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Antimicrobial Resistance Patterns of Bacteria Isolated and Identified from Cow's Milk at Different Sampling Points in Jimma Town, South-Western Ethiopia

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Abstract

Milk and milk products play an important role in human nutrition throughout the world including Ethiopia. However, bacteriological milk quality is potentially affected during its production and processing level, until consumption. The aim of this study was to isolate and identify bacteria using bacteriological parameters from fresh cows' milk at different sampling points and determine antimicrobial resistance patterns of selected bacterial pathogens in fresh milk samples, in Jimma town. A total of 348 milk samples from udder, milking bucket, storage containers and vendors were randomly collected. *Staphylococcus aureus (47.5%), Staphylococcus intermidus (28.75%), Staphylococcus epidermidus (28.13%), Escherichia coli (26.9%) and Psudomonas aeruginosa (16.25%)* were the major bacterial isolates identified. The disc diffusion assay of 68 isolates against six antimicrobials revealed the highest resistance to Ampicillin (82.05%) followed by Amoxicillin (75%), Tetracycline (69%), Tylosin (47.05%), Streptomycin (32.4%), and Trimethoprim+ sulfamethoxazole (30.9%). The results of the current study indicated that the cow milk produced and distributed in the study area contaminated with different bacterial isolates and also developed resistance even with the extent to multidrug resistance that poses impact on public health. Community engagement and awareness creation on proper use of antimicrobials agents and multi-disciplinary intervention/studies are recommended as a containment of antimicrobial resistance bacteria in milk producing animal, environment and consumers.

INTRODUCTION

Milk and milk products play an important role in human nutrition throughout the world including Ethiopia. It's the first and the only food for the offspring of mammals as is almost complete food [1]. It is a balanced form of food for building and maintaining the human and animal body as well [2]. However, milk and milk products are highly susceptible to variety of microorganisms and serves as an excellent culture medium for the growth and multiplication of several microorganisms due to their complex biochemical composition and high water content [3].

The safety of dairy products with respect to food-borne diseases is a great concern around the world. This is especially true in developing countries like Ethiopia, where production of milk and various milk products takes place under unsanitary conditions and poor production practices [4-6].

Microorganism may contaminate milk at various stages of procurement, processing and distribution. This contamination could arise from the cow's udder, barn, milk collection equipment, feed, soil, faces, grass, long duration of transportation, various ingredients added to dairy products and dairy farm workers [7-10]. Bacterial contamination of milk not only reduces the nutritional quality but also consumption of such milk threatens health of the society [11]. Bacteriological safety of milk continues to be a topic of concern in the dairy industry and public health communities. In general, in order to provide safe and healthy milk products, the Hazard Analysis and Critical Control Points (HACCP) system should be implemented starting from milk collection, through processing and storage. Microbial exposure assessments are critical components of the risk analysis [12].

Ethiopia, as a developing country, faces many challenges in producing quality products that are safe for consumption. In the country, there is no standard hygienic condition followed by producers during milk production [4]. The hygienic conditions are different according to the production system, adapted practices, level of awareness, and availability of resources [13]. Hygienic quality control of milk and milk products in Ethiopia is not usually conducted on routine basis. Apart from this, door-to-door raw milk delivery in the urban and peri-urban areas is

commonly practiced with virtually no quality control at all levels [14].

In Jimma town, milk and milk products represent an important place in the nutrition of consumers as well as nutrition and income of producers [15]. Although milk and milk products represent an important place, there is paucity of information on quality of raw cow's milk in Jimma town. Therefore, this study was designed to fill this gap with the following objectives:

1. To isolate and identify major bacterial pathogens in fresh cow milk samples at different sampling points.
2. To determine antimicrobial resistance patterns of the bacterial isolates.

MATERIALS AND METHODS

Study area

The study was conducted in Jimma town of Oromia Regional State, South-Western, Ethiopia. Jimma is located at 355 km away from Addis Ababa, Ethiopian. Mixed agricultural activity, mainly coffee production and livestock production are important as well and detailed (Figure 1). Milk is produced in small dairy farms established in the city and sold to collection centers and milk retailers and/or to consumer [16].

Study population

The study animals were dairy cattle's from selected dairy farms in Jimma. The average herd size in study farms were 12 lactating cows, ranges minimum 6 to maximum 16 lactating Holstein-Friesian cross-breed cows managed under indoor and outdoor management system. The estimated daily milk yield was 7 to 9.5 liters/cow/day. The milk collection centers and vendors were used for bulk milk sampling. Milk is delivered to the nearest milk collection centers of their association twice a day. From collection centers milk sold to the local consumers, cafeterias or selling point. Jimma town has one dairy cooperative, 8 collection centers and 35 vendors. The milk collection center owners and the vendors were informed on the purpose of the study prior to sample collection through the dairy co-operative. The time of milk collection from the centers was either during the morning from 8:30 to 9:30 AM or in late afternoon from 4:00 to 5:30 PM.

Study design

A cross-sectional study was carried out. Bulk milk samples were collected from three points of milk suppliers (dairy farms, collection centers and vendors) which were expected to be the major risk areas where contamination can take place as many people may share the pooled product. Raw milk samples were taken from the udder, milking bucket, storage containers at collection center and vendor 3 times from each point.

Sampling procedures

The sampling frame of all dairy farms was obtained from Jimma town dairy producers and marketing cooperative societies. From a total of 52 small holder dairy farms, a recruitment exercise was made to identify a willing dairy farm owners, from these 25 of them were selected randomly. The randomly selected farms that supply their milk to the selected four collection centers were

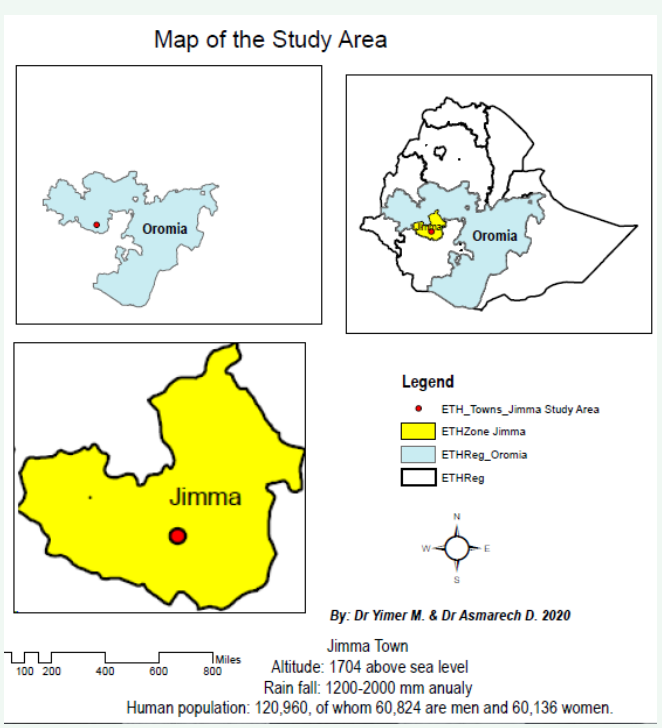

Figure 1 Geographic location of study area.

used as sources of samples for this study. Twelve vendors were randomly selected which receives milk mostly from the selected farms and also milk from various production farms. From each farm, three individuals milking cows were randomly selected at randomly selected farms and quarter milk samples were directly collected into sterile screw bottles following the protocol described in National Mastitis Council guidelines [17]. Samples were collected from all sampling points at every two month intervals for a period of 6 months. A total of 348 milk samples were collected from the various sampling points (Table 1).

BACTERIOLOGICAL PARAMETERS

Milk samples collection and transportation

Milk samples were collected from different sampling points: i.e., directly from the teat during milking, milking bucket at farm level, storage containers at milk collection center, and vendor. Before sampling of milk, the udder and teats were cleaned and dried using 68% ethyl alcohol. Then milk was collected after discarding the first 3 streams of milk. Prior to sampling from milking bucket and transport containers, milk samples in the bulk containers were agitated and samples were taken from the top of the bulk milk with a sanitized dipper. Approximately, 25 ml of bulk milk was collected aseptically in sterile plastic containers [18]. At all levels of sampling, the sampling bottles were capped, labeled with a permanent marker and placed in ice box and transported to the laboratory. The collected samples were handled aseptically in the laboratory to prevent/minimize the contamination. All samples were kept in an icebox and transported to the Mastitis and Milk Quality Laboratory of Jimma University, School of Veterinary Medicine and kept under refrigeration at 4°C until processed for microbiological analysis.

Table 1: Number of samples of raw milk collected from each sampling point.

Sampling period	Udder	Bucket	Collection center	Vendor	Total
August-October	75	25	4	12	116
November-December	75	25	4	12	116
January-February	75	25	4	12	116
Total	225	75	12	36	348

The milk samples were cultured within 24 hours as described by [19].

Bacterial isolation and identification

A loopful of the milk sample was streaked onto blood agar base enriched with 7% heparinzed sheep blood and MacConkey agar. The plates were aerobically incubated at 37ºC and examined for bacterial growth after 24-48 hours. From culture positive plates, typical colonies were subjected to Gram's stain to study their gram reaction and cellular morphology. Similar colonies of pure culture were transferred from blood agar into nutrient agar plate to get fresh colonies. From this, a series of biochemical tests that aided final identification of various bacteria were conducted following standard methods [19].

Staphylococcus species were identified based on colonies morphology (golden yellow, white, and white to yellow), hemolysis pattern, Gram's staining (shape and cell arrangements), and fermentation of manitol and by biochemical tests like catalase test, pigment production, coagulase test, *Deoxyribonuclease* (DNase) test, and further confirmed by Polymixin susceptibility test.

Streptococcus species were identified based on Gram's staining, catalase production, hemolysis pattern, differential growth characteristics on Edward's medium, CAMP test (Christie, Atkins, and Munch-Peterson), and aesculin hydrolysis.

Coliform organisms (gram negative organisms) were identified based on the growth characteristics on MacConkey agar, Gram's stain reaction, and biochemical test includes catalase test, oxidase test, fermentation patterns from lactose, hydrogen sulfide production (H_2S), indole production, methyl red (MR), Voges-Proskauer (VP) reaction, citrate utilization, motility and acid production from sucrose and glucose.

Antimicrobial resistance pattern test

Antimicrobial resistance pattern of the isolates was performed on Mueller Hinton agar by the Kirby Bauer disk diffusion method using standard procedure of the Clinical and Laboratory Standards Institute [20]. The antimicrobials tested were, Ampicillin (AMP^{10}), Amoxicillin (AMC^{30}), Streptomycin (STP^{100}), Tetracycline (TET^{30}), Tylosin ($TYLO^{150}$) and Trimethoprim+sulfamethazole ($TR+SU^{1.25/23.75}$). The antimicrobials tested for resistance pattern in this study were, those which were proved to be often available and routinely used in the study areas for the treatment of animals. The test was performed on four isolates namely; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and Coagulase negative staphylococcus. Three to five well isolated colonies were taken with a sterile cotton swab and transferred to a tube containing 5 ml of sterile saline solution. Within 15 minutes, the saline solution was plated on Mueller-Hinton agar plates with sterile cotton swabs. The dry surface of a MH plate was inoculated by streaking the swab over the entire sterile agar surface. The streaking procedure was repeated two more times, and the plate was rotated approximately 60°C each time to ensure an even distribution of inoculum. Thereafter, six different antimicrobial discs were distributed evenly on the MH plate so that they were no closer than 24 mm from center to center. Using sterile forceps and gently pressed down with the point of a sterile forceps to ensure complete contact with the agar surface. Plates were then incubated at 37°C for 18-24 hours. After the final incubation time, the zone of inhibition around each disc was measured, with the help of a ruler, which was held on the back of the inverted petri plate and the results were interpreted as 'Resistant', 'Intermediate' and 'Susceptible' by comparing with recorded diameters of strains according to the general guidelines prepared [20].

Data management and analysis

Microsoft Excel spread sheet was used for raw data base establishment and management. Descriptive statistics such as minimum, maximum, mean, percentage, and frequency distributions were used to compute the data by using Statistical Package for Social Science [21]. The Log_{10} transformation of bacterial count was done before the analysis of bacterial counts. The analysis of variances test (ANOVA) was used to show the association between mean of bacterial counts at different sampling points, and the effect of sources on bacterial counts. The significance of differences ($p<0.05$) of the mean microbial count was evaluated with one way ANOVA. Relationship between different factors for microbial contamination in raw milk was computed against TBC and CC. For all analysis, statistical significance was established at 95% confidence interval and $p<0.05$.

RESULTS

Bacterial isolates from milk samples

From 160 milk samples, 16 major bacterial isolates were identified from udder (S1), milking bucket (S2), storage containers at collection center (S3) and vendor (S4). The major bacterial isolates were *Staphylococcus aureus* (47.5%), *Staphylococcus intermidus* (28.75%), *Staphylococcus epidermidus* (28.13%), *Escherichia coli (*26.9%), *Pseudomonas aeruginosa* (16.25%), *Enterobacter aerogenes* (9.38%) and *Streptococcus agalactiae* (7.5%) (Table 2).

Antimicrobial resistance patterns of isolates

Antimicrobial resistance patterns based on types of isolates category: Sixty eight selected bacterial isolates obtained in this study were subjected to antimicrobial resistance

Table 2: Bacteria isolates from raw milk samples at different sampling points.

Bacterial isolated	S1(n=69)	S2 (n=51)	S3 (n=12)	S4 (n=28)	Total (%) (n=160)
S. aureus	28 (17.5)	23 (14.4)	9 (5.62)	16 (10.0)	76 (47.5)
S. hyicus	10 (6.25)	5 (3.12)	2 (1.25)	4 (2.5)	21 (13.12)
S. intermidus	18 (11.25)	12 (7.5)	7 (4.38)	9 (5.62)	46 (28.75)
S. epidermidus	20 (12.5)	10 (6.25)	6 (3.75)	9 (5.62)	45 (28.13)
S. chromogenes	8 (5.0)	11 (6.87)	2 (1.25)	4 (2.5)	25 (15.6)
S. simulans	3 (1.9)	4 (2.5)	0 (0)	1(0.63)	8 (5.0)
S. agalactiae	6 (3.8)	4 (2.5)	1 (0.63)	1 (0.63)	12(7.5)
S. dysagalactiae	3 (1.88)	1 (0.63)	2 (1.3)	0 (0)	6 (3.8)
S. uberis	1 (0.63)	2 (1.3)	0 (0)	1 (0.63)	4 (2.5)
E. coli	21 (13.12)	11 (6.9)	3 (1.88)	8 (5)	43 (26.9)
P. aeruginosa	12 (7.5)	7 (4.375)	4 (2.5)	3 (1.88)	26 (16.25)
Aeromonues spp	7 (4.38)	5 (2.36)	3 (1.88)	0 (0)	15 (9.38)
E. aerogenes	4 (2.5)	5 (3.12)	3 (1.88)	3 (1.88)	15 (9.38)
Proteus mirablis	2 (1.3)	0 (0)	1 (0.63)	2 (1.3)	5 (3.13)
Proteus vulgaris	1(0.63)	0 (0)	1(0.63)	0(0)	2 (1.25)
Kleb.pneumonae	4 (2.5)	2 (1.3)	0 (0)	2 (1.3)	8 (5)

Key: S1=Udder, S2= Bucket, S3= Storage containers at collection center, and S4=Vendors, n= number of raw milk samples

test. From total 68 bacterial isolates subjected to antimicrobial resistance test, 66 (97%) of them showed resistance to one or more antimicrobials. All (100%) of *Staphylococcus aureus (S. aureus)*, (94.7%) of *Escherichia coli (E. coli)*, (100%) of Coagulase negative staphylococcus (CNS*)*, and (90%) of *Pseudomonas aeruginosa (P. aeruginosa)* isolates were found to be resistant to one or more antimicrobials. The isolates showed highest resistance to Ampicillin (82.05%) followed by Amoxicillin (75), Tetracycline (69%), Tylosin (47.05%), Streptomycin (32.3%) and Trimethoprim+sulfamethoxazole (30.9%) (Table 3).

Antimicrobial resistance patterns of different bacterial isolates

Staphylococcus aureus isolates were highly resistant to Amoxicillin (83.3%) and Ampicillin (66.7%). However, they were highly susceptible to Trimethoprim+sulfamethoxazole (91.7%) and to some degree to Tylosine (50%). Similarly *E. coli* were found to be highly resistant to Amoxicillin (84.2%) followed by tetracycline (78.9%). But *E. coli* were highly susceptible to Trimethoprim+sulfamethoxazole (73.7%) and Streptomycin (68.4%). In this study, CNS was found to be highly resistance to Ampicillin (86.7%), Amoxicillin (80%) and tetracycline (73.4%). From 10 isolates of *P. aeruginosa,* high resistance was observed primarily to Ampicillin (80%) followed by Trimethoprim (70%) and tetracycline (70%) (Table 4).

Antimicrobial resistance patterns of isolates based on sources

Staphylococcus aureus isolated from farms (n=12), from collection centers (n=4) and from vendors (n=8), all 24 (100%) showed resistance to one or more antimicrobials. No resistance *S. aureus* strain was isolated from collection centers and vendors milk for Trimethoprim-sulfamethoxazole. All isolated *Escherichia coli* from farms and venders were showed resistance to one or more antimicrobials. All 15 CNS from the three sources were showed resistance to one or more antimicrobials. Similarly *Pseudomonas aeruginosa* isolates from collection centers and from vendors showed resistant to one or more antimicrobials (Table 5).

Multi-drug resistance pattern

Multi-drug resistance (MDR) was also observed on majority of bacterial isolates. From (n= 68 bacterial isolates), (91.1%) were resistant to two or more antimicrobials. However, (97%) isolates were resistant to one and more antimicrobials. Result showed only one isolates was resistance to all antimicrobials (Table 6).

DISCUSSION

The safety of dairy products with respect to food-borne diseases is a great concern around the world in general and in developing countries like Ethiopia in particular, where the production of milk and milk products take place under unsanitary conditions and poor production practices [4,7]. Food born zoonotic disease can get into or pose risk to the consumers through consumption of unsafe cow milk. This study found out poor bacteriological quality of milk and those bacterial isolates were observed highly resistant antimicrobials. In order to avoid such constraint, provision of milk and milk products of good hygienic quality is desirable from consumer health point of view [22].

In this current study, 160 cow milk samples collected were positive for bacteriological test, the most prevalent bacteria in raw milk were *Staphylococcus aureus, Staphylococcus epidermidus* (CNS), *Staphylococcus intermidus*, *Escherichia coli, Pseudomonas aeruginosa*, *Enterobacte aerogenes*, *Aromenous spps*, *streptococcus agalactiae* and *streptococcus dysagalactia*e.

Table 3: Overall resistance patterns of isolates to different antimicrobials (n=68).

Number (%) of resistant isolates
Isolates TET[30] AMP[10] AMC[30] TYLO[150] STP[100] TR+SU[1.25/23.75] R≥1 drugs
S. aureus (n=24)14 (58.4) 24 (100) 20(83.3) 10 (41.7) 11 (45.8) 2 (8.3) 24 (100)
E. coli (n=19) 15 (78.9) 12 (63.15) 16(84.2) 11 (57.9) 4 (21) 5 (26.3) 18 (94.7)
CNS (n=15) 11 (73.3) 13 (86.7) 12 (80) 9 (60) 3 (20) 7 (46.7) 15 (100)
P. aero (n=10) 7 (70) 8 (80) 3 (30) 2 (20) 4 (40) 7 (70) 9 (90) Total (n=68) 47 (69.1) 49 (82.05) 51 (75) 32 (47.05) 22 (32.4) 21 (30.9) 66 (97)
Key: AMP: Ampicillin, AMC: Amoxicillin, TYLO: Tylosin, STP: Streptomycin, TET: Tetracycline, and TR+SU: Trimethoprim+sulfamethoxazole, R≥1: Resistance to 1 and more drug

Table 4: Antimicrobial resistance patterns of bacterial isolates to different antimicrobials (n=68) (%).

Isolates Ampicillin Amoxicillin Tylosin Streptomycin Tetracycline Trimethoprim Resistant
(AMP[10]) (A MC[30]) (TYLO[150]) (STP[100]) (TET[30]) TR+SU [1.25/23.75] R ≥1
R I S R I S R I S R I S R I S R I S
S. aureus24 0 0 20 0 4 10 2 12 11 4 9 14 0 10 2 0 22 24
(n=24) (100) (0) (0) (83.3) (0) (16.7) (41.7) (8.3)(50) (45.8) (16.7) (37.5) (58.3) (0) (41.7) (8.3) (0) (91.7) 100
E. coli 12 2 5 16 0 3 11 0 8 4 2 13 15 0 4 5 0 14 18
(n=19) (63.2) (10.5) (26.3) (84.2)(0) (18.8) (57.9) (0) (42.1) (21) (10.5) (68.4) (78.9) (0) (21) (26.3) (0) (73.7) 94.7
CNS 13 2 0 12 0 3 9 0 6 3 0 12 11 0 4 7 0 8 15
(n=15) (86.7) (13) (0) (80) (0) (20) (60) (0) (40) (20) (0) (80) (73.4) (0) (26.7) (46.7) (0) (53.4) 100
P. aero 8 0 2 3 0 7 2 0 8 4 0 6 7 0 3 7 0 3 9
(n=10) (80) (0) (20) (30) (0) (70) (20) (0) (80) (40) (0) (60) (70) (0) (30) (70) 0 (30) 90
Total 49 2 18 51 0 17 22 2 34 22 6 40 47 4 17 21 0 47 66
(n=68) 72 2.9 26.5 75 0 25 32.4 29.4 50 32.4 8.8 58.8 69 5.9 25 30.9 0 69 97
Key: R=Resistant, I= Intermediate, S= Susceptible, *P. aero= Pseudomonas aeruginosa*

Table 5: Clinical breakpoints used in the Kirby Bauer disk diffusion method to test the total antimicrobial resistance of *S. aureus*, *E. coli*, CNS and *P. aeruginosa* strains isolated from milk samples sources (3 sources)

Isolates Sources Antimicrobials
AMP AMC STP TET TYLO TR+SU R ≥1
n % n % n % n % n % n % *S. aureus*
FM (n=12) 12 100 9 75 4 33.4 6 50 7 58.3 2 16.7 12 100
CC (n=4) 4 100 3 75 4 100 4 100 3 75 0 0 4 100
VD (n=8) 8 100 8 100 3 37.5 4 50 0 0 0 0 8 100
Total (n= 24) 24 100 20 83.4 11 45.8 14 58.4 10 41.7 2 8.4 24 100
E. coli FM (n=8) 6 75 7 87 3 37.5 5 62.5 8 100 3 37.5 8 100
CC (n=5) 3 60 4 80 0 0 4 80 1 20 0 0 4 80
VD (n=6) 3 50 5 83.4 1 16.7 6 100 2 33.4 2 33.4 6 100
Total (n=19) 12 63. 16 84.2 4 21 15 78.9 11 57.9 5 26.3 18 94.7
CNS FM (n=6) 5 83.4 5 83.4 0 0 5 83.4 5 83.4 3 50 6 100
CC (n=3) 3 100 3 100 0 0 1 33.4 1 33.4 1 33.4 3 100
VD (n=6) 5 83.4 4 40 3 50 5 83.4 3 50 3 50 6 100
Total (n=15) 13 86.7 12 80 3 20 11 73.4 9 60 7 46.7 15 100
P. aeruginosa FM (n=4) 3 75 1 25 0 0 2 50 0 0 3 75 3 80
CC (n=3) 3 100 2 66.7 1 33.4 3 100 1 33.4 2 66.7 3 100
VD (n=3) 2 66.7 0 0 3 100 2 66.7 1 33.4 2 66.7 3 100
Total (n=10) 8 80 3 30 4 40 7 70 2 20 7 70 9 90
Key: FM = Farm, CC= Collection center, VD= Vendor, n=number of isolates

Table 6: Multi-drug resistance patterns of isolates to antimicrobials.					
No. of Antimicrobials *S. aureus* CNS *E. coli P. aeruginosa* whole bacteria (%)					
(n=24)	(n=15)	(n= 19)	(n= 10)	(n= 68)	
AMC/AMP 24 (100)	15 (100)	18 (94.7)	9 (90)	66 (97.0)	
TET/AMC/AMP 21 (87.5)	14 (93.4)	18 (94.7)	9 (90)	62 (91.1)	
TET/TYLO/AMC/AMP 18 (75)	13 (86.7)	16 (84.2)	8 (80)	31(45.59)	
AMC/AMP/TET/TYLO 0 (0)	1 (6.67)	0 (0)	0 (0)	1 (1.47)	
Key: AMC/AMP: one and more, TET/AMC/AMP: Two and more, TET/TYLO/AMC/AMP: Three and more, and AMC/AMP/TET/TYLO: Resistance to all antimicrobials, AMC: Amoxicillin, AMP: Ampicillin, TET: Tetracycline, TYLO: Tylosin, No.: number of antimicrobials					

The isolation of *S. aureus, Streptococci*, *E. coli* and other organisms are commonly incriminated as causes of sub clinical and clinical mastitis in the cow could source from environment [23]. The isolation of *Staphylococcus* species and coliform microorganisms can cause spoilage of the milk when present in raw milk [7].

The overall prevalence of *Staphylococcus aureus* identified in the current study was (47.5%). This finding was in line with the previous reports from different parts of Ethiopia by [24] (42.5%), [25] (40%) and 41.3%, [26, 27] (41.1%). However the current result was higher than the finding of [28] (19.5%) from Addis Ababa and Adama, [29] (26.7%) from Mekele, [30] (20.3%) from Bahir Dar town, [31] (34%) from Kersa District, Jimma Zone, [32] (17.2%) from Egypt, and [33] (15.5%) and (19.6%). The possible reason of high incidence of *S. aureus* is due to the presence of sub clinical mastitis, combined with limited veterinary services, poor hygienic practices and the inability to refrigerate milk prior to consumption.

In the present study, the prevalence of *Staphylococcus aureus* (14.4%) from bucket milk and (5.62%) from collection centers which was little beat closer to the finding of [34] who reported (8%) in the bucket milk and (10%) in tanks milk samples were contaminated with *Staphylococcus aureus*. The results found from the dairy cows teat (17.5%) was comparable with the finding of [30] (20.3%) in dairy farms from Bahir Dar town, [31] (13.9%) from fresh milk directly collected from udder in Jimma town and [7] (15.8%), all from Ethiopia. But the current result was lower than the previous findings in Ethiopia as reported by [35] (21.8%) from transportation container at selling point, (25.7%) from bucket and (26.9%) from storage container at milk collection center in Hawassa. The reason for the difference in prevalence could be the in geographic region of the area sampled, sample size, storage, types of medium used for isolation, and a high proportion of these bacteria in milk relates to poor hygiene practices.

Escherichia coli is a normal inhabitant of the intestines of animals and humans and it is ubiquitous but its recovery from food may be of public health concern due to the possible presence of enteropathogenic and/or toxigenic strains which lead to sever gastro intestinal disturbance and most dangerous among them are enterohemorrhagic *E. coli* strains, especially serotype O157:H7 [36]. So the occurrence of pathogenic strains of *E. coli* in milk products, which could be dangerous for consumers and affect health in the present study. In this study, the prevalence of *E. coli* (13.12%) from udder, (6.9%) from bucket, (1.88%) from storage container and (5%) from upon arrival at selling point were identified. It was found that the presence of *Escherichia coli* in each critical point was an indicative of the fact that there was lack of proper hygienic milk handling procedures, poor source of water or lack of milking personnel hygiene, which was very much in line with the previous work [37-39] who reported that using contaminated or poor quality water, lack of milking personal hygiene, poor cleaning utensils and unhygienic food processing can be means of milk contamination with *Escherichia coli.*

Overall, 71% of the bacterial isolates identified in this particular study area belonged to coliform bacteria. Among which, *Escherichia coli* (26.9%), *Pseudomonas aeruginosa* (16.25%), *Enterobacter aerogenes* (9.25%), and *Aeromoneous spps* (9.25%) were the dominant one. These are suggestive of poor sanitary and hygienic condition of the farms and transportation system which confirms previous report by [9]. The high presence of *Escherichia coli* in the milk samples also imply that faecal contamination could have occurred and sub-clinically ill cows might have served as the causes of the microbial contamination. Traditional practices are likely to contribute to the contamination of the milk and proliferation of the micro-organisms. The implication is that there is high risk of acquiring foodborne diseases since most peoples have the habit of consuming raw or unpasteurized milk is still considered good for health. The existence of coliform bacteria may not necessary indicate a direct faecal contamination of milk, but precisely is an indicator for poor sanitary practices during milking and further handling processes. In the other way the detection of coliform bacteria and pathogens in milk indicates a possible contamination of bacteria either from the udder, milk utensils or water supply used [23].

The antimicrobial resistance patterns determination of *staphylococcus aureus* isolated from milk samples in our study showed that 100% of the organisms were resistant to one or more tested antimicrobials and 87.5% were multi drug resistant. The high resistance of *S. aureus* to Amoxicillin (83%) and Ampicillin (75%) in this study was in-line with other reports [40,41]. The resistance of *S. aureus* to Amoxicillin and Ampicillin may be attributed to the frequent use of beta-lactam groups Lactacloxa in most farms in Jimma. Other possible reason might be due to the production of beta-lactamase that inactivates penicillin and closely related antibiotics. It is believed that around 50% of mastitis causing *S. aureus* strains produce beta-lactamase [42]. The present study has demonstrated the existence of alarming levels of resistance to *S. aureus* to commonly used antimicrobial agents in the study farms due to misuse of these antibiotics or commonly given antimicrobial growth promoters. This could be similar to studies in other countries [43,44] who suggested a possible development of resistance from prolonged and

indiscriminate usage of some antimicrobials. It is therefore, very important to implement a systemic application of an *in vitro* antibiotic susceptibility test prior to the use of antibiotics in both treatment and prevention of intra-mammary infections.

The resistance of *S. aureus* to Tetracycline (58.85%) in this study was in-line with the findings in Ethiopia by [45] (51.5%) in Jimma and apart from Ethiopia, by [46] (55.9%) in Nigeria. But the current finding was tends to be lower than the findings in Ethiopia by [33] (66.7%) in Addis Ababa, [47] (73.5%) in Dessie, and [48] (97%) who reported from Egypt. But this findings was higher than the reports by [49] (30.8%) in Ethiopia, and apart from Ethiopia, [50] (30%) in India and [51] (30%) in India. The resistant development was due to the fact that tetracycline is the most commonly used antimicrobial in the treatment of infections in the livestock sector in Ethiopia. The other reason for difference in the resistance pattern of *staphylococcus* could be explained by the fact in the difference of study area and empirical use of broad-spectrum antibacterial, size of inoculums (turbidity), sample size, the test mediums, incubation conditions and the bacterium degree of resistance. The *S. aureus* resistance to streptomycin (45%) was higher than the findings of [52] (12.1%) from Switzerland, and [53] from Brazil. The difference in these finding reports might be due to different uncontrolled application culture of antimicrobial agents of various classes for therapeutic intervention of diseases in animals, and for prophylactic measures, size of inoculums of the tests, antimicrobial concentration in the disc, and study area period differences.

The antimicrobial resistance test revealed a high resistance of *E. coli* (94.7%) to one or more antimicrobials and of which resistance to Amoxicillin (84.2%), Tetracycline (78.9%) and Ampicillin (63.15%) more observed than Tylosin (57.9%), Streptomycin (21%) and Trimethoprim (6.3%). The current resistant of *E. coli* to Amoxicillin was in line with finding of [54] who reported (75%) raw milk from Burkina Faso. Resistance of *E. coli* to Ampicillin was lower than 93.5% by [35] from Awassa, Ethiopia and 73.0% by [40] from Trinidad. The resistant of *E. coil* to Tetracycline (78.9%) was more or less similar to the finding of [55] who reported (81%) from Southern Rift Valley Region of Ethiopia but lower than the reports of [56] from Tigery, Ethiopia. Furthermore, multi-drug resistance was also observed in several bacteria isolates with (91.2%) of the bacterial isolates showed multi-drug resistance patterns. This means that many of the antimicrobial agents that are used in livestock production are not suitable any more. There are several factors which might account for the observed multi-drug resistance, this include antibiotic concentration (low/high), long-term exposure, organism type, antibiotic type and host's immune status.

CONCLUSION AND RECOMMENDATIONS

- Clean milk could only be obtained if effective sanitary measures are taken starting from the point of milk withdrawn from the cow until it reaches the consumers. The results obtained in this study showed that milk available to consumer in Jimma town has poor quality. Wide variety of bacterial species were identified in milk. High bacterial loads, the presence of several pathogenic bacteria in milk samples not only affect the milk quality but also definitely pose a safety issue to consumer. The antimicrobial resistance test (antimicrobial susceptibility test) of the isolates in this study revealed that there was high level or alarming report of antimicrobial resistance even multi drug resistance were observed. This needs high attention of all stakeholders in order to reduce the ever increasing burden of AMR. Based on the above conclusion the following recommendations are forwarded:Awareness should be created along dairy cow owners, collection centers and vendors on the importance of good hygienic milk production to reduce the level of bacterial contamination using the available limited resource.
- Careful selection and prudent use of antimicrobial agent is recommended in the treatment of cows to reduce antimicrobial resistance.
- Further studies are encouraged to investigate the presence and extent of bacterial load and their antimicrobial resistance profile from milk or milk producing animal, environment and consumers to mitigate the threats of AMR through one health approach.

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