understanding of the milking and hygienic practice. In this study among the farmers, 20.45 % had a habit of drinking raw milk and 79.54 % of them didn't have awareness about food born disease associated with consumption of raw milk. This results is agree to a study done by Tsige, (2018) around Arsi Negelle town, which is 21.7% of the raw milk consumption and 62% of them have no awareness about milk borne disease among farmers. Though the results showed relatively a lower percentage of raw milk consumption, still these individuals are at a greater risk of contracting food born intoxication infection than those who do not consume raw milk.

In addition, three factors on the farm level were assessed as probable variables related to the higher frequency of samples positive for *E.coli*. There was statistically significant ( $P < 0.05$ ) associations between body condition, age and breeds of the animals with positive isolates. This finding was comparable with the finding of Iqbal *et al.* 2004 (40.7%). However, it is much higher than the finding of (Biruke and Shimeles, 2015) (18.6%). This prevalence of *Escherichia coli* is presumably due to the fact that *E. coli* is the commonest environmental contaminants, which is closely associated with hygiene condition of the animals as well as the environment. It becomes pathogenic whenever the hygienic conditions of the animal or environment become poor. Moreover, the existence of high concentration of *E. coli* in milk also indicates the relatively poor quality of milk, related with substandard hygiene of the farm management, milk collection and processing system. The isolation of *E. coli* is of public health significance as this bacterium is known to cause serious gastrointestinal disorders in both young and adult humans (FAO and WHO, 2004).

Concerning the type of examined milk samples, the high prevalence of *E. coli* in raw milk may be attributed in Bishoftu dairy farms is since milk is mainly transported directly to the dairy plant for processing meanwhile market milk is usually collected from small farms or farmers therefore it will be liable to cross contamination by different ways as mixed fresh clean milk with mastitis milk, unclean hands of workers, unclean utensils and unhygienic water supply for washing the utensils could be the source for accelerating the bacterial contamination. This idea agreed with conditions for contamination of raw milk at different critical points due to less hygienic practices (Reta *et al.*, 2016; Gwida and EL-Gohary, 2013).

The present study showed that the resistance of *E.coli* to Penicillin G (85%), vancomycin(60%), chloramphenicol (50%), and (45%) streptomycin observed in milk samples. Comparably *E.coli* isolate resistance result was reported by Igbinsosa *et al.*, (2021) in Benin city, Nigeria, which revealed 100% penicillin G and ampicillin; 94.5% chloramphenicol, 89.5 % erythromycin, 78.9% Oxy tetracycline and sulfamethoxazole.

In the present findings, 85% of Gentamycin was sensitive to *E.coli* infection followed by kanamycin (50%), streptomycin (45%), vancomycin (40%) and 32.5% chloramphenicol. This finding was in line with the findings of Igbinsosa *et al.*, (2021) in Benin city, Nigeria, reporting *E.coli* isolates were 100% sensitive to Gentamycin and Ofloxacin. Comparable with the present findings, Frehiwot M *et al.*,(2023) in Adami Tulu Jida, komobolcha District, reported that, 100% resistance was observed for ampicillin, cephalothin and rifampin and on the other hand 100% susceptibility was observed for chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid, kanamycin and tetracycline.

In the present observation, Of the 25 isolates of *E.coli*, 11 isolates ( 27.5%) were resistance to three classes of antimicrobials; six isolates= ( streptomycin, vancomycin, penicillin), 1 isolates for vancomycin, penicillin, and kanamycin) and four isolates for (chloramphenicol, penicillin, streptomycin) frequent multidrug resistance pattern were exhibited. Antibiotic resistance pathogenic *E.coli* isolates has been a challenge to both animal and public health.

In this study, 62.5% MDR was observed. The test isolates were resistant to vancomycin, penicillin, streptomycin, chloramphenicol and kanamycin). This is in agreement with the report of Bekele *et al.* [2014] and Atinafe [2017]. Multidrug resistance occurred due to the misuse of antimicrobial agents or due to genetic mutation [Mendelson M.,2011]. On contrary, all isolates were susceptible to the most commonly used antimicrobials including chloramphenicol, ciprofoxacin, gentamicin [Kibert *et al.*, 2011] and tetracycline. However, Hiko *et al.* [2008],

Bekele et al. [214] and Haile et al. [2022] were reported resistance to tetracycline which is the most commonly used antimicrobials in Ethiopia, which is contrary to the present study. But, Mohammed et al. [2014] reported susceptibility to tetracycline which is in line with the present study

Consistently, Igbinsosa *et al.*, (2021) in Benin city, reported that, multidrug resistance (MDR) profiles of 41 other *E. coli* isolates shows that 40 (97.6%) were resistant to 3 antibiotics (AMPR, PENR, CHLR) in 2 antimicrobial classes with MAR index of 0.13. The extensive drug resistance profiles include resistance of 25 (60.9%) to 11 antibiotics (AMPR, PENR, CHLR, ERYR, SULR, AMXR, AMCR, CTXR, SAMR, IMIR, TMPR) in 7 antimicrobial classes with MAR index of 0.46 .

The antibiotic resistance rates in this study is slightly different from an earlier report by Msolo et al., (2016) which indicated a resistant rate of 85% for penicillin G, 45% for chloramphenicol, 70% for erythromycin, and 74% for sulfamethoxazole. The 100% resistance to penicillin observed in our study agrees with the study of Alam et al., (2017) who reported high rate (100%) of resistance to penicillin among *E. coli* O157 isolates cultured from raw milk marketed in Chittagong, Bangladesh. The high susceptibility rate (100%) to Gentamicin and Ofloxacin for genotypically confirmed *E. coli* O157:H7 isolates obtained in this study is different from a report by Alam et al., (2017) where 50% rate to gentamicin and ofloxacin was reported. High resistance rates to penicillin and tetracyclines in our study agrees with the antibiotic susceptibility test study by Reuben and Owuna (2013) on *E. coli* O157 isolates recovered from milk samples.

This result was higher than the finding of Igbinsosa *et al.*, (2021), who reported 2.6 % of multiple drug resistance to pathogenic *E. coli* isolated from cow milk in Benin city. This might be due to the variation in the type and frequency of use of these antibiotics for the treatment and prevention of prevailing bacterial diseases. Multiple antibiotic-resistance pathogenic *E. coli* strains have been isolated from milk obtained from dairy animals in many part of the world (Lemma *et al.*, 2021).

In a similar study by Tadesse et al., (2017), *E. coli* showed high resistance rates to ampicillin (70%), sulfamethoxazole-trimethoprim (60%), clindamycin (80%), erythromycin (60%), chloramphenicol (50%), and kanamycin (50%), which is slightly different from the findings of our study. Antibiotic resistance could be due to abuse of antibiotics in both human medicine and for agricultural purposes, predominantly in disease suppression and advancement of growth in animal production. The high susceptibility of *E. coli* to kanamycin in our study is different from the study of Tadesse et al., (2017) which reported 50% resistance rate, although the study reported high susceptibility rates to some antibiotics such as gentamicin (100%), ofloxacin (100%), and ciprofloxacin (90%), which is similar to the findings in our study.

## 5. CONCLUSION AND RECOMMENDATIONS

The findings of our present study clearly indicated that safety and quality of fresh and fermented milk in Banbasi were unsatisfactory. Higher 35% *E. coli* contamination rate was detected in milkers' hand swab followed by 18.75% (milk samples); (14.67%) udder swab; and 13.33% in container swab, of milk marketing channels, which was significantly associated  $P < 0.05$ . The overall prevalence of *E. coli* from the milk production channels were 18.22%. This indicates that *E. coli* is one of the major problems of dairy cows in milk production that contaminated and reduced the quality of milk. Besides, all *E. coli* isolates exhibited bright pink color with lactose fermentation on MacConkey agar plates, metallic sheen on Eosin Methylene Blue agar plate and gram-negative, pink-colored, small rod-shaped organisms arranged in single with pairs or short chains on Gram's staining and Indole -positive, methyl red -positive, voges- proskauer- negative and simmon's citrate- negative. Consistently, udder washing, age, breed, parity, lactation stage, pregnancy status, milking hygiene, blind teat, teat lesion, body condition, and drainage system were *E. coli* associated risk factor in the milk production channels were significant ( $P < 0.05$ ) whereas previous udder infection, floor type, and herd size were not significantly associated ( $P > 0.05$ ).

Besides, 85% of penicillin G, followed by 60% vancomycin; 50% chloramphenicol; and 42.5% streptomycin were resistance whereas 85% of Gentamycin, 50% kanamycin, 45% streptomycin, 40% vancomycin, and 32.5% chloramphenicol were sensitive to *E. coli* isolates. Similarly, 27.5% of the tested isolates were revealed multi drug resistance to three classes of antibiotic discs, 2.5% of isolates for four classes of drugs, and 32.5% of isolates for two classes of drugs. The disease has public health importance and it also harm the health and wellbeing of human being. Therefore, the results of the present study provided that *E. coli* quality and safety of raw milk was unsatisfactory. These findings stress the need for an integrated control of *E. coli* from farm production on to consumption of food of animal origin.

In light of the above conclusive remarks, the following recommendations are forwarded:-

- Awareness should be created on milk handling practice, storage and milking process to Dairy farmer and milk collectors.
- Proper raw milk storage, milk pasteurization, and hygiene and sanitary of milk handling across milk production channels.
- Increasing knowledge and awareness of Dairy cow owners on milk consumption cultures, handling, and processing across marketing channels.
- The professionals should apply different methods for prevention and control of the disease.
- The professionals should inform the public about the relevance of milk pasteurization before consumption to avoid food born infection
- Regular resistance follow-up, using antimicrobials sensitivity tests helps to select effective antibiotics and to reduce the problems of drug resistance developments towards commonly used antimicrobials.

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