PREVALENCE, ANTIMICROBIAL SUSCEPTIBILITY PROFILE AND PUBLIC HEALTH SIGNIFICANCE OF *ESCHERICHIA COLI* O157: H7 FROM RAW COW MILK IN AND AROUND ASSOSA TOWN, BENISHANBGUL GUMUZ ,WESTERN, ETHIOPIA

Asmamaw Aki Jano

Assosa, Regional Veterinary Laboratory, P.O. Box 326, Assosa, Ethiopia; asmamawaki@gmail.com, phone: +251-902330029

ABSTRACT: Across-sectional study was conducted on Isolation, Identification and Antimicrobial susceptibility profile of *E.coli O157:7* and its public health Impact in milk supply chain of Assosa District, Benishangul Gumuz, western Ethiopia from October 2024 to April 2025 in Dairy cows, with the objectives to isolate and identify pathogenic *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 pathogenic Escherichia coli isolates. 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*. The study revealed that 5.72% of the collected raw milk was contaminated with E. coli. Higher (12.90%) E.coli contamination was reported in milkers' hand swab followed by (6.06%) milk samples, (5.2%) container swab and 1.85% udder swab. In this study, there was significant ($p< 0.05$) association between the udder washing practices, sample types, herd size, milking hygiene, and teat lesion of the cow with the isolates of pathogenic E.coli. In other way, previous udder infection, floor type, age, breed, stage of lactation, pregnancy status, and blind teat risk factors was not significant ($P>0.05$). Majority (95%) of drug resistance prevalence was reported in penicillin G, followed by (82%) Oxacillin; ciprofloxacin (72%), 64% chloramphenicol; and 39% streptomycin and 32% of tetracycline. Whereas higher (86%) of drug sensitivity or susceptibility was recorded in Gentamycin, followed by 64% tetracycline, 50% streptomycin, and 36% chloramphenicol and 27% ciprofloxacin. In this study 45% MDR were recorded in three classes of drugs and four classes (4.5%) of antibiotic discs. The presence and consumption of raw milk may constitute a public health hazard and reduced milk quality due to pathogenic E. coli. Thus health professionals should create awareness about milk handling practice, storage and milking process to Dairy farmer, milk handlers', 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.

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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 a*l., 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 extraintestinal illnesses in humans (Ahmida, 2020). Raw milk reaches consumers with a higher coliform content (Adzitey *et al*., 2018).
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 E. coli O157:H7 infection related to consumption of raw milk is high, indicating that there is risk of E. coli O157:H7 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 sever 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 Dairy farms in Nejo, 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 Nejo 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 *Escherichia coli O157:H7* from raw cow milk and determine likely sources of contamination in dairy farms of marketing channels and milking handlers and equipment..

1.4.2. Specific Objectives

- To isolate and identify *Escherichia coli O157:H7* from raw cow milk in a dairy farm.
- To determine antimicrobial susceptibility patterns of *Escherichia coli O157:H7* isolates.
- To assess the source of raw cow milk contamination and the extent to which milking activities and milking equipment are a cause of contamination.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

The study was conducted in Assosa town, Benishangul Gumuz Regional State (BGRS), western Ethiopia. Currently, Assosa town has two administrative districts (district-1 and district-2). Each district as five “ketenas”. According to Benishangul Gumuz Regional State Metrological Center report, (2020), the town is located at 10º 00’and 10º 03’ north latitude and 34º 35’ and 34º 39’ east longitude. The total population of the town is 62,632 of which 32, 100 are males and 30,532 are females (CSA, 2020). The total area of the town is 2361.34 hectares with an altitudinal difference that ranges between 1461- 1641 meters above sea level (BGRSEIB, 2020). The mean annual

temperature of Assosa town ranges from a minimum of 14-33^{0}C. However, there is a slight variation of temperature by month. February to May is the hottest months while November to December is the cold months. The average annual rain falls recorded during the last nine months were 1,119 mm (BGRSMSC, 2020). The rainy season starts in March and extends to November with the highest concentration in June, July, and August. The population size of different livestock species in Assosa town are cattle 569, goat 1545, sheep 739, poultry 17676 donkeys 122, and pig 8 total 20,659 livestock populations are found in the town (Assosa Town Administrative Office of Agriculture, 2020).

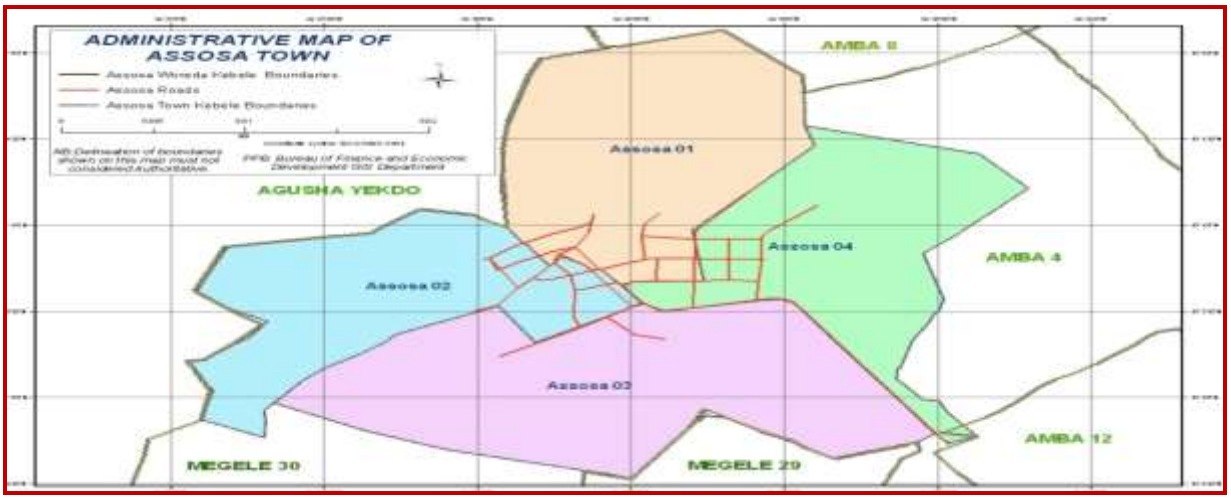

Figure 1: Administrative map of Assosa town (ATAO, 2021).

2.2. Study Population

The study animals were apparently healthy lactating cows. The milk samples were collected from different milking channels such as udder milk, bulk milk, and swab samples from milk handlers, milking environment and milk utensils of in and around Assosa Town.

2.3. Study Design and Sampling Method

A cross-sectional study was conducted from October 2024 to April 2025 to isolate and identify *E. coli O157:H7* from raw cow milk of dairy farms and milk distributing channels. Based on Assosa district agriculture office data, dairy farms and production channels were chosen purposively and a simple random sampling method was used to take each individual samples.

2.4 Sampling Size Determination

The total sample size for *E. coli O157: H7* isolation and identification was assigned according to statistical formula (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 O157: H7* prevalence of raw cow milk, the expected prevalence of this study was taken as 50%. Therefore, the total sample size for this study was calculated as follows:

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

Where: n = the total sample size

P = expected prevalence (50%)

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

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

Therefore, to isolate *E. coli O157: H7* a total of 384 samples was taken from three parts (udder milk =108, milk=99, 115= container swab and 62 hand swab samples from milk handlers, milking environment and milk utensils).

2.5. Sample Collection Procedure

Samples from the lactating dairy cows, as well as swab samples from the milk handlers, milking environment, and milk utensils were aseptically collected. Before sampling, the udders and teats were carefully cleansed, dried, and gently wiped with cotton swabs moistened with 70% ethyl alcohol. The first 3-4 streams of milk was discarded and about 10 ml of milk was collected from the udder aseptically and put in a sterile screw-top universal bottle. Swab samples were collected from milk handlers, the milking environment, and milking of the milk production channels. The swab samples were taken using sterile swabs and kept in sample bottles containing sterile physiological saline solution to prevent desiccation.

Then, the milk and swab samples were labeled and documented. Sample code, sampling date, and sample type are all assigned to all collected samples. The samples were transferred using an icebox to Assosa Regional Veterinary, 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).

2.6. Questionnaire Survey

A structured questionnaire with closed-ended questions was used to collect data on lactating cows and potential risk factors thought to impact the possible source of *E. coli O157:H7* contamination in dairy farms. Data on intrinsic factors such as age categorized as young (≤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 months), parity level categorized as few (1-2calves), mid (3-4 calves), and many (≥4 calves) were collected for this purpose. Data on potential extrinsic risk factors were collected from the interviews of dairy workers and observations. In addition, observational checklists were used to rate the hygiene of milk and milk product utensils, milk handlers (Yes or No), and dairy farm hygiene (good or poor). The survey was written in English and then translated into the local language.

2.7. Laboratory Analysis

2.7.1. Isolation and Identification of E. coli O157:H7

Isolation and Identification of *E. coli O157:H7* from swabs, bulk, and teat milk samples were done using routine culture and biochemical analysis. Approximately 1ml of milk was suspended in 9 ml of buffered peptone water. Samples were water spout-formed and incubated overnight at 41°C. After BPW, 50µl of product was streaked onto MacConkey agar for primary isolation of *E. coli* and incubated aerobically at 37°C for 24 hours. Plates were observed for the growth of *E. coli* (pink colony; lactose fermenter). 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, TSI and Simmons's citrate test was done.

The Isolation of *E. coli O157*:*H7* was carried out as earlier described (De-Boer *et al*., 2000; 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. These were later sub-cultured (twice) to obtain pure colonies. The Non-Sorbitol-Fermenting (NSF) *E. coli* (colorless or pale colonies) would be considered as *E. coli* O157:H7 strains whereas pinkish colored colonies (Sorbitol-Fermenters) would be considered as non O157:H7 *E. coli* strains (Ivbade *et al*., 2014). NSF isolates would be again subjected to Latex *E. coli* O157:H7 agglutination test for confirmation using a latex agglutination test kit (*E.coli O157:H7* latex test, Oxoid®, UK) as described by the manufacturer. The positive isolates were stored in 15% glycerol at −20 °C until further processed.

2.7.2 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). Streptomycin (10µg), Gentamycin (10µg), Chloramphenicol (30 µg), Tetracycline (30µg), Ciprofloxacillin (5 µg), and Penicillin (10 g) and Oxacillin-1 (1µg) are used to test the antibiotic susceptibility of *E. coli O157:H7* isolates. In a nutshell, the bacteriawas 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, 2023).

3.8. Data Processing and Analysis

Data was processed and analyzed using computer softwares for statistical data analysis. The collected, questionnaire data of associated risk factors was stored into MS excel spread sheets to create a database and processed (data was checked for accuracy). The coded and processed data was entered and analyzed using STATA version 17. Differences were considered statistically significant at 5% level of significance. Means was said to be significantly different when $P \leq 0.05$.

3. RESULTS

3.1 Occurrence of pathogenic E.coli

Out of the total lactating cows examined, 58/384 (15.10%) E.coli prevalence was identified, which was statistically significant (P<0.05). Consistently, higher E.coli (20.96%) isolation rate was recorded in milkers' hand swab followed by 20.86%, 12.04% and 8.08% *E.coli* container swab, udder swab and milk samples, respectively (Table 2).

Besides this, higher (12.90%) E.coliO157:H7 infection was registered in milkers hand swab while 6.06%, 5.22%, and 1.85% of pathogenic E.coli was identified in milk samples, containers swab and udder swab respectively (Table 3).

In the present study, *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, Methyl red and Indole test of the *E. coli* isolates were positive but the Voges proskaur 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. Accordingly, the non-sorbitol-Fermenting (NSF) *E. coli* (colorless or pale colonies) were considered as *E. coli* O157:H7 strains. NSF isolates were again subjected to Latex *E. coli* O157:H7 agglutination test, which result with agglutinating positive reaction (Table 1).

Table 1. Results of various biochemical tests performed on E.coli O157:H7 **isolates**

Biochemical tests	***Bacterial isolate***
	E.coli O157:H7
Nutrient agar	smooth, circular, white to grayish colonies
MacConkey agar	lactose fermenter, pink to dark pink or red colonies
Sorbitol *MacConkey agar*	pale colony(colorless colonies)-NSF
Sorbitol	Not ferment sorbitol(colorless colonies)
Gram staining	Gram negative, pink or reddish appearance
Catalase	Positive
Oxidase	Negative
Triple sugar iron agar (TSI)	Slant=yellow; butt= yellow; no H_2S production , gas production (Y/Y/H_2S-)
Indole test	Positive
Methyl red	Positive
Voges-proskauer test	Negative
Simmon's Citrate utilization	negative
Oxidation-Fermentation test	Fermentative
Glucose	positive
Lactose	positive (lactose fermenter)
Sucrose	positive

Table 2. Prevalence of *E.coli* with sample types

Sample Type	Examined sample	No. positive	Prevalence	CHI2	P-value
milk samples	99	8	8.08	9.39	0.024
Udder swab	108	13	12.04		
Hand swab	62	13	20.96		
Container swab	115	24	20.86		
Total	**384**	**58**	**15.10**		

Although the highest prevalence of *E.coli* was recorded in hand swab, there was statistically significant association of *E.coli* to the sample types. The overall prevalence of the *E.coli* in the present study was 15.10% .

Table 3. Prevalence of *E.coli O157: H7* with sample types

Sample Type	Examined sample	No. positive	Prevalence	CHI2	P-value
milk samples	99	6	6.06	9.22	0.027
Udder swab	108	2	1.85		
Hand swab	62	8	12.90		
Container swab	115	6	5.21		
Total	**384**	**22**	**5.72**		

As described in Table 3, 22 isolates were non - sorbitol fermenting E.coli (E.coli O157:H7), and hence higher (12.90%) infection were recorded in hand swab followed by 6.06% (milk samples), 5.21% (containers swab), and 1.85% (udder swab).

3.3 Risk factor associated with prevalence of E.coli O157: H7 infection

Prevalence of E.coli O157 infection related to the specific risk factors were determined as the proportion of affected cows out of the total examined. As indicated in (Table 4), the questionnaire survey and observation data result shows association of sample type, udder washing, herd size, milking hygiene, teat lesion, are amongst the potential risk factors, which are associated with pathogenic *E.coli* infection in dairy cows. Accordingly, *E.coli* prevalence showed significant variation among different udder wash groups (p = 0.002), herd size (0.02); milking hygiene (0.002), teat lesion (0.0003). However, floor system, herd size, BCS, Drainage, age factors, breed factors, PS, and previous mastitis treatment history have no significant difference with *pathogenic E.coli* (p>0.05).

Table 4: **Multivariate Binary Logistic Regression of attribute risk factors with *E.coliO157***

Risk factors	Categories	No. examined	No (%) positives	OR	ChI2	P-value
Age(years)	≤3 (year)	23	0 (0%)	1.12	1.97	0.37
	4-7 years	253	14(5.53%)			
	> 7 years	108	8(7.40%)			
Breed	Cross	117	4(3.42%)	1.66	1.68	0.19
	Zebu	267	18(6.74%)			
Parity	1-3	210	12(5.71%)	1.29	4.74	0.09
	4-6	164	8(4.87%)			
	≥6	10	2(20%)			
Lactation Stage (m)	Early (≤3)	125	9(7.2%)	0.90	1.89	0.59
	Mid (4-6)	85	3(3.56)			
	Late (7-9)	50	4(8%)			
	Dry (>9)	124	6(4.83%)			
Pregnancy Status	Pregnant	60	2(3.33%)	0.63	0.76	0.38
	Non	323	20(6.19%)			
Previous udder infection	Infected	352	20(5.68%)	0.68	0.03	0.84
	Non	32	2(6.25%)			
Floor type	Concrete	158	5(3.16%)	1.90	3.29	0.07

	Muddy (soil)	226	17(7.52%)			
Milking hygiene	Good	240	7(2.92%)	0.41	9.49	0.002*
	Poor	144	15(10.42%)			
Blind teat	No	306	18(5.88%)	0.90	0.05	0.82
	Yes	78	4(5.12%)			
Teat lesion	No	356	17(4.77%)	3.36	8.75	0.003*
	Yes	28	5(17.85%)			
BCS	Good	196	9 (4.59%	0.65	0.93	0.33
	Poor	188	13(6.91%)			
Udder shape	Pendiculous	78	5(6.41)	0.70	0.10	0.75
	High up	307	17(5.54%)			
Drainage	*Yes*	*170*	*7(4.11)*	0.56	1.49	0.22
	No	*213*	*15(7.04)*			
Herd size	*Small*	*178*	*13(7.30)*	0.88	8.18	0.02*
	Medium	*190*	*6(3.15)*			
	Large	*16*	*3(18.75)*			
Udder washing	*Yes*	*240*	*7(2.92)*	0.25	9.49	0.002*
	No	*144*	*15(10.42)*			

*Chi2=chi-square; OR= odd ratio

3.4 Antimicrobial Susceptibility Test

22 E.coli O157:H7 isolates were subjected to antimicrobial susceptibility tests. Penicillin (95.45%) followed by Oxacillin (81.81%), Ciprofloxacin (72.72%); chloramphenicol (63.63%); streptomycin (50%) and tetracycline (31.81%) were drugs to which a large proportion of pathogenic *E.coli* isolates' resistance. As it is indicated in Table 9, most isolates (95% and 82%) were resistance to penicillin and Oxacillin drugs respectively. All 22 testes species of pathogenic E.coli were highly susceptible to Gentamycin (86.36%) followed by Tetracycline (63.63%); Streptomycin (50%) ; Chloramphenicol (36.36%) and Ciprofloxacin (27.3%) (Table 5).

Table 5: Resistance and susceptible of *E. coli O157* isolates to different antimicrobials (n = 22).

Antimicrobial agents	Disc content (µg)	No. of Isolates	Resistance	Intermediate	Susceptible
			No (%)	No (%)	No (%)
Streptomycin	S10	22	9(39.13)	2(9.09)	11(50)
Gentamycin	CN10	22	3(13.63)	0(0)	19(86.36)
Penicillin G	P-10	22	21(95.45)	0(0)	1(4.54)
Chloramphenicol	C-30	22	14(63.63)	0(0)	8(36.36)
Tetracycline	TE 30	22	7(31.81)	1(4.54)	14(63.63)
Ciprofloxacin	CIP5	22	16(72.72)	0(0)	6(27.27)
Oxacillin	OX-1	22	18(81.81)	0(0)	4(18.18)

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

3.4.1 Multi drug resistance of E.coli

In this study, overall 21(95%) of the drugs were developing resistance. 10 isolates of E.coli showed resistance to two classes of antimicrobial drugs. From this isolates, (5) five oxacillin and penicillin, two (2) for chloramphenicol and penicillin and three (3) for ciprofloxacin and streptomycin. Four (4) isolates were resistance to three classes of antimicrobial (oxacillin, penicillin and ciprofloxacin); three isolates of each for (chloramphenicol, penicillin and oxacillin) and one for streptomycin, penicillin and oxacillin) and (penicillin- chloramphenicol, tetracycline). And one again for (oxacillin- ciprofloxacin- chloramphenicol) The maximum multiple drug resistance registered for one isolates were resistance to four classes of antimicrobials as indicated in Table 6.

Table 6. Multi drug resistance/MDR/ pattern among E.coli isolates

No. AMR	AMR patterns	No. isolates	No. of isolates (%)
Two	CHL, PG	2	2 (9.09)
	OX, PG	5	5 (22.72)
	S, CIP	3	3(13.63)
THREE	CHL, PG, OX	3	3(13.63)
	OX, CIP, PEN	4	4(18.2)
	S, PG, OX	1	1(4.54)
	PG, CHL,TE	1	1(4.54)
	OX,CIP, CHL,	1	1(4.54)
FOUR	CIP, CHL, OX, PG	1	1(4.54)

Key: no= number; %=percent; CIP= ciprofloxcillin, OX=Oxacilin, S= Streptomycin; PG=penicillin G; CHL= chloramphenicol.

3.5 Questionnaire survey

The issues of public health significance arising from pathogenic *E.coli* and possible sources of contamination of milk, hand washing, utensil cleaning, milk storage, animal health status, waste disposal, disinfectant, udder hygiene, milkers' hand and milk container contamination with *E.coli* were assessed using semi- structured questionnaire survey from Assosa livestock owners. Besides this, public health significant risk factors associated with *E.coli* infection in lactating cows were assessed .

With respect to milk consumption habit, 40%, 16.66%, 20% and 23.33% form of milk consumption were boiled, Ayib, raw and ergo respectively. 70% of participants had milk consumption habit while 30% of respondent interviewers had no habit of consuming practices of milk. 76.66 % of respondents did not use refrigerator for milk while 23.33% of participants use refrigerator for milk storage. 90.90% of participant dairy farmers use of soap, detergent to clean milk container wheras 9.09% of lactating cow owners did not use detergents for cleaning milk containers.

Only (36.66%) of the dairy farmers were aware of the occurrence of food borne diseases due to raw milk consumption and (33.33%) of them have aware of E.coli food poisoning associated with consumption of raw milk and milk products. 76.66 % dairy farmers consuming milk and milk products, have the chance of acquiring illness whereas those dairy farmers consuming milk have the 23.33% of chance not to acquiring illness.

With regard to demographic information of study livestock owners, As Table 8 described, dominant livestock owners (60%) were male participated and indicated as E.coli was risk in lactating cows as compared to female domain. Higher (60%) participants of 30- 50 years age categories were interviewed followed by (23.33%) participants of greater than 50 years age level and (16.66%) of 15-30 years respondents respond as E.coli in milk was risk to consumers. Majority (63.33%) of participants was married and 36.66% were single. Regarding education level of dairy cow interviewers, The consumption of raw milk is relatively higher among primary education categories(71%) of respondents than uneducated (illiterate) dairy farmers (20%); followed by (6.66%) secondary education; (3.33%) college level respondants respond as pathogenic E.coli was public health risk in the lactating cows through production channels. In Nejo districts, concerning role in milk handling, 33.33% milker and 30% transporters were majority respondents followed by 26.66% seller and 10% processor(**Table 8**).

Table 8. Demographic Survey of Livestock owners associated with pathogenic E.coli (n=30)

Factors	Categories	Frequency	Percentage%
Sex	Female	12	40%
	male	18	60%
marital status	married	19	63.33%
	single	11	36.66%
Age	20-30 years	5	16.66%
	30-50 years	18	60%
	>50 years	7	23.33%
Education	Illiterate	6	20%
	Primary	21	71%
	Secondary	2	6.66%
	College	1	3.33%
Role in milk handling	Milker	9	30%
	Processor	3	10%
	Transporter	10	33.33%
	Seller	8	26.66%
Awareness on milk borne illness	Yes	11	36.66
	No	19	63.33
Awareness on E.coli food poisoning	yes	10	33.33
	no	20	66.66
Acquiring illness	Yes	23	76.66
	No	7	23.33
Form of milk consumption	Raw	6	20
	Ergo	7	23.33
	Ayib	5	16.66
	Boiled	12	40
Milk consumption habit	Yes	21	70
	No	9	30
Milk storage	Refrigerator (+40c)	7	23.33
	room temperature	23	76.66

With respect to knowledge of dairy cow owner, 80% participants agree as cleanliness of hands is important for safe milk handling; 86.6% of lactating cow owners agree utensils used for milk handling should be washed thoroughly after each use. 90% respondents agree as milk should be stored at a cool temperature to prevent spoilage. 25(83.33%) of respondents agree as pasteurization kills harmful bacteria in milk, 73.33% of respondents agree as Animals with visible signs of illness should not be milked for human consumption. 63.33% of participants agree to the statements of Flies and other insects can contaminate milk. 66.66% of respondents agree the use of clean water is crucial in all stages of milk handling; 90% of cow owners agree Proper disposal of waste milk and cleaning materials is important. 76.66% of respondents agree to Personal hygiene, such as wearing clean clothing, is important when handling milk. Consistently, 83.33% of participants agree to Hand washing with soap and water is sufficient, even without using sanitizers(Table 9).

Table 9. Knowledge of Dairy cow owners with regards to public health (n=30)

Factors	Agree%	Disagree%	Unsure%
Cleanliness of hands is important for safe milk handling.	24 (80%)	1 (3.33%)	5(16.6%)
Utensils used for milk handling should be washed thoroughly after each use	26(86.6%)	0(0%)	4(13.33%)
Milk should be stored at a cool temperature to prevent spoilage	27(90%)	1(3.33%)	2(6.66%)
Pasteurization kills harmful bacteria in milk	25(83.33%)	2(6.66%)	3(10%)
Animals with visible signs of illness should not be milked for human consumption	22(73.33%)	3(10%)	5 (16.66%)
Flies and other insects can contaminate milk.	19(63.33%)	6(20%)	5(16.66%)
The use of clean water is crucial in all stages of milk handling.	20(66.66%)	6(20%)	4(13.33%)
Proper disposal of waste milk and cleaning materials is important.	27(90%)	0(0)	3(10%)
Personal hygiene, such as wearing clean clothing, is important when handling milk.	23(76.66%)	3(10)	4(13.33)
Hand washing with soap and water is sufficient, even without using sanitizers.	25(83.33)	3(10)	2(6.66)

With regards to attitudes of Lactating cow respondents, 85% of participant respondents agree as hygienic milk handling practices are important for public health, 90% confident to handle milk hygienically; while70% of respondents dis agree to statement- adhering to hygienic practices is time- consuming and un necessary. 90% of dairy cows' owners respondents agree as Training on hygienic milk handling practices is beneficial. Consistently, 95% of respondents agree as implementing hygienic practices increases the quality of milk (Table 10).

Table 10. Attitudes of the Dairy cattle owners in line with Public Health (N=20)

Statements	Agree %	Dis agree %	not sure %
Hygienic milk handling practices are important for public health.	17(85)	0(0)	3(15)
I am confident in my ability to handle milk hygienically.	18(90)	0(0)	2(10
Adhering to hygienic practices is time-consuming and unnecessary.	5(25)	14(70)	1(5)
Training on hygienic milk handling practices is beneficial.	18(90)	0(0	2(10)
Implementing hygienic practices increases the quality of milk.	19(95)	0(0)	1(5)

With regards to Dairy cow owners' handling and Hygienic practices, higher (55%) of the Dairy cow owners wash hand with soap and water before handling milk, followed by 40% respondents use hand sanitizers and 5% donot wash hands regularly before handling milk. 55% of participants wash utensils with soap and water after each use, while (35%) Use a disinfectant on utensils and (10%) Rinse utensils with water only. Majority of (55%) store milk in a refrigerator (+4^{0}c; followed by 40% milk at room temperature and (5%)Use other cooling methods. With respect to animal health, 35% of participant respondents, only milk healthy animals whereas 55% Isolates sick animals before milking followed by (10%) milk all animals regardless of their health status,

Dominant (65%) participant livestock owners dispose of waste milk properly, and 35% dispose of waste milk with other garbage. Similarly, 100% of lactating cow owners, wear clean clothes when handling milk (Table 11).

Table 11: Practices of Dairy cattle owners with respect to Public health (n=20)

Factor	Categories	Freq.	response rate%
Hand Hygiene	Wash hand with soap and water before handling milk	11	55
	Use hand sanitizer.	8	40
	Don't wash my hands regularly before handling milk.	1	5
Utensil Cleaning	Wash utensils with soap and water after each use	11	55
	Use a disinfectant on utensils.	7	35
	Rinse utensils with water only.	2	10
Milk Storage	Store milk in a refrigerator(+4^{0}c	11	55
	Store milk at room temperature.	8	40
	Use other cooling methods	1	5
Animal Health	Only milk healthy animals	7	35
	Milk all animals regardless of their health status	2	10
	Isolate sick animals before milking.	11	55
Waste Disposal	Dispose of waste milk properly	13	65
	Dispose of waste milk with other garbage	7	35
Clothing	Wear clean clothes when handling milk.	18	100
	Wear the same clothes I wear for other activities	0	0

4. DISCUSSION

4.1 Prevalence of E.coliO157

In the present study, out of (N=384) lactating cows' raw milk samples collected and processed by bacteriologically method, 58/384(15.10%) and 22/384 (5.72%) *E.coli* and ***E.coli O157:H7*** prevalence were identified respectively, which was significant (P<0.05). Higher pathogenic E.coli (12.90%) was recorded in milkers' hand swab followed by milk sample (6.06%), milk container swab (5.2%) and udder swab (1.85%). The present finding were agreed with the previous findings of Solomon *et al.,* 2020) in Sebta town (9.9%). As compared to the present findings, higher report were reported by Gebremedhin, (2018) in mekelle (25%); Edilu *et al*, (2022) in West Shewa (33.8%); Melaku *et al*., (2013) in Haramaya (30.97%); Rundasa *et al*., (2019) in Bishoftu 42%; Melese *et al*., (2016) in Jigjiga (58%); Adem *et al*., (2016) in Haramaya (43.2%).

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, Methyl red and Indole test of the *E. coli* isolates were positive but the Voges-Proskauer 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.

4.2 E.coli O157:H7 infection Associated Risk factors

With regards to E. coliO157:H7 associated risk factors, 5.72% of the samples were contaminated with pathogenic E.coli O157:H7. In this findings, the prevalence of E.coli O157 infection was significantly influenced by age categories (P >0.05). Higher (7.40%) pathogenic *E.coli* infection with age categories was recorded with greater than 7 years age; followed by (5.53%) in 4-7 years age and in 3 years 0(0%) which was non- significant (P>0.05). Similar result was reported by Shimelis (2014) in Selale /Fitche). 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 (6.74%) pathogenic E.coli infection was recorded in cross breeds followed by 3.42% 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 late lactation 8% as compared to early 7.2% ; mid 4.87% and mid lactation stage 3.52% was non- significant (p>0.05). The finding of higher infection in cows in late lactation stage followed by early, dry, and mid lactation stages in the study concurs with previous reports. In cows most new infections occur during the late 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 pathogenic *E.coli* isolates were significantly different among parity groups. Early birth of cows with 1-3 parity has 5.71% of infection followed by cow with 4-6 parity or frequency of birth which was (4.87%) of infection and highest in greater than 6 birth rate (20%) which was 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 a*l., (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 a*l., 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 7.52% prevalent while 3.16% was registered in concrete floor which has non- significant (p>0.07). 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 0.56 times more likely to be harbor pathogenic 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 hygiene practice (p=0.002). Cows at farms with poor milking hygiene standard are severely affected (10.42%) than those with good milking hygiene practices (2.92%). 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 serous 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 awarness about milk borne disease among farmers. Though the results showed relatively a lower percentage of raw milk consumption, still these individuals are at a a greater risk of contracting food born intoxication infection than those who donot 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 a statistically non- significant ($P > 0.05$) association 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).

4.3 Antimicrobial Sensitivity test result

The present study showed that the resistance of *E.coli* to Penicillin G (95%), Oxacillin (82%), ciprofloxacillin(72%), chloramphenicol (63.63%), (39.13%) streptomycin; and (31.81%) tetracycline observed in milk samples. Comparably E.coli isolate resistance result was reported by Igbinosa *et al*., (2021) in Benin city, Nigeria, which revealed 100% penicillin G and ampicillin; 94.5% chloramphenicol, 89.5 % erythromycin, 78.9% Oxytetracycline and sulfamethoxazole.

In the present findings, 86.36% of Gentamycin was sensitive to E.coli infection followed by Tetracycline (63.63%), streptomycin (50%), (27.3%) ciprofloxacin, 36.36% chloramphenicol and (18.2%) Oxacillin. This finding was in line with the findings of Igbinosa *et al*., (2021) in Benin city, Nigeria, reporting E.coli isolates were100% 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 21 (95.45%) isolates of E.coli, 10 isolates (35.71%) were resistance to three classes of antimicrobials: four isolates= (oxacillin, ciprofloxacilin, penicillin), three isolates for (chloramphenicol, penicillin, and oxacillin) and one isolates for : (chloramphenicol, penicillin, tetracycline) ; (streptomycin, penicillin, oxacillin) and also (oxacillin, ciprofloxacillin, chloramphenicol) and one isolates four classes of drugs, 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, 50% Multi drug resistance was observed in three and above classes of drugs. The test isolates were resistant to chloramphenicol, penicillin, streptomycin, oxacillin and ciprofloxacillin). 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., 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*.

(2014) 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.

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 0157: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 Igbinosa *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 Nejo district were unsatisfactory. Higher 16.39% pathogenic *E.coli* contamination rate was detected in milkers' hand swab followed by 7.5% (milk samples); (5.92%) container swab; and 3.70% in udder swab, of milk marketing channels, which was significantly associated P<0.05). The overall prevalence of pathogenic *E.coli* from the milk production channels were 7.29%. This indicates that pathogenic *E.coli* is one of the major problems of dairy cows in milk production that contaminated and reduced the quality of milk. Besides, all pathogenic *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 and sorbitol mac conkey (non-sorbitol fermenting E.coli=colorless=pale colony). Consistently, sample source, udder washing, parity, milking hygiene, teat lesion, and drainage system were E.coli associated risk factor in the milk production channels were significant (P<0.05) whereas previous udder infection, body condition, breed, age, floor type, and herd size were not significantly associated (P>0.05).

Besides, 96% of penicillin G, followed by 82% Oxacillin; 71% chloramphenicol and ciprofloxacillin; and 42.5% streptomycin were resistance whereas 82% of Gentamycin, 64% Tetracycline, 46% streptomycin, and 28.6% chloramphenicol and ciprofloxacillin were sensitive to pathogenic E.coli isolates. Similarly, 35.71% of the tested isolates were revealed multi drug resistance to three classes of antibiotic discs, 3.57% of isolates for four 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 pathogenic E.coli quality and safety of raw milk was unsatisfactory. These findings stress the need for an integrated control ofpathogenic 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 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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