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Research Article

Isolation, identification and antimicrobial susceptibility pattern of *Salmonella, E. coli, and S. aureus* from selected dairy farms in Bedele and Nekemte Districts, Western Ethiopia

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Abstract

Background: Bacterial diseases transmitted through food pose a serious threat to human and animal health. *Salmonella, E. coli,* and *S. aureus* are among the major foodborne pathogens. It is becoming a worldwide problem to date. In this regard, there is a lack of information among farms in western Ethiopia. Therefore, the study was conducted to isolate, identify, and assess the antimicrobial susceptibility profile of *Salmonella, E. coli,* and *S. aureus* from selected dairy farms in the study area.

Methodology: A cross-sectional study was done from December 2018 to June 2019 on small-scale dairy farms from Bedele and Nekemte town, Western Ethiopia with the objective of isolating and identifying *Salmonella, E. coli,* and *S. aureus* from lactating cows, milkers' and milking equipment at farms and to determine the antimicrobial susceptibility of the isolates. A total of 383 samples consisting of cow milk, feces, cow nasal swabs, milkers' hand swabs, milkers nasal swabs, bucket swabs, and floor swabs were collected from 20 dairy farms. The samples were examined for the presence of *Salmonella, E. coli,* and *S. aureus* following standard techniques and procedures. The agar disc diffusion method was used for the antimicrobial susceptibility testing.

Results: The overall occurrence of *Salmonella, E. coli,* and *S. aureus* was 2.35%, 11.75%, and 2.35% respectively. Out of the 9 *Salmonella* isolates, 5(4.95%), 3(2.97%), and 1(5%) were isolated from udder milk, rectal feces, and floor swab respectively. *S. aureus* isolate was highest in udder milk 3(2.97%), followed by cow nasal swab 2(1.98%), feces 1(0.99%), bucket swab 1(5%), floor swab 1(5%) and milker's nasal swab 1(5%). *E. coli* was highest in milk sample 19(18.81%), followed by fecal samples 16(15.84%), bucket swab 5(25%), floor Swab 4(20%) and 1(0.99%) in cow nasal swab. All *Salmonella* isolates were 100% sensitive to nalidixic acid, however 55.55%, 22.22%, and 11.11, respectively, were resistant to cefoxitin, tetracycline, and gentamycin. Tetracycline, cefoxitin, and streptomycin resistance were observed in 33.33%, 9.52%, and 19.05% of *E. coli* isolates, respectively. On the other hand, all isolates were 100% sensitive to nitrofurantoin, ceftriaxone, nalidixic acid, and ciprofloxacin. The antibiotic susceptibility profiles of *S. aureus* showed that 55.55% and 11.11% were resistant to Penicillin G and Erythromycin, respectively and all isolates were 100% sensitive to cefoxitin, nitrofurantoin, and gentamicin.

Conclusion: The current study shows lower prevalence, lower antimicrobial resistance, and higher susceptibility for most antimicrobials.

Recommendation: Stringent control measures, such as treatment of positive cases with effective medications and preventative measures including strict hygiene standards, such as cleaning of the floor, pens, and milking equipment, as well as adequate hand washing throughout the milking process, should be adopted.

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Introduction

Food-borne disease is common in developing countries, particularly in Africa, due to inadequate food safety regulations, a weak regulatory framework, a lack of financial resources to invest in safer equipment, and a lack of knowledge of food handlers [1]. Changes in eating habits, mass catering, hazardous food storage conditions, and poor hygiene practices all contribute to the spread of food-borne illnesses. Contaminated feces, eggs, meat, milk, and milk products all contribute to the spread of zoonotic pathogens. Contaminated feces, eggs, meat, milk, and milk products all increase the risk of zoonotic pathogen transmission [2]. Foodborne diseases are a public health issue in both developed and developing countries. Over 250 different foodborne diseases have been identified. The majority of these diseases are caused by infections produced by various bacteria, viruses, and parasites. Poisonings are another type of disease caused by hazardous poisons or substances, such as poisonous mushrooms [3]. In countries where foodborne illness was investigated and documented, pathogens such as *S. aureus*, *Campylobacter*, *E. coli*, and *Salmonella* species were identified as prominent causes. These organisms were previously known to induce acute gastroenteritis and may produce a more serious septicemic disease, usually in the very young, the elderly, or immune-weakened individuals [1].

Foodborne bacterial diseases pose a significant threat to human and animal health. *Salmonella* is one of the zoonotic food-borne pathogens that may be found in food. Salmonellosis is the most prevalent foodborne bacterial disease in the world, causing severe bacterial enteric disease in both humans and animals [4]. Increasing proportion of *Salmonella* isolates currently demonstrate resistance to various antimicrobial drugs in both developing and developed countries. Salmonellosis in animals is classified into several subcategories. *Salmonella Abortus ovis* in sheep, *Choleraesuis* in pigs, *Gallinarum* in poultry, *Abortus equi* in horses, and *Dublin* in cattle are the primary *Salmonella* serovars that cause disease. These serovars are primarily responsible for abortions or acute gastroenteritis in their hosts. *Salmonella* serovars that cause disease in several animal and human hosts include *S. Typhimurium*, *S. Enteritis*, *S. Hadar*, and *S. Infantis [5]*. The use of antimicrobial agents in food animals leads to the emergence of antibiotic-resistant *Salmonellae*, which are zoonotic bacteria and are transferred to humans, typically through the food supply [6].

Escherichia coli is a common food contaminant and a good sign of fecal contamination. The presence of *E. coli* in milk products suggests the existence of enter pathogenic microorganisms that pose a risk to public health [7]. *E. coli* is one of several pathogenic bacteria that can contaminate milk and some dairy products, and it is regarded as a reliable sign of contamination by manure, soil, and contaminated water [8]. *E. coli* is a natural inhabitant of animal and human intestines, but its recovery from food may be a public health issue because of the existence of enter pathogenic and/or toxigenic strains that cause severe gastrointestinal disturbances. Other toxigenic bacteria, such as *E. coli* O157: H7, induce life-threatening syndromes [9]. *Staphylococcus aureus* is famous for developing antibiotic resistance. The development of multidrug resistance in *S. aureus* is a global issue. Because of its ability to form an exopolysaccharide capsule, *S. aureus* develops drug resistance more rapidly, and its location in the micro abscess limits drug access to infected cells [10]. Milk and dairy products have been linked to diseases associated with milk collection and normal processing conditions, which may allow bacteria in dairy cows and the dairy environment to be introduced directly into the milk. The highly nutritious milk medium, once introduced, promotes fast microbial growth. As a result, the risk of food-borne disease and intoxication from milk and dairy products is a problem [11].

Antimicrobial resistance is becoming an increasingly serious danger to global public health. In developing countries, the situation is worse because, in addition to the increased use of antibiotics and their easy availability without a prescription, inadequate sanitation surrounding premises contributes to the development of resistant strains. It is now well established that clinically significant bacteria are defined not only by a single drug resistance but also by multiple antibiotic resistance [12]. The increased use of antibiotics in the poultry, fishery, and livestock production industries to treat and prevent infections, or as growth promoters has greatly contributed to the growth in antibiotic resistance in potential food-borne pathogens (*Salmonella*, *E. coli*, *S. aureus*, *Shigella*, *Campylobacter*, and etc.) in past years. The widespread use of antibiotics in agriculture has contributed significantly to the emergence and spread of antibiotic-resistant food-borne pathogens in humans as a result of poultry and dairy product consumption [10].

Foodborne infections have been identified as a significant public health and economic hazard in both developed and developing countries. As a result, microbial food safety has developed as a major global concern for consumers, industry, researchers, and regulatory authorities. Microbial contamination is one of the primary causes of food spoilage throughout the world [13]. The World Health Organization (WHO) estimates that up to 30% of the population in developed countries suffers from food-borne diseases each year, while up to 2 million deaths are estimated in underdeveloped countries [14]. Food-borne diseases are frequent in underdeveloped nations like Ethiopia due to poor food handling and sanitation practices; lack of food safety regulations; weak regulatory systems; a lack of financial resources to invest in safer equipment; and a lack of education for food handlers [15]. In recent years, a number of studies in Ethiopia have observed the occurrence of *Escherichia coli* in foods of animal origin, primarily meat and milk [16-19]. Because of the large animals' extensive farming methods in those locations, the majority of the investigations were done in central Ethiopia. Estimation and quantification of *Escherichia coli* occurrence at the national level might assist responsible entities in the prevention and control of its occurrence in foods before they reach end consumers, hence decreasing its effect [7].

In Ethiopia, the first published antimicrobial preliminary study on AMR for several microbial agents was published in 1970. Since then, AMR reports from various antimicrobial surveillance and research have revealed the fast development and dissemination of resistant strains [20]. According to

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Sibhat, et al. [21], 18 (20.7%) of the 87 isolates of *Salmonella* serovars Newport (*n* = 14), Anatum (*n* = 3), and Eastbourne (*n* = 1) were resistant to two or more antimicrobials. S. Newport was multidrug-resistant (15.6%) and resistant to streptomycin, sulphisoxazole, and tetracycline among the antimicrobial-resistant *Salmonella* serovars [22]. *Staphylococcus aureus* isolates from bovine mastitic milk exhibit significant levels of resistance to ampicillin, penicillin, polymixin B, and streptomycin [23-26].

Due to the substantial proportion of Ethiopians who live in close proximity to their animals, there is a risk of the transfer of resistant *Staphylococcus aureus* from animal to human via milk intake. To facilitate more suitable treatment decisions, reduce morbidity and mortality related to resistant infections, and maintain antibiotic efficacy, the knowledge of AMR in a country must be summarized and synthesized. Updating national treatment recommendations requires evidence that has been appropriately summarized and synthesized [20].

Another major concern for human health today is antimicrobial resistance caused by antibiotic usage in livestock production, as well as human disease situations in developing countries. Penicillin, streptomycin, gentamycin, and oxytetracycline are the most common antibiotics used in Ethiopia to treat animal and human infections [20,27]. Despite the need for a better understanding of antibiotic use in Ethiopia, this resistance variation may be due to the indiscriminate use of antimicrobials in animal production without prescription in the animal and human health sectors, which may favor selection pressure that increased the advantage of bacteria maintaining resistance genes [27]. Ethiopia is one of the countries that continues to use antimicrobial drugs in dairy production, mostly for therapeutic purposes [20]. Unnecessary use of antimicrobial drugs in dairy production has been speculated to induce selection pressure for antimicrobial resistance; this method can be transmitted from animals to humans primarily through the food chain. Antibiotic selection pressure allows the microbe to survive as a resistant strain. Not only will resistant bacteria grow and produce resistant offspring, but they may also horizontally transfer the resistance gene to other microbes in other hosts and geographic regions [28].

Antibiotic-resistant microorganisms spread from animal to human via food. Because antibiotic-resistant bacteria can be transferred to humans through food supply owing to unsanitary and conventional milk production and processing procedures, the problem of milk safety remains a difficulty. If these safety concerns are not addressed, the high nutritional makeup and neutral pH of milk may transport numerous antibiotic-resistant foodborne bacteria [27]. As a result, antibiotic resistance linked with foodborne pathogens should be closely examined in this sector. However, there is little information on the isolation and identification of foodborne *Salmonella* species, *E. coli*, and *S. aureus*, as well as their antibiotic susceptibility pattern in western Ethiopian dairy cows. As a result, the study focused on the isolation, identification, and antibiotic susceptibility profile of selected foodborne bacterial pathogens linked to dairy cow production in western Ethiopia.

Materials and Methods

Study area

The research was carried out in the Bedele and Nekemte districts of western Ethiopia from November 2018 to May 2019. Bedele is 483 kilometers west of Addis Ababa, at latitude 08° 26'N and longitude 036° 97' E. The district's elevation spans from 1400 to 2010 meters above sea level, and it receives more than 1400mm of annual rainfall, with typical annual temperatures ranging from 12.5 to 27.5 degrees Celsius. The climate in the area is subtropical, with moderate temperatures during the day and night. At 09°55' N latitude and 036°33'E longitude [29]. Nekemte is 331 kilometers west of Addis Ababa. The district's elevation ranges from 2,088 meters above sea level, with annual rainfall ranging from 1450 to 2150 millimeters. 15 °C to 27 °C are the mean lowest and highest yearly temperatures, respectively [30]. The study area's communities rely heavily on agriculture and various dairy production systems (intensive and semi-intensive production). Cross, foreign, and indigenous cow breeds are employed on dairy farms in both locations.

Study population

The study populations are lactating dairy cows in Bedele and Nekemte districts and the study animals were apparently healthy dairy cows in small-scale dairy farms located in the selected study areas. The study population comprises exotic, crossbreeds in small-scale dairy farms in which those animals are managed semi-intensively. The farms were selected randomly based on the availability and accessibility of study animals from both districts. Accordingly, 8 farms were selected from Nekemte and 12 farms were selected from Bedele. Due to constraints of the transportation system and other resources, the number of farms selected for this study was higher in the Bedele district than in Nekemte as Bedele Regional Lab is residing in Bedele, and farms located in the Bedele are more accessible than Nekemte's one. All animals fulfilling the inclusion criteria or apparently healthy animals were considered and also it depends on farm owners' willingness. The study populations were divided according to their location of districts Bedele town and Nekemte town. Farm equipment used for milking (milk bucket), farm floor, and personnel (milkers) were part of the study.

Study design

A cross-sectional study was conducted to isolate and identify *Salmonella, E. coli*, and *S. aureus* using traditional culture and biochemical methods, as well as to assess their antibiotic susceptibility patterns. During the research period, each farm was only visited once. Udder milk, milkers' hand swabs, feces, milkers' nasal swabs, cow nasal swabs, floor swabs, and bucket swabs were among the samples obtained from each farm. Prior to sample collection, a cooperation letter was written to the livestock and fisheries resource development bureaus in the Bedele and Nekemte districts, and sampling from each dairy farm was done in partnership with animal health personnel from the bureau.

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Sampling and sample collection

During the milking processes, all samples were taken aseptically from lactating cows. The samples were coded correctly. Animals, persons, and utensils were the sources of the sample. Udder milk (n = 101), feces (n = 101), cow nasal swab (n = 101), milkers' hand swab (n = 20), milkers nasal swab (n = 20), buckets swab (n = 20), and floor swab (n = 20) were the sample types collected in quantity. A total of 383 samples were taken from the animals (n = 303), the personnel (n = 40), the farm floor (n = 20), and the milking utensils (n = 20). Dairy cow samples were taken from lactating cows that are considered to be apparently healthy. Fresh feces samples were taken straight from the rectum of apparently healthy lactating dairy cows into a sterile universal bottle using disposable gloves.

Milk samples were collected after the teats were scrubbed vigorously with a pledge of cotton moistened with 70% ethyl alcohol and the first two to three streams of milk were discarded. The nearest teats were sampled first, then toward far ones to reduce contamination. The collecting vial is held as near horizontal/inclined as possible by turning the teat to a near horizontal position. Approximately 10 ml of milk was collected aseptically from all teats in a sterile test tube. The milker's hand swabs, milker's nasal swabs, tank swabs, and buckets swabs are collected before the beginning of the milking process by using sterile cotton swabs. Then samples were immediately transported under cold chain conditions with an ice box to the Bedele Regional Veterinary Laboratory of Bacteriology laboratory for microbiological analysis. Upon arrival, samples were processed separately.

Laboratory diagnostic techniques

Isolation and identification of *E. coli:* For purification, the samples were streaked on the surface of MacConkey agar plates to differentiate lactose fermenting and non-lactose fermenting bacteria, and those lactose fermenting bacteria with a pinkish color colony were sub-cultured on the surface of Eosin Methylene Blue agar (EMB agar). The indole test, citrate test, and triple sugar iron (TSI) slant agar test were used to analyze the biochemistry of *E. coli* isolates [31].

Isolation and identification of *Salmonella*

Pre-enrichment: Because the quantity of *Salmonellae* in asymptomatic animals' feces, ambient samples, animal feed, and food is generally low, pre-enrichment mediums such as buffered peptone water are frequently used to aid isolation. This allowed tiny numbers of *Salmonellae* to proliferate and aid in reviving *Salmonellae* that had been sub-lethally harmed, which had been killed by the toxic action of selective enrichment medium [32].

Selective enrichment

Salmonella enrichment media are liquid or semi-solid agar medium with additions that allow *Salmonella* to thrive while preventing the development of other bacteria. Tetrathionate, Müller–Kauffmann broth, selenite cystine, brilliant green broth, Rappaport–Vassiliadis broth, and modified semi-solid Rappaport–Vassiliadis (MRSV) agar are examples of selective enrichment media [32].

Selective plating and colony selection

These are solid, selective agars that allow for variable degrees of differential growth. They stop bacteria other than *Salmonella* from growing and provide information on some of the most important metabolic differences, such as non-lactose fermentation and hydrogen sulphide (H_2S) generation. After 24 to 48 hours of culture at 37 °C, the findings are read. With the probable exceptions of Proteus, Pseudomonas, Citrobacter, and Hafnia, *Salmonellae* produce distinct colonies on such media that are typically recognizable from the colonies of other bacteria on the plate. *Salmonellae* that ferment lactose were identified on rare occasions, and H2S generation was inconsistent. Semi-solid motility medium was more efficient in detecting such unusual bacteria [32]. The enriched sample was combined (Vortexed) and streaked 3-mm loop full (10µl) Rappaport broth on Xylose Lysine Deoxycholate (XLD) agar overnight at 37 ˚C. (18-24 hours). Red colonies with or without a black center, as well as nearly entirely black colonies, will be picked up after 24 hours of incubation and deemed presumptive *Salmonella*. The purified *Salmonella* cultures were preserved in nutrient broth for biochemical assays and other procedures to confirm their identity [33].

Biochemical characterization of *Salmonella* isolates

Biochemical assays are used to identify *Salmonella* isolates. L-lysine decarboxylation, Indole, Methyl red, Voges proskaeur The isolates were then subjected to the following procedures to determine their biochemical activity. On TSI, urea hydrolysis and H2S generation are seen [32].

Indole test

In 2 ml of tryptone water, 0.2 ml of Kovac's reagent is introduced to a two-day-old isolate growth. The presence of a pink ring (layer) on the surface is seen as a favorable reaction. The yellow layer, on the other hand, is negative, as it is in *Salmonella [32].*

Methyl- Red (MR) test

In one ml of Methyl Red-Voges-Proskauer Broth, five to six drops of 0.02 percent Methyl Red reagent are added to a two-day growth of the isolate (MR-VP Broth). As with *Salmonella*, the appearance of a pink or bright red tint is considered positive [32].

Voges - Proskauer (VP) test

To a 5-day growth of the isolate in 5 ml of MR-VP Broth, three ml of a 5 percent solution of - naphthol in absolute ethanol and one ml of 40 percent KOH are added. A positive test is indicated by the appearance of a pink tint in the combination [32].

Urease test

Slants of urea agar are inoculated and incubated for 24 hours at 37 °C. The emergence of a pink hue in the slant indicates a

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favorable response. The emergence of a yellow tint, which is a characteristic of *Salmonella*, indicates a response [32].

Hydrogen sulphide production on TSI agar

By stabbing the butt and streaking the slope of the slant, the TSI agar slants are densely infected. For 16 hours, the tubes were incubated at 37 °C. H_2S generation (a characteristic feature of *Salmonella*) was shown by the black staining of the butt and slant, whereas acid production was indicated by the yellow color of the butt and slant [32].

Isolation and identification of *Staphylococcus* species

The process flow chart shows how *Staphylococcus aureus* was isolated and identified. Gram staining, catalase test, sugar fermentation, and coagulase tests are used to validate the presumptive identification of Staphylococci organisms [31].

Catalase test

Isolates are isolated and combined with a drop of 3 percent H_2O_2 on a clean glass slide using a sterile loop from the agar slant. Bubbles of oxygen are freed within seconds if the organism is positive, while the catalase-negative isolates do not create bubbles. *Staphylococci* are cocci that have catalase activity [31].

Mannitol Salt Agar (MSA)

The colonies that tested positive for *Staphylococcus* in the Gram-staining reaction and catalase test were streaked on mannitol salt agar plates, incubated at 37 °C for 24-48 hours, and checked for growth and changes in the medium color. Growth and a change in pH in the medium (from red to yellow hue) were used to confirm the presence of *Staphylococci*. The acidic metabolic product of mannitol is detected using the phenol red pH indicator. *S. aureus* fermentation of mannitol results in a yellow staining of the medium. After 24 hours of incubation, colonies that generate a faint or delayed yellow color are classified as *S. intermedius*, whereas colonies that fail to create any change on the medium are classified as *S. hyicus* and Coagulase-negative *Staphylococcus [31]*.

Coagulase test

In sterile tubes, the tube coagulase test is conducted. The chosen *Staphylococcus* was subcultured into brain heart infusion broth and incubated for 24 hours at 37 degrees Celsius. Then, 0.5 ml of broth culture and 0.5 ml of sterile rabbit plasma were combined in a thin sterile tube, along with a reference tube containing 0.5 ml of sterile Brain Heart Infusion broth and 0.5 ml of rabbit plasma, and incubated at 37 °C for 4 and 24 hours, with clot formation noted. When compared to the control, any coagulation of plasma is considered positive at any of the values [31].

Antimicrobial susceptibility test

The antimicrobial susceptibility test was performed according to the Clinical and Laboratory Standards Institute's agar disc diffusion technique. In brief, the bacteria are suspended in 0.85 percent sterile normal saline solution in a 0.5 Mac-Farland standardized suspension. A sterile cotton swab was dipped in the standardized bacteria suspension and then streaked uniformly across the Mueller-Hinton agar surface. The paper discs are then put on the agar surface, each impregnated with a specific concentration of antibiotics. The susceptibilities of the isolates were tested for the following antibiotic discs: Ceftriaxone (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Nalidixic acid (30µg), Streptomycin (10µg), Cefoxitin (30µg), Penicillin G (10µg), Azithromycin (30µg), Nitrofurantoin (300µg), Erythromycin (15µg) and Tetracycline (30µg) were placed at least 15 mm apart and from the edge of the plates to prevent overlapping of the inhibition zones and incubated in an inverted position at 37 °C for 24 hours. After incubation for 24 hours, clear zones of inhibition are produced by the bacterial growth and diffusion of the antibiotics and these were measured in millimeters using a caliper and interpreted as susceptible, intermediate, and resistant [34]. The antimicrobial susceptibility of *Enterobacteriaceae* and *S. aureus* to different drugs is presented in Tables 1,2.

Data analysis

Data were entered into a Microsoft Excel spreadsheet and analyzed by using the SPSS software package (SPSS 20.0 for Windows 7, SPSS Inc, Chicago, Illinois). Descriptive analysis is used to describe the result of proportion analysis. Proportion is estimated as the number of samples detected positive for *Salmonella*, *E. coli*, and *S. aureus* isolated from the total sample analyzed. Chi-square tests were done to study the association between *Salmonella*, *E. coli* and *S. aureus* isolates and variables

Table 1: Antimicrobial susceptibility test interpretive criteria for Enterobacteriaceae *[34]*.

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

Key: S: Susceptible; I: Intermediate; R: Resistance

Table 2: Antimicrobial susceptibility test interpretive criteria for *S. aureus* [34].

Antimicrobial Agent	Disk concentration	Zone Diameter: Interpretive Criteria (nearest whole millimeter)		
		S	I	R
Cefoxitin (CXT)	30 µg	≥22	---	≤21
Ciprofloxacin (CPR)	5 µg	≥21	16–20	≤15
Nitrofurantoin (NIT)	300 µg	≥17	15–16	≤14
Gentamicin (GEN)	10 µg	≥15	13–14	≤12
Azithromycin (AZM)	30 µg	≥18	14–17	≤13
Erythromycin(ERY)	15 µg	≥23	14–22	≤13
Streptomycin (S)	10 µg	≥15	12–14	≤11
Tetracycline (TET)	30 µg	≥15	12–14	≤11
Penicillin (PG)	10 µg	≥29	---	≥28

Key: S: Susceptible; I: Intermediate; R: Resistance

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considered (sampling area, sample source, and sample type). The significance level was set at 0.05.

Results

Proportion of bacteria isolated from dairy farms

A total of 383 samples originating from dairy farms were analyzed by conventional culture method for the detection of *Salmonella, E. coli,* and *S. aureus*. Bacteriological examination was conducted on all samples from selective dairy farms (udder milk, bucket swabs, milkers' hand swabs, milkers' nasal swabs, cow nasal swabs, floor swabs, and cow rectal feces). Out of the total sample analyzed 9 (2.35%) *Salmonella* were isolated from dairy farms. Of this positive result, the isolation of *Salmonella* was highest in udder milk 5 (4.95%), followed by rectal feces 3 (2.97%), and in-floor swab 1(5%). The frequency of isolation of *Salmonella* varied between sample types and ranged from 2.97% to 5%. The highest prevalence was observed in floor swabs (5%), however, the difference was statistically not significant ($p > 0.05$) (Table 3).

Out of the total samples, *S. aureus* was isolated from 9 (2.35%) samples from the dairy farms. Of these positive cases, the isolation of *S. aureus* was the highest in udder milk 3 (2.97%), followed by cow nasal swab 2 (1.98%), in feces 1 (0.99%), in bucket swab 1 (5%), in floor swab 1 (5%) and milker's nasal swab 1(5%) The frequency of isolation of *S. aureus* varied between sample types and ranged from 0.99% to 5%. The highest prevalence was observed in bucket swabs, floor swabs, and milkers' nasal swabs (5%), however, the difference was not significant ($p > 0.05$) (Table 3).

Out of all total samples collected and processed, 45 were positive for *E. coli,* with an overall prevalence was 11.75%. Of these positive cases, the isolation of *E. coli* was the highest in milk sample 19(18.81%), followed by fecal samples 16 (15.84%), in bucket swab 5 (25%), in floor swab 4 (20%) and 1 (0.99%) in cow nasal swab as presented in Table 3. The frequency of isolation of *E. coli* varied between sample types and ranged from 0.99% to 18.81%. The highest prevalence was observed in udder milk (18.81%), however, the difference was statistically not significant ($p > 0.05$) (Table 3).

Antimicrobial susceptibility profiles of isolates

All the 9 isolates of *Salmonella* were tested against 8(eight) commonly used antimicrobials. The antimicrobial susceptibility profiles of the isolates showed that the isolates were 55.5%, 22.2%, and 11.1% resistant to cefoxitin, tetracycline, and gentamycin, respectively. On the other hand, all isolates were 100% sensitive to nalidixic acid (Table 4).

From all 45 isolates of *E. coli*, only 21 isolates were randomly selected and tested against 8 commonly used antimicrobials. The antimicrobial susceptibility profiles of the isolates showed that the isolates were 33.33%, 9.52%, and 19.05% resistant to tetracycline, cefoxitin, and streptomycin respectively. On the other hand, all isolates were 100% sensitive to nitrofurantoin, ceftriaxone, nalidixic acid, and ciprofloxacin (Table 5).

All the 9 isolates of *S. aureus* were tested against 9 commonly used antimicrobials. The antibiotic susceptibility profiles of the isolates showed that the isolates were 5(55.5%) and 1(11.1%) resistant to Penicillin G and Erythromycin, respectively. On the other hand, all isolates were 100% sensitive to cefoxitin, nitrofurantoin, and gentamicin (Table 6).

Of the total isolates ($n = 9$) derived from all sources subjected to antimicrobial susceptibility testing, 11.11% ($n = 1/9*100$) of the isolates were multidrug-resistant (i.e., resistant to three or more of the antimicrobials tested). The one isolate from the milk sample was shown resistant to four antimicrobial drugs Gen, CPR, CXT, and TET (Table 7).

Table 3: Proportion of *Salmonella*, *E. coli*, and *S. aureus* species isolated from dairy farms.

Sample source	Sample of type	No of samples	*Salmonella* Positive (%)	*E. Coli* Positive (%)	*S. aureus* Positive (%)	χ2 (*p* - value)
Dairy Farms	Udder milk	101	5(4.95%)	19(18.81%)	3(2.97%)	1.08(0.78)
	Rectal feces	101	3(2.97%)	16(15.84%)	1(0.99%)	
	Bucket swab	20	-	5(25%)	1(5%)	
	Milkers' hand swab	20	-	-	-	
	Floor	20	1(5%)	4(20%)	1(5%)	
	Milkers Nasal Swab	20	-	-	1(5%)	
	Cow Nasal Swab	101	-	1(0.99%)	2(1.98%)	
Total		383	9(2.35%)	45(11.75%)	9(2.35%)	

Table 4: Antimicrobial Sensitivity test results of *Salmonella* isolates from dairy farms.

Types of antimicrobials	Disc concentration (µg)	Number of isolates tested	Resistant (%)	Intermediate (%)	Susceptible (%)
Nitrofurantoin	300	9	0 (0)	2 (22.2%)	7 (77.8%)
Tetracycline	30	9	2 (22.2%)	0 (0)	7 (77.8%)
Ciprofloxacin	5	9	1 (11.1%)	3 (33.3%)	5 (55.5%)
Ceftriaxone	5	9	0 (0)	2 (22.2%)	7 (77.8%)
Cefoxitin	30	9	5 (55.5%)	0 (0)	4 (44.4%)
Streptomycin	10	9	0 (0)	5 (55.5%)	4 (44.4%)
Nalidixic acid	30	9	0 (0)	0 (0)	9 (100%)
Gentamycin	10	9	1 (11.1%)	5 (55.5%)	3 (33.3%)

Table 5: Antimicrobial Sensitivity test results of *E. coli* isolates ($N = 21$) from dairy farms in the study area.

Types of antimicrobials	Disc concentration (µg)	Number of isolates	Resistant (%)	Intermediate (%)	Susceptible (%)
Nitrofurantoin	300	21	0(0)	0(0)	21(100%)
Tetracycline c	30	21	7(33.33%)	1(4.76%)	13(61.9%)
Ciprofloxacin	5	21	0(0)	0(0)	21(100%)
Ceftriaxone	5	21	0(0)	0(0)	21(100%)
Cefoxitin	30	21	2(9.52%)	0(0)	19(90.47%)
Streptomycin	10	21	4(19.05%)	2(9.52%)	15(71.42%)
Nalidixic acid	30	21	0(0)	0(0)	21(100%)
Gentamycin	10	21	0(0)	5(23.8%)	16(76.19%)

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Table 6: Antimicrobial Sensitivity test results of *S. aureus* isolates from dairy farms.

Types of antimicrobials	Disc concentration (µg)	Number of isolates	Resistant (%)	Intermediate (%)	Susceptible (%)
Penicillin G	10	9	5(55.5%)	0(0)	4 (44.4%)
Azithromycin	30	9	0(0)	1 (11.1%)	8 (88.9%)
Erythromycin	15	9	1 (11.1%)	1 (11.1%)	7 (77.8%)
Ciprofloxacin	5	9	0 (0)	2 (22.22%)	7 (77.8%)
Cefoxitin	5	9	0 (0)	0 (0)	9 (100%)
Tetracycline	30	9	0 (0)	6 (66.67%)	3 (33.3%)
Nitrofurantoin	300	9	0 (0)	0 (0)	9 (100%)
Streptomycin	10	9	0 (0)	1 (11.11%)	8 (88.9%)
Gentamycin	10	9	0 (0)	0 (0)	9 (100%)

Table 7: Multiple drug resistance profile of *E. coli, S. aureus*, and *Salmonella* isolates.

No. of drugs showing resistance	Frequency	No. and% of isolates			Total%
		E. coli	*S. aureus*	*Salmonella*	
1	12	5(23.8%)	4(44.44%)	3(33.33%)	30.8%
2	6	4(19.05%)	1(11.11%)	1(11.11%)	15.38%
3	0	0(0)	0(0)	0(0)	0(0)
4	1	0(0)	0(0)	1(11.11%)	11.11%
Total	19	9(42.9%)	5(55.56%)	5(55.56%)	48.84%
% with MDR	1	0(0)	0(0)	1(11.11%)	11.11%

Key: No: Number; MDR: Multidrug Resistance

Discussion

Infection with *Salmonella*, *E. coli*, and *S. aureus* in dairy cattle is still a big issue across the world. Significant economic losses were incurred as a result of infected animals' mortality and poor growth, as well as the danger of transmission to people via the food chain or direct animal contact. As a result, detecting animals contacting humans and equipment is critical for controlling *Salmonella*, *E. coli*, and *S. aureus* on-farm and their spread to the general public [2,35,36].

The frequency of *Salmonella* in apparently healthy lactating dairy cows (udder milk & fecal samples) in this research (7.9%) is lower than in a similar report from Gondar town (12.5%) [37]. Even if the finding is lower, lactating cows are potential sources of *Salmonella* infection for dairy farm workers and the general public. The current investigation found a fecal *Salmonella* prevalence of 3.97% among lactating dairy cattle, which is lower than the fecal *Salmonella* isolation rate of 7.7% in lactation cows and in contact people in Addis Ababa dairy farms [38,39].

The current result also agrees with a report of a 1.56% prevalence of *Salmonella* fecal shedding in dairy cattle in Egypt by Mohamed, et al. [40] and 0.7 percent in dairy cows in the United States (USA) by Callaway, et al. [41] The current investigation also demonstrated that the variation in the volume and relative occurrence of *Salmonella* isolates between the current and previous studies in different locations of Ethiopia might be related to differences in risk variables that lead to *Salmonella* occurrence. These are host-related risk variables such as age, breed, animal physiological condition, feeding techniques, and immunization status [36,42]. Environmental risk factors include hygiene and management practices; stocking density; feed type and volume; readily available water sources; use of contaminated utensils; and housing [43].

Although a relatively low proportion of *Salmonella* (2.35%) was isolated and identified in the current study compared to previous studies, this could pose significant health risks to humans and animal species by provoking salmonellosis in high-risk groups such as newborns, infants, the elderly, and immune-compromised individuals who are susceptible to *Salmonella* infections at a lower infective dose than healthy adults. As a result, it is a source of *Salmonella* infection through the eating of infected dairy products, which is particularly significant in Ethiopia, where dairy products are regularly ingested without appropriate boiling.

In Ethiopia, *E. coli* is regarded as a major threat to dairy development and public health. *Escherichia coli* is regarded as not only a sign of fecal contamination but also of inadequate hygiene and sanitary practices during milking and subsequent handling. This study also suggests that *E. coli* is the most significant dairy development obstacle in the study area. The frequency of *E. coli* in lactating cows, milkers, and dairy farm environments was reported to be 11.75% in the Bedele and Nekemte districts. This research is lower than the findings of Mohanty, et al. which found (21%) from India [44].

The current finding was in agreement with the findings of Lye, et al. [45] and Addo, et al. [46], who reported 8.75% and 11.2%, respectively, from Malaysia and Ghana. The present finding, however, is higher than the one published by Bedasa, et al. [47], who reported a prevalence of 3.5% in food of animal origin in Bishoftu, Central Ethiopia. This difference in findings between the current study and previous investigations might be related to differences in environmental conditions, management, and hygiene standards. This study was also undertaken to determine the frequency of *Staphylococcus aureus* in dairy farms, as well as their antibiotic susceptibility. The capacity of *Staphylococcus aureus* to appear in a variety of environments may disclose their resilience properties. Bucket swabs (5%), udder milk (2.97%), fecal (0.99%), floor swabs (5%), and cow nasal swabs (1.98%) were among the samples collected in diary settings. The results of the current investigation were consistent with those reported by Bitew, et al. [48] in Bahir Dra, 3.9 percent in Adama by Abera, et al. [49], and 6.25 percent in Egypt by Thaker, et al. [50].

The current finding was lower than the report of Abebe, et al. (15.5%) [51] and Reta, et al. (24.2%) [52] on *S. aureus* in raw milk samples in Ethiopia. El-Gedawy, et al. [53] found 51% and 17% in Egypt, and 52% and 18.2% in Turkey, according to Ekici, et al. [54]. This variation in *S. aureus* prevalence between reports could be attributed to differences in farm management practices, study methods, and agro-climatic conditions, as well as the fact that milk always contains microorganisms derived from the milk ducts in the udder, even when drawn under aseptic conditions. Furthermore, pollutants from milking tools, human handlers, dirty ambient conditions, and improper udder preparation may cause bacterial contamination of raw milk.

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Antimicrobial use in animal production systems has long been suspected to be a cause of the emergence and dissemination of antimicrobial-resistant pathogens. Improper use of antimicrobials in both human and veterinary medicine has contributed to the development and dissemination of antimicrobial-resistant pathogens [36,38]. In the current study, resistance to three or more antimicrobials was observed only on one *Salmonella* isolate resistant to gentamycin, ciprofloxacin, cefoxitin, and tetracycline. This is lower than studies conducted in Ethiopia by Addis, et al. (10.7%) [38] and Tadesse and Dabassa (80% to Nalidixic acid, 35% each to Tetracycline and Kanamycin and 30% to Amikacin Gentamycin, 25% each to Chloramphenicol and Streptomycin and 5% to Ciprofloxacin) [55] and Fadlalla, et al. (46.8% to 10 antimicrobial drugs) [56] in Sudan. A study in Alexandria Egypt by Mohamed, et al. [40] reported that 85.7% of *Salmonella* species isolated from dairy cattle were sensitive to tetracycline. This result agrees with the current study in which 77.78% of the isolates were sensitive to tetracycline. This study also reported that 100% of *Salmonella* species isolated from dairy cattle were sensitive to nalidixic acid. This finding also disagreed with a report in Sudan by Fadlalla, et al. [56] in which *Salmonella* isolates from humans and cattle were 100% susceptible to ciprofloxacin whereas our current study found 55.5% of the tested *Salmonella* was susceptible to ciprofloxacin.

In the current study, all *E. coli* isolates were found to be 100% susceptible to nitrofurantoin, ceftriaxone, nalidixic acid and ciprofloxacin followed by 90.47%, 76.19%, 71.42%, and 61.9% of the isolates were susceptible to cefoxitin, gentamicin, streptomycin and tetracycline, respectively (Table 5). The current study is relatively in line with findings reported by Reuben and Owuna [57] who reported all *E. coli* isolates were 89.5% of the isolates were susceptible to gentamicin from Nigeria. Bagre, et al. [58] found all *E. coli* isolates were 100% susceptible to gentamicin, from Burkina Faso. Salehi and Bonab [59] also reported all *E. coli* isolates were 100% susceptible to gentamicin from Iran. Similar studies conducted in Ethiopia by Tesfaye, et al. [60] and in Nigeria by Wariso, et al. [61] found similar susceptibility rates to the current study. High sensitivity to ciprofloxacin, gentamicin, and nitrofurantoin, has been recorded from previous studies conducted in India [621]. In this study, nitrofurantoin, ceftriaxone, nalidixic acid, and ciprofloxacin were found to be the most effective antimicrobials against *E. coli* isolates. The overall resistance of *E. coli* isolates in this study was low this indicates antimicrobial drug usage in the study area may be lower than in the other areas, due to the agro-climatic condition of the areas.

Table 6 summarizes the antimicrobial resistance profile of *S. aureus* isolates from cow milk, milk-related equipment, and farm floor swab samples. As a result, all *S. aureus* isolates in this investigation were completely resistant to cefoxitin, nitrofurantoin, and gentamicin. According to some investigators, the increasing incidence of penicillin G-resistant isolates might be linked to extended, inappropriate, and indiscriminate use [55]. The resistance pattern of *S. aureus* to penicillin G and erythromycin was found to be 55.6% and 11.1%, respectively, in the current study, which is lower than the findings of Sori, et al. (87.2%) [63] in Ethiopia and Landin (80%) [64] in Sweden, but strongly agrees with Gooraninejad. *et al* .(57%) in Iran [65].

This study also disagreed with reports from other researchers indicating *S. aureus* isolates were resistant to ciprofloxacin (90.9%), ceftriaxone (63.6%), and Penicillin-G (81.8%). A similar result was most frequently observed for penicillin (100%) followed by erythromycin (Wang. et al. (95.7%) [66] in China, and penicillin G by Beyene (100%) [67] in Ethiopia, whereas the percent were found higher when compared to report by Hamid, et al. [68] of 94.4 and 50% resistance for penicillin and ceftriaxone, respectively of *S. aureus* isolates with particular emphasis on penicillin G. The possible explanations for the high record of most drug Susceptible *S. aureus* in dairy farms may be due to the limited use of antibiotics in dairy farms of the study area.

Conclusions and Recommendations

Food-borne bacterial infections, such as *Salmonella*, *E. coli*, and *S. aureus*, are the most common causes of disease in humans and animals when contaminated foods are consumed, particularly dairy products. Infected milk had a higher prevalence of *E. coli* isolates than *Salmonella* and *S. aureus* isolates. *E. coli* isolates were shown to be extremely sensitive to nitrofurantoin, ceftriaxone, nalidixic acid, and ciprofloxacin whereas just a few isolates were resistant to tetracycline and streptomycin based on antimicrobial susceptibility patterns. When compared to previous research in other places, there was a reduced incidence of *S. aureus*.

S. aureus was 100% sensitive to cefoxitin, nitrofurantoin, and gentamicin. The occurrence of *Salmonella* at the dairy farm level showed that dairy cattle and their environment are important sources of contamination. Nalidixic acid could be considered a first-choice drug as the isolates of *Salmonella* were susceptible to this drug. Even if, the current finding shows that lower prevalence and lower antimicrobial resistance, it is better to give attention to further improvement of hygienic measures as well as antimicrobial drug usage of dairy farms, to safeguard the public from the risk of *S. aureus*, *E. coli*, and *Salmonella* pathogens which are causing food poisoning and acquiring multi-drug resistant isolates in study area. Because many people regularly drink raw milk with no further heat processing, it is a critical public health concern because milk is a vehicle for food-borne diseases.

Based on the abovementioned conclusion, the following recommendations should be considered: Everyone involved in the milk and dairy production chain should be certified in sanitary procedures to ensure raw milk quality.

- To protect customers from zoonotic infections, thorough animal and environmental hygiene should be followed in order to break the bacteria's continued transmission in farms
- Physicians in the area should consider nitrofurantoin, ceftriaxone, nalidixic acid, and ciprofloxacin as the first

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choice of drugs in the treatment of clinical diseases associated with *E. coli* also cefoxitin, nitrofurantoin, and gentamicin for *S. aureus* and nalidixic acid for *Salmonella.*

- In order to prevent antimicrobial resistance in the study area physicians as well as veterinarians should create awareness among dairy farm owners about antimicrobial resistance occurrence and its effect.

Availability of data and materials

On reasonable request, the corresponding author made the data sets utilized in the current study accessible.

Ethical clearance

Animal study ethical clearance was obtained from Addis Ababa University College of Veterinary Medicine, with reference number VM/ERC/01/06/10/2018. All of the animal owners and attendants were informed about the purpose of the study, as well as the significance and benefit of the research in terms of immediate and future values. While collecting samples, safe handling protocols were followed.

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Author contributions

All authors made significant contributions to the conception and design, data acquisition, data analysis, and data interpretation; participated in the drafting of the article or critically revised it for important intellectual content; agreed to publish in the current journal; and gave final approval of the version to be published.

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