1874-2858/20

*Send Orders for Reprints to reprints@benthamscience.net*

234

The Open Microbiology Journal

Content list available at: https://openmicrobiologyjournal.com

RESEARCH ARTICLE

# Isolation and Characterization of *Staphylococcus aureus* From Food of Bovine Origin in Mekelle, Tigray, Ethiopia

Million Weldeselassie[1], Getachew Gugsa[2,*], Ashwani Kumar[3], Yisehak Tsegaye[4], Nesibu Awol[2], Meselu Ahmed[2], Nigus Abebe[4] and Habtamu Taddele[4]

[1]*Shire Agricultural, Technical Vocational and Education Training College, Tigray, Ethiopia.*
[2]*School of Veterinary Medicine, Wollo University, Amhara, Ethiopia*
[3]*College of Agriculture and Veterinary Medicine, Addis Ababa University, Addis Ababa, Ethiopia*
[4]*College of Veterinary Medicine, Mekelle University, Mekelle, Tigray, Ethiopia.*

**Abstract:**

***Background:***

Among Food-borne diseases, *S. aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food.

***Objectives:***

The study aimed to isolate and characterize *S. aureus* from raw milk, yogurt and meat and determine its antibiotic sensitivity pattern.

***Materials and Methods:***

A cross-sectional study was conducted from December 2014 to June 2015 in Mekelle. A total of 284 samples were collected purposively. Enumeration of total viable bacteria count (TVBC), bacteriological isolation and identification, antimicrobial susceptibility testing, as well as PCR amplification of *fem A* and *mec A* genes were performed. Chi-square ($\chi^2$) and one way ANOVA tests were used for analysis.

***Results:***

Overall TVBC mean was found to be $1.29x10^8$cfu/ml/g. The highest TVBC ($1.38x10^8$cfu/ml) was from the yogurt sample and the lowest ($1.26x10^8$cfu/g) was from meat. The overall prevalence of coagulase positive *S. aureus* (CoPS) was 39.1% (111) and of the smaples, 51(56.04%), 38(26.20%) and 22(45.83%) were isolated from meat, raw milk, and yogurt, respectively. There was a statistically significant difference ($p<0.05$) among the different sample types and sources in the prevalence of *S. aureus*. Almost half of the CoPS isolates were sensitive to Tetracycline, Gentamycin, and Kanamycin, but resistant to Amoxicillin (96.9%) and Penicillin G (93.8%). Moreover, 93.75% of the isolates developed multidrug resistance. All isolates carried the *fem A* gene and among these isolates, 12 (37.5%) carried *mec A* gene.

***Conclusion:***

The present study revealed that foods of bovine origin of the study area are found to be having less bacteriological quality, high prevalence of CoPS and development of drug resistance.

**Keywords:** Antimicrobial, Bacteriological, Bovine, Food, Mekelle, PCR, *S. aureus*.

| Article History | Received: January 25, 2020 | Revised: May 11, 2020 | Accepted: May 12, 2020 |
|---|---|---|---|

## 1. INTRODUCTION

To date, around 250 different food-borne diseases have been described, and bacteria are the causative agents of two-thirds of food-borne disease outbreaks [1]. Factors that contribute to outbreaks of the bacterial food-borne diseaseinclude, obtaining food from unsafe sources, contaminated raw food items, improper food storage, poor-personal hygiene during food preparation, inadequate cooling and reheating of food items and a prolonged time-lapse between preparing and consuming food items [2]. Among the bacteria predominantly involved in these diseases, *Staphylococcus aureus* (*S. aureus*) is a leading cause of gastroenteritis resulting from the

* Address correspondence to this author at the School of Veterinary Medicine, Wollo University, Amhara, Ethiopia; Tel: +251 914 73 92 42;
E-mail: gugsag@yahoo.com

consumption of contaminated food. Staphylococcal food poisoning is due to the absorption of Staphylococcal enterotoxins pre-formed in the food [3]. *S. aureus* is a ubiquitous organism frequently isolated from raw milk that is manually drawn from individual animals, bulk raw milk and naturally, from the milk of dairy cattle suffering from mastitis [4]. Similarly, meat and its products are important reservoirs for many of the food-borne pathogens including *S. aureus* [5].

Despite advances in food science and technology, food-borne diseases remain one of the major public health and economic problems all over the world [6]. Up to one-third of the population of developed countries may be affected by food-borne diseases each year. The problem is likely to be even more widespread in developing countries like Ethiopia because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment, and lack of education for food handlers [7, 8]. In Ethiopia, both food shortage and lack of appropriate food safety assurance systems are problems that have become obstacles to the country's economic development and public health safety [9, 10]. Data regarding food-borne diseases are extremely scarce at a national level and a few studies conducted in the different parts of the country showed the poor sanitary conditions of catering establishments and presence of pathogenic organisms like Campylobacter, Salmonella, *S. aureus*, *Bacillus cereus* and *Escherichia coli* [11 - 15]. Therefore, there is a pressing need to systematically investigate this recurring food-borne disease complex and to initiate appropriate intervention measures for the said problems. In the study area, only a few BiOLOG identification and biochemical characterizations of *S. aureus* from different food sources were conducted by some researchers [16, 17], and [15]. Moreover, in the study area, there is no published and/or accessible research work done on molecular characterization of *S. aureus*. Hence, the objectives of the current study were to isolate and characterize *S. aureus* from raw milk, yogurt and meat of different sources in the study area and to determine the antibiotic sensitivity pattern of the isolates.

## 2. MATERIALS AND METHODS

### 2.1. Study Area

The study was conducted from December 2014 to June 2015 in Mekelle city. Mekelle is the capital city of Tigray Regional State, situated about 783 Kms North of Addis Ababa at 38.5° East longitude and 13.5° North latitude at an altitude of 2300 above sea level. The city has a total population of 215,546 [18], 308 cafeterias, 292 restaurants, 258 supermarkets and an active urban-rural exchange of goods which has 30000 micro- and small enterprises [19]. The weather condition is hot and dry. The mean annual rainfall of the area is 628.8 mm and an annual average temperature of 21ºC [20].

### 2.2. Study Design and Study Population

A cross-sectional study on *S. aureus* was conducted from December 2014 to June 2015 on raw milk, yogurt and meat samples collected from different sources and parts of Mekelle, Tigray, Ethiopia. The study populations comprised of purposively selected slaughtered cattle and milked dairy cows found in Mekelle.

### 2.3. Sampling Technique and Sample Collection

A total of 284 samples of bovine raw milk (n=145), yogurt (n=48) and meat (n=91) were collected using a purposive sampling technique from Mekelle. These samples were collected based on the willingness of the owners. Raw milk samples were aseptically collected directly from the teats of lactating cows (n=100), whole seller (n=17), and cafeterias (n=28). Similarly the yogurt samples were collected from dairy farms (n=26) and cafeterias (n=22) using a sterile universal bottle. However, the raw meat samples were collected from abattoirs (n=55), butcher shops (n=16) and restaurants (n=20) during slaughtering and selling. Then afterward, the sections of meat samples were aseptically removed and placed in separate sterile plastic bags to prevent spilling and crosscontamination. Both samples were transported to the Veterinary Microbiology Laboratory of College of Veterinary Medicine, Mekelle University using icebox and stored at 4$^{o}$C until the performance of laboratory experiments.

### 2.4. Enumeration of Total Viable Bacteria Count

One ml of milk and yogurt or 1 g of meat samples were homogenized into 9 ml of sterile peptone water using vortex mixer and from the 10$^{-}$fold dilutions of the homogenates, 1 ml of $10^{-6}$ dilution of the homogenates were plated on standard plate count agar (HiMedia, India) for the targeted organism using pour plate method. The plates were incubated at 37°C for 48h. Then after, plates containing 25-250 colonies were counted using the illuminated colony counter. The counts for each plate were expressed as colony-forming unit of the suspension (cfu/g/ml) [21].

### 2.5. Bacteriological Isolation and Identification

Isolation of *S. aureus* was attempted according to the method described [21] with slight modification. A part of each sample (10 ml or g) was enriched in sterile peptone water (HiMedia, India) (90 ml) and was incubated at 37°C for 24 h. A loopful of the enriched sample was aseptically streaked onto 5% Sheep Blood Agar (Oxoid Ltd., Basingstoke, Hampshire, England) and the plates were incubated aerobically at 37°C and examined after 24h of incubation for growth. The colonies were provisionally identified on the basis of staining reaction with Gram's stain, cellular morphology and hemolytic pattern on blood agar. The representative colonies were sub-cultured on Manitol Salt Agar (MSA) (Oxoid, UK) and incubated at 37°C. Then the colonies that grew on MSA were sub-cultured on nutrient broth and agar media and cultures were preserved and maintained for characterizing the isolates. Growth on MSA, catalase test, oxidase test, oxidation-fermentation test, detection of hemolysis, DNase test (Spot inoculation and Line streak inoculations) and tube coagulase test were performed [21]. Bacterial strain that was used as quality control organism in this study was the standard strain of *S. aureus* American Type Culture Collection (ATCC) 25923. The samples that were considered as coagulase positive *S. aureus* (CoPS) were further characterized.

### 2.6. Polymerase Chain reaction Amplification of *femA* and *mecA* genes of *S. aureus*

The *S. aureus* genomic DNA extraction and purification were performed as per the protocol provided by Thermo Scientific, GeneJET Genomic DNA Purification Kit for Gram positive organisms and the total genomic DNA was checked by running on 1.0% agarose gel. The total genomic DNA containing tubes were stored in the fridge for use as the template DNA. All antimicrobial susceptibility tested isolates were screened for the presence of the *mec A* and *fem A* genes by multiplex PCR according to the procedure given [22] using the following specific primers: F-5'GTA GAA ATG ACT GAA CGT CCG ATA A3' and R-5'CCA ATT CCA CAT TGT TTC GGT CTA A3'- for *mec* A gene (having a band size of 310 bp) and F-5'-AAA AAA GCA CAT AAC AAG CG-3' and R-5'GAT AAA GAA GAA ACG AGC AG-3' for *fem A* gene (*S. aureus* species specific and encoding a factor responsible for methicillin resistance and having an amplicon size of 132bp). Each PCR reaction mixture (50µl) was prepared from 5µl of 10X reaction buffer, 5µl of template DNA, 1µl of each primer, 3µl of 10mM dNTP mixture, and 1µl of Taq polymerase. The remaining volume was nuclease-free deionized water. Amplification was carried out in a Tianlong PCR Thermocycler with thermal cycling conditions of an initial denaturation at 94°C for 6 min followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 30 s and extension at 72°C for 45 s, and with final extension at 72°C for 6 min. Finally PCR products were separated by running on a 1.5% (w/v) agarose gel containing 0.5 g/ml ethidium bromide. Electrophoresis was conducted in a horizontal equipment system for 55 min at 110 V using 1X TAE buffer (40 mM Tris, 1 mM EDTA and 20 mM glacial acetic acid, pH 8.0). The amplicons were visualized under UV-light gel doc and their molecular weights were estimated by comparing with100 bp DNA molecular weight marker (Solis BioDyne, Tartu, Estonia) [23 - 25].

### 2.7. Antimicrobial Susceptibility Testing

The *S. aureus* isolates were screened for *in vitro* antimicrobial susceptibility using the agar disk diffusion method according to the procedure given [26] on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, Hampshire, England). The following seven different antibiotic discs, with their concentrations given in parentheses, were used in the antibiogram testing: Gentamycin (CN)(10 µg), Polymyxin B (PB) (300 µg), Vancomycin (VA)(30 µg), Penicillin G (P)(10 µg), Amoxicillin (Amx)(2 µg), Tetracycline (TE)(30 µg) and Kanamycin (Ka)(30 µg) (Oxoid Company, Hampshire, England). After 18 to 24h of incubation, the clear zones (inhibition zones of bacterial growth around the antibiotic discs (including the discs) diameters for individual antimicrobial agents were measured and then translated into Sensitive (S), Intermediate (I), and Resistant (R) categories according to the interpretation table of the Clinical and Laboratory Standard Institute [27].

### 2.8. Data Management and Analysis

All collected data were entered into Microsoft Excel Sheet and analyzed through the Statistical Package for Social Sciences [28]. Accordingly, descriptive statistics such as percentages and frequency distribution were used to determine the prevalence and mean of the total viable count of microbial load in the food items and Chi-square ($\chi^2$) test was applied to assess the association. The mean of the total viable count of microbial load in the food items was compared with one way ANOVA.

## 3. RESULTS

### 3.1. Total Viable Bacterial Count (TVBC)

The overall total viable bacterial count mean was found to be $1.29x10^8$cfu/ml/g. The highest total viable bacterial count ($1.38x10^8$cfu/ml) was found from the yogurt sample and the lowest ($1.26x10^8$cfu/g) was from meat. The TVBC mean in meat samples collected from the butcher shop was higher followed by that from the restaurant. The highest viable bacterial count among the raw milk samples was found for the samples collected from whole sellers but in yogurt samples, the higher total viable bacterial count was from that of the cafeteria (Table **1**).

### 3.2. Prevalence and Antimicrobial Susceptibility Profile of *S. aureus*

Out of the total 284 raw milk, meat, and yogurt food samples collected from different sources and examined, 111(39.1%) were found CoPS and of these 111 isolates, 51(56.04%), 38(26.20%) and 22(45.83%) were isolated from meat, raw milk and yogurt, respectively (Table **2**).

**Table 1. Total viable bacterial count means with their sample type and site of collection.**

| Sample Type | Site of Collection | Range of Viable Count CFU/g/ml | | |
|---|---|---|---|---|
| | | Minimum | Maximum | Mean |
| Meat | Abattoir | $0.4x10^8$ | $3 x10^8$ | $1.14 x10^8$ |
| | Butchery | $0.4x10^8$ | $3 x10^8$ | $1.55x10^8$ |
| | Restaurant | $0.7x10^8$ | $3.x10^8$ | $1.28x10^8$ |
| | Total | $0.4x10^8$ | $3 x10^8$ | $1.26 x10^8$ |
| Raw milk | Farm | $0.2 x10^8$ | $2 x10^8$ | $0.726 x10^8$ |
| | Whole seller | $1 x10^8$ | $2 x10^8$ | $1.58 x10^8$ |
| | Cafeteria | $0.4x10^8$ | $2 x10^8$ | $1.51 x10^8$ |
| | Total | $0.2 x10^8$ | $2 x10^8$ | $1.30 x10^8$ |

*(Table 1) contd.....*

| Sample Type | Site of Collection | Range of Viable Count CFU/g/ml | | |
|---|---|---|---|---|
| | | Minimum | Maximum | Mean |
| Yogurt | Farm | $0.3x10^8$ | $0.6x10^8$ | $0.48x10^8$ |
| | Cafeteria | $0.6x10^8$ | $3x10^8$ | $1.54 x10^8$ |
| | Total | $0.3x10^8$ | $3x10^8$ | $1.38x10^8$ |
| | Overall total | $0.2x10^8$ | $3x10^8$ | $1.29x10^8$ |

**Table 2. Prevalence of *S. aureus* among the different sample types.**

| Sample Type | No. of Examined Samples | No. of Positive Samples | $\chi^2$ | *p-value* |
|---|---|---|---|---|
| **Meat** | 91 | 51 (56.04%) | 22 | 0.000 |
| **Raw milk** | 145 | 38 (26.20%) | | |
| **Yogurt** | 48 | 22 (45.83%) | | |
| **Total** | **284** | **111 (39.1%)** | | |

The prevalence CoPS from different sources of meat samples was found 68.75%(11), 49.09%(27), and 65%(13) from butcher shop, abattoir, and restaurant, respectively; and 17%(17), 29.4%(5) and 57.14%(16) for raw milk samples collected from farm, whole seller, and cafeteria, respectively. Similarly, the prevalence of CoPS from different sources of yogurt samples was found to be 50%(13) and 40.9%(9) from farm and cafeteria, respectively. There was a significant difference ($p < 0.05$) among the different sample types and sample sources in the prevalence of *S. aureus* (Tables **2** and **3**).

The antibiogram test results of the 32 CoPS isolates revealed that almost half of the CoPS isolates were sensitive to Tetracycline (53.1%), Gentamycin (50%), and Kanamycin (50%). But the high levels of resistance were recorded for Amoxicillin (96.9%), Penicillin G (93.8%), and Vancomycin (59.4%). Moreover, 93.75% of the isolates developed multidrug resistance (Table **4**).

Besides the antibiogram test, the PCR amplification result indicated that all antimicrobial tested CoPS isolates carried the *fem A* gene, which has a band size of 132 bp and among these isolates, 12 (37.5%) carried *mec A* gene (MRSA), which has a band size of 310 bp.

## 4. DISCUSSION

The total viable bacterial count is commonly employed to indicate the sanitary quality of foods. In the present study, the overall TVBC mean was $1.29x10^8$cfu/ml/g. The high mean value of microbial load ($1.38x10^8$cfu/ml) was found in yogurt samples. This might be due to yogurt made, which is done by natural fermentation under ambient temperature with no defined starter cultures to initiate the fermentation processes and it is also stored for long period. On the other hand, the microbial load of fermented milk samples could vary from sample to sample based on the original milk sample [29]. However, in this investigation, no statistically significant difference was recorded between cafeterias and farms. According to a study [30], in most households of Ethiopia, no attempts are made to control the fermentation process of milk and its products are manufactured under traditional systems and generally have poor quality and do not meet the acceptable quality requirements set by various regulatory agencies. The mean value of the microbial loads in meat was less than that of raw milk in the current findings but higher than that reported before [15], which showed $4.3x10^6$/g in the street meat sales in the same study area. This is due to less hygienic handling of meat and no maintenance of a hygienic environment in their working area such as butcher shops, abattoirs and in some of the abattoirs, there is no clear division of slaughtering process. According to a study [15], most of the butcher shop workers handle money while serving food. Since money is full of microbes, it can contaminate the food. Handling of foods with bare hands may also result in cross-contamination.

The current findings were consistent with the previous findings [31] which reported the presence of high mean values of microbial load in hanging meat ($5.5x10^7$) and minced meat ($6.5x10^7$), and $10^6$-$10^{10}$ cfu/g in raw meat and its environment has also been reported in retail shops in Pakistan [32]. The raw milk TVBC mean was higher than the report [33] which reported $1.0x10^6$-$1.5x10^7$ in raw milk sold in Nigeria and this exceeds the standard limit ($1x10^5$cfu/ml) recommended [34]. It is stressed that the values above this limit are indications of serious faults in product hygiene and another study [35] pointed out that bacterial count exceeding $10^5$/g in delicatessen food products is indicative of dangerous contamination.

Generally due to poorly organized farm to table production chain, poor hygienic practices in dairy farms, poor milking hygiene, poor standard sanitary operational procedures practiced by abattoir personnel, food contamination in food establishments (cafeterias, restaurant and whole sellers) are some of the risk factors which could contribute to the high bacterial load obtained in the current finding.

**Table 3. Prevalence of *S. aureus* among different collection sources and their sample types.**

| Sample Type | Site of Collection | No. of Examined Samples | No. of Positive Samples | $\chi^2$ | *p-value* |
|---|---|---|---|---|---|
| **Meat** | Abattoir | 55 | 27 (49.09%) | 40.1 | 0.000 |
| | Butchery | 16 | 11(68.75%) | | |
| | Restaurant | 20 | 13 (65%) | | |
| | **Total** | **91** | **51 (56.04%)** | | |
| **Raw milk** | Farm | 100 | 17 (17%) | 40.1 | 0.000 |
| | Whole seller | 17 | 5 (29.4%) | | |
| | Cafeteria | 28 | 16 (57.14%) | | |
| | **Total** | **145** | **38 (26.20%)** | | |

*(Table 3) contd....*

| Sample Type | Site of Collection | No. of Examined Samples | No. of Positive Samples | $\chi^2$ | *p-value* |
|---|---|---|---|---|---|
| **Yogurt** | Farm | 26 | 13(50%) | 40.1 | 0.000 |
| | Cafeteria | 22 | 9 (40.9%) | | |
| | **Total** | **48** | **22 (45.83%)** | | |

**Table 4. In vitro antimicrobial sensitivity pattern of *S. aureus* isolates.**

| Antimicrobial agents | Interpretations | | |
|---|---|---|---|
| | **Sensitive** | **Intermediate** | **Resistant** |
| **Gentamycin** | 16(50%) | 4(12.5%) | 12(37.5%) |
| **Penicillin G** | 2(6.2%) | 0(0%) | 30(93.8%) |
| **Amoxicillin** | 1(3.1%) | 0% | 31(96.9%) |
| **Vancomycin** | 13(40.9%) | 0(0%) | 19(59.4%) |
| **Tetracycline** | 17(53.1%) | 4(12.5%) | 11(34.4%) |
| **Kanamycin** | 16(50%) | 5(15.6) | 11(34.4%) |
| **Polymyxin B** | 0(0%) | 17(53.1%) | 15(46.9) |

The present study revealed that 39.1% (111/284) of the overall prevalence of CoPS and of these 111 isolates, 51(56.04%), 38(26.20%) and 22(45.83%) were isolated from meat, raw milk and yogurt, respectively. This overall prevalence of CoPS was nearly comparable with the findings of studies conducted [36] in Asella, South Eastern Ethiopia (35.71%) [17], in Northen Ethiopia (36%) [37], in and around Wolaita Sodo, Southern Ethiopia (37.14%) [38], in the region of Tirupathi, India (39.09%) [39], in Adama town, Ethiopia (42.14%), and [40] in Urban and peri-urban areas of Debre-Zeit, Ethiopia *(*44%). However, the present finding was higher than the reports [41] in Addis Ababa, Ethiopia (9%) [42], (9.1%) [43], in Addis Ababa, Ethiopia (10%) [16], in selected Districts of Tigray (9.8%) [44], in Mityana District, Uganda (10%) [45], in Zaria and Kaduna, Nigeria (12.6%) [46], in Nigeria (12.63%) [47], in South Italy (12.9%) [48], around Addis Ababa, Ethiopia (16.2%) [49], in Fars, Chahar Mahalva Bakhtiari and Ghom, provinces, Iran (17.9%) [50], in Fieri Region in Albania (18%) [51], in and around Gondar town, North Western Ethiopia (18.44%) [52], in and around Asella, Ethiopia (19.3%) [53], in South-West Uganda (20.3%) [54], in Bahir Dar town and its environs, Ethiopia (20.3%) [55], in Addis Ababa, Ethiopia (20.8%) [56], in Sebeta, Central Oromia, Ethiopia (23.4%) [57], in Bangladesh (25.53%) [58], in Adama, Ethiopia (28.1%) [59], in and around Addis Ababa, Ethiopia (28.8%) [60], in Pokhara, Nepal (29.7%) [61], in Plateau State, Nigeria (30.9%) [62], in Faizabad, India (31.78%) [63], in Wolayta Sodo, Ethiopia (32.14%) [64], in Mafikeng town, North-West Province of South Africa (32.5%), and [65] from Northern Plains of India (32.8%). But it was lower than the reports [66] in Southern Assam, India (47.86%) [67], in Hawassa area, Ethiopia (48.75%) [68], in the Selale/Fitche area, North Showa, Ethiopia (51.56%) [45], in Zaria and Kaduna, Nigeria (52.42%), and [69] in Hawassa town, Ethiopia (53.5%). The variation in the prevalence of *S. aureus* from the different studies might be due to differences in sample size and type, isolation techniques, husbandry practices, awareness and skill of the farm owners, animal health delivery systems, and geographic region of the sampled area.

The antibiogram test results of the 32 CoPS isolates revealed that almost half of the CoPS isolates were sensitive to Tetracycline (53.1%), Gentamycin (50%), and Kanamycin (50%). But the high levels of resistance were recorded for Amoxicillin (96.9%), Penicillin G (93.8%), and Vancomycin (59.4%). Moreover, 93.75% of the isolates developed multidrug resistance. Besides the antibiogram test, the PCR amplification result indicated that all antimicrobial tested CoPS isolates carried the *fem A* gene and among these isolates, 12 (37.5%) carried *mec A* gene (MRSA). This finding was in line with the findings of authors [70] who reported 100% resistance to Penicillin G and Amoxicillin and 42.7% to Cefoxitin [39], who reported 94.4% resistance to Penicillin G [48], who observed 92.2% resistance to Penicillin G and 33.3% to Oxacillin [71], in Sohag Governorate, Egypt who reported 43.1% resistance to Oxacillin and 83.7% to one or more antimicrobial agents [57], who reported 100% resistance to Penicillin and Amoxicillin [63], who reported 93.3% resistance to Penicillin G [72], who observed 100% resistance to Penicillin G and Amoxicillin, and 42.7% to Cefoxitin [64], who illustrated a large proportion (60%-100%) of resistance to Penicillin G and Ampicillin [66], who reported 87.5% resistance to Penicillin G [65], who reported 96% resistance to Penicillin G and 93% to Ampicillin [37], who observed 100% resistance to Penicillin G and 71.8% multidrug resistance [61], who reported 35.6% resistance to Oxacillin [52], who reported 95.5% resistance to Penicillin G [68], who reported 36.6% MRSA [38], who reported 86.04% resistance to Penicillin G and 74.42% to Ampicillin [45], who reported 100% resistance to Penicillin G and 46.8% to Oxacillin, and 44.6% to vancomycin, detected *mec* A by polymerase chain reaction in 4 of the 18 MRSA isolates, and 88.9% multi-drug resistance [46], who reported 100% of resistance to Penicillin G, 65% to Amoxicillin, and 40% to Oxacillin [56], who reported 98.5% resistance to Penicillin G [55], who reported 95.3% resistance to Penicillin G, and 100% of multidrug resistance, and [52] who reported 95.5% resistance to Penicillin G and 95.5% of multidrug resistance. However, the current finding of MRSA was higher than the reports of [66] (8.93%) [38], (13.95%) [47], (8.3%) [45], (4.8%), and [73] (5.9%). The detection of *mec A* by the PCR is considered a gold-standard technique for oxacillin resistance detection [74]. The resistance of *S. aureus*

to Penicillin and closely related antibiotics might be attributed to the production of β-lactamase, an enzyme that inactivates penicillin and closely related antibiotics. Around 50% of mastitis-causing *S. aureus* strains produces β-lactamase [75]. Moreover, the development of antimicrobial resistance might be as a result of repeated therapeutic and/or indiscriminate use of them in the dairy and fattening farms, particularly penicillin and oxytetracycline for the treatment of mastitis cases as well as prophylaxis in the study area at large.

## CONCLUSION AND RECOMMENDATIONS

The present study revealed that foods of bovine origin of the study area are found to be of less bacteriological quality. Moreover, there is a high prevalence of CoPS as well as MRSA in the different sample sources. This might be due to poor sanitary conditions and improper handling practices of foods. The antibiogram test results revealed that almost half of the CoPS isolates were sensitive to Tetracycline, Gentamycin, and Kanamycin. But almost all the isolates developed resistance to Amoxicillin and Penicillin G. Moreover, nearly all the isolates developed multidrug resistance. The development of antimicrobial resistance might be as a result of repeated therapeutic and/or indiscriminate use of them in the dairy and fattening farms. This higher percentage of multidrug resistance pattern indicates an alarming situation for designing prevention and control measures. However, the current study only targeted *mec A* and *fem A* genes for molecular characterization. In general, the low level of bacteriological quality, detection of CoPS and MRSA in different samples and development of drug resistance indicate that the products are not appropriate at all for human consumption. Hence, it poses a serious economic, animal welfare, food safety and public health problem. Therefore, strict hygiene should be implemented by creating awareness among different workers and managers of different food sources regarding transmission, zoonotic importance and control and prevention strategies of the disease; and dispensing of non-prescribed drugs and indiscriminate use of antibiotics should be avoided. Eventually, further studies on molecular characterization and sequencing of MRSA should be conducted by targeting other important genes in addition to the targeted genes in the study area and at large in the country.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## HUMAN AND ANIMAL RIGHTS

Not applicable.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article is available from the corresponding author [G.G.] on reasonable request.

## FUNDING

None.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

## ACKNOWLEDGEMENTS

The authors acknowledge the College of Veterinary Medicine, Mekelle University for financial support. We would like to extend our acknowledgement to owners, managers and workers of the different dairy farms, abattoir, butcher shop, restaurant, milk whole seller, and cafeteria of the study site for their keen interest and cooperation during the collection of the samples, and to the College of Veterinary Medicine Staff members who directly or indirectly helped us during the research period.

## REFERENCES

[1] Bhatia A, Zahoor S. *Staphylococcus aureus* Enterotoxins: A review. J Clin Diagn Res 2007; 1: 188-97.

[2] Linda du T Irma V. Food practices associated with increased risk of bacterial foodborne disease of female students in self-catering residences at the Cape Peninsula University of Technology. J Fam Ecol Consu Sci 2005; 33: 74-88.

[3] Le Loir Y, Baron F, Gautier M. Staphylococcus aureus and food poisoning. Genet Mol Res 2003; 2(1): 63-76. [PMID: 12917803]

[4] Asperger H, Zangerl P. Staphylococcus aureus.Encyclopedia of Dairy Science. San Diego: Academic Press 2003; pp. 2563-9.

[5] Leedom JM. Milk of nonhuman origin and infectious diseases in humans. Clin Infect Dis 2006; 43(5): 610-5. [http://dx.doi.org/10.1086/507035] [PMID: 16886155]

[6] Legnani P, Leoni E, Berveglieri M, Mirolo G, Alvaro N. Hygienic control of mass catering establishments, microbiological monitoring of food and equipment. Food Control 2004; 15: 205-6. [http://dx.doi.org/10.1016/S0956-7135(03)00048-3]

[7] FAO/WHO. Food Safety Risk Analysis a Guide for National Food Safety Authorities FAO. Rome: FAO Food and Nutrition 2006; p. 87.

[8] WHO Regional Office for Africa. Developing and Maintaining Food Safety Control Systems for Africa Current Status and Prospects for Change Second FAO/WHO Global Forum of Food Safety Regulators. Bangkok, Thailand. 2004; pp. 12-4.

[9] FAO/WHO. Joint FAO/WHO Food Standards Programmed FAO/WHO Coordinating Committee for Africa, Seventeenth Session. Rabat, Morocco. 2007.

[10] Ayalew H, Birhanu A, Asrade B. Review on food safety system: Ethiopian perspective. Afr J Food Sci 2013; 7: 431-40. [http://dx.doi.org/10.5897/AJFS2013.1064]

[11] Bayleyegn M, Daniel A, Woubit S. Sources and distribution of Salmonella serotypes isolated from food animals, slaughterhouse workers and retail meat products in Ethiopia. Ethiop J Health Dev 2003; 17: 63-70.

[12] Abera K, Ashebir M, Aderajew A, Ayalew T, Bedasa B. The sanitary condition of food and drink establishments in Awash-Sebat Kilo Town, Afar Region. Ethiop J Health Dev 2006; 20: 201-3.

[13] Knife Z, Abera K. Sanitary conditions of food establishments in Mekelle Town, Tigray, North Ethiopia. Ethiop J Health Dev 2007; 21(1): 3-11.

[14] Tefera W, Daniel A, Girma Z. Prevalence of thermophilic Campylobacter species in carcasses from sheep and goats in an abattoir in Debre Zeit area, Ethiopia. Ethiop J Health Dev 2009; 23: 230.

[15] Haileselassie M, Taddele H, Adhana K, Kalayou S. Food safety knowledge and practices of abattoir and butchery shops and the microbial profile of meat in Mekelle City, Ethiopia. Asian Pac J Trop Biomed 2013; 3(5): 407-12. [http://dx.doi.org/10.1016/S2221-1691(13)60085-4] [PMID: 23646306]

[16] Abebe M, Tafese B. Zoonotic bacterial pathogens isolated from food of bovine in selected Woredas of Tigray, Ethiopia. World Appl Sci J 2014; 31(11): 1864-8.

[17] Haftu R, Taddele H, Gugsa G, Kalayou S. Prevalence, bacterial causes, and antimicrobial susceptibility profile of mastitis isolates

from cows in large-scale dairy farms of Northern Ethiopia. Trop Anim Health Prod 2012; 44(7): 1765-71.
[http://dx.doi.org/10.1007/s11250-012-0135-z] [PMID: 22476790]

[18] Central Statistic Authority CSA. Federal Democratic Republic of Ethiopia Population Census Commission. Summary and Statistical Report of population and Housing 2007.

[19] Bryant C. Investment Opportunities in Mekelle. Ethiopia: Tigray State 2011.

[20] TRHDA. Tigray Regional Housing and Development Agency. Housing Report of 2007/2008 2008.

[21] Quinn PJ, Markey B K, Carter ME, Donnelly WJC, Leonard FC, Maguire D. Veterinary Microbiology and Microbial Disease. 1st ed. Blackwell Science Ltd UK 2002.

[22] Johnson WM, Tyler SD, Ewan EP, Ashton FE, Pollard DR, Rozee KR. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. J Clin Microbiol 1991; 29(3): 426-30.
[http://dx.doi.org/10.1128/JCM.29.3.426-430.1991] [PMID: 2037659]

[23] Vannuffel P, Gigi J, Ezzedine H, *et al.* Specific detection of methicillin-resistant Staphylococcus species by multiplex PCR. J Clin Microbiol 1995; 33(11): 2864-7.
[http://dx.doi.org/10.1128/JCM.33.11.2864-2867.1995] [PMID: 8576335]

[24] Pérez-Roth E, Claverie-Martín F, Batista N, Moreno A, Méndez-Alvarez S. Mupirocin resistance in methicillin-resistant *Staphylococcus aureus* clinical isolates in a Spanish hospital. Co-application of multiplex PCR assay and conventional microbiology methods. Diagn Microbiol Infect Dis 2002; 43(2): 123-8.
[http://dx.doi.org/10.1016/S0732-8893(02)00388-7] [PMID: 12088619]

[25] Vannuffel P, Heusterspreute M, Bouyer M, Vandercam B, Philippe M, Gala JL. Molecular characterization of *femA* from *Staphylococcus hominis* and *Staphylococcus saprophyticus*, and *femA*-based discrimination of staphylococcal species. Res Microbiol 1999; 150(2): 129-41.
[http://dx.doi.org/10.1016/S0923-2508(99)80030-8] [PMID: 10209768]

[26] Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966; 45(4): 493-6.
[http://dx.doi.org/10.1093/ajcp/45.4_ts.493] [PMID: 5325707]

[27] CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI document M100-S24,. Wayne PA. 2014.

[28] Statistical Package for Social Science, Inc. (SPSS). SPSS for Window (Version 115). Chicago, IL, USA: SPSS 2002.

[29] Assefa E, Beyene F, Santhanam A. Isolation and characterization of inhibitory substance producing lactic acid bacteria from ergo, Ethiopian traditional fermented milk. LRRD 2008; 20(3)

[30] Ashenafi M. The microbiology of Ethiopian foods and beverages: A review. SENET: Ethiop J Sci 2002; 25(1): 97-140.
[http://dx.doi.org/10.4314/sinet.v25i1.18076]

[31] Tesfay K, Berihun A, Habtamu T, Abrha B. Assessment of bacteriological quality of sold meat in the butcher shops of Adigrat, Tigray, Ethiopia. AJH 2014; 3(3): 38-44.

[32] Hassan Ali N, Farooqui A, Khan A, Khan AY, Kazmi SU. Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. J Infect Dev Ctries 2010; 4(6): 382-8.
[http://dx.doi.org/10.3855/jidc.599] [PMID: 20601790]

[33] Olatunji EA, Jubril AE, Okpu EO, Olafadehan OA, Ijah UJ, Njidda AA. Bacterial assessment and quality analysis of raw milk sold in Gwagwalada area council of the federal capital territory (FCT) Abuja, Nigeria. F S Q M 2012; 7: 1-4.

[34] Bergdoll MS. Staphylococcal Enterotoxin.Foodborne Infections. New York: Academic Press 1988; pp. 444-90.

[35] Mohammad A, Rajput IR, Khaskheli M, Faraz S, Devrajani K, Fazlani SA. Evaluation of microbial quality of goat meat at local market of Tando Jam. Pak J Nutr 2010; 9(3): 287-90.
[http://dx.doi.org/10.3923/pjn.2010.287.290]

[36] Birhanu A, Diriba L, Iyob I. Study of bovine mastitis in Asella government dairy farm of Oromia regional state, South Eastern Ethiopia. Int J Curr Res Acad Rev 2013; 1(2): 134-45.

[37] Biniam TD, Biruk TB, Tesfaye ST, Ashenafi KW. Isolation and identification of methicilin resistant *Staphylcoccus aureus* (MRSA) from bovine mastitic milk in and around Wolaita Sodo, Southern Ethiopia. J Vet Sci Res 2017; 2(3)000136

[38] Sudhanthiramani S, Swetha CS, Bharathy S. Prevalence of antibiotic resistant *Staphylococcus aureus* from raw milk samples collected from the local vendors in the region of Tirupathi, India. Vet World 2015; 8(4): 478-81.
[http://dx.doi.org/10.14202/vetworld.2015.478-481] [PMID: 27047118]

[39] Abera M, Demie B, Aragaw K, Regassa F, Regassa A. Isolation and identification of *Staphylococcus aureus* from bovine mastitic milk and their drug resistance patterns in Adama town, Ethiopia. J Vet Med Anim Health 2010; 2(3): 29-34.

[40] Fanta D, Kohei M, Akafte T, Delia G. Contamination of informally marketed bovine milk with *Staphylococcus aureus* in urban and peri urban areas of Debre-Zeit, Ethiopia AJDFMP 2013; 1(1): 008-11.

[41] Bishi A. Cross-sectional and longitudinal prospective study of bovine mastitis in peri-urban and urban dairy production systems in the Addis Ababa region, Ethiopia, (unpublished MSc thesis, Faculty of Veterinary Medicine, Addis Ababa University and Freje Uiversity, Berlin). 1998.

[42] Riva A, Borghi E, Cirasola D, *et al.* Methicillin-resistant *Staphylococcus aureus* in raw milk: prevalence, SCCmec typing, enterotoxin characterization, and antimicrobial resistance patterns. J Food Prot 2015; 78(6): 1142-6.
[http://dx.doi.org/10.4315/0362-028X.JFP-14-531] [PMID: 26038904]

[43] Hussein N, Yehualashet T, Tilahuan G. Prevalence of mastitis in different local and exotic breeds of milking cows, Ethiopia. J Agric Sci 1997; 16: 53-60.

[44] Mugenyi KC. Prevalence, control and antimicrobial susceptibility patterns of bovine mastitis causing bacteria in Mityana District, (unpublished MSc Thesis , Makerere University). 2014.

[45] Gali AU, Junaid K, Veronica JU, Mohammed B, Jacob KPK. Methicillin-resistant *Staphylococcus aureus* (MRSA) in fresh and fermented milk in Zaria and Kaduna, Nigeria. IJDRT 2013; 3(3): 67-75.

[46] Gali AU, Junaidu K, Veronica JU, Mohammed B, Jacob KPK. Occurrence of vancomycin-resistant *Staphylococcus aureus* (VRSA) in fresh and fermented milk in Nigeria: A preliminary report. J Public Health Epidemiol 2014; 3(8): 054-8.

[47] Basanisi MG, La Bella G, Nobili G, Franconieri I, La Salandra G. Genotyping of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk and dairy products in South Italy. Food Microbiol 2017; 62: 141-6.
[http://dx.doi.org/10.1016/j.fm.2016.10.020] [PMID: 27889140]

[48] Abebe M, Daniel A, Yimtubezinash W, Genene T. Identification and antimicrobial susceptibility of *Staphylococcus aureus* isolated from milk samples of dairy cows and nasal swabs of farm workers in selected dairy farms around Addis Ababa, Ethiopia. Afr J Microbiol Res 2013; 7(27): 3501-10.

[49] Forough A, Ebrahim R, Amir S, Hassan M, Majid R, Manochehr M. Antimicrobial resistance of *Staphylococcus aureus* isolated from bovine, sheep and goat raw milk. Glob Vet 2012; 8(2): 111-4.

[50] Kapllan S, Jorida T, Renata K, *et al.* Incidence of coagulase positive *Staphylococcus aureus* in raw cow milk produced by cattle farms in Fieri Region in Albania. JLS 2013; 7(4): 390-4.

[51] Firaol T, Sefinew A, Awoke T. Aerobic microorganisms isolated from mastitic bovine milk and their antimicrobial susceptibility profiles, Ethiopia. JAES 2013; 13(7): 920-5.

[52] Fufa A, Tekest A, Fikru G, *et al.* Staphylococcus: Isolation, identification and antimicrobial resistance in dairy cattle farms, municipal abattoir and personnel in and around Asella, Ethiopia. JVST 2016; 7: 383.

[53] Asiimwe BB, Baldan R, Trovato A, Cirillo DM. Prevalence and molecular characteristics of Staphylococcus aureus, including methicillin resistant strains, isolated from bulk can milk and raw milk products in pastoral communities of South-West Uganda. BMC Infect Dis 2017; 17(1): 422.
[http://dx.doi.org/10.1186/s12879-017-2524-4] [PMID: 28610560]

[54] Molalegne B, Arega T, Tadele T. Study on bovine mastitis in dairy farms of Bahir Dar and its Environs. J Anim Vet Adv 2010; 9(23): 2912-7.
[http://dx.doi.org/10.3923/javaa.2010.2912.2917]

[55] Beyene T, Hayishe H, Gizaw F, *et al.* Prevalence and antimicrobial resistance profile of *Staphylococcus* in dairy farms, abattoir and humans in Addis Ababa, Ethiopia. BMC Res Notes 2017; 10(1): 171.
[http://dx.doi.org/10.1186/s13104-017-2487-y] [PMID: 28454589]

[56] Ayele Y, Gutema FD, Edao BM, *et al.* Assessment of *Staphylococcus aureus* along milk value chain and its public health importance in Sebeta, central Oromia, Ethiopia. BMC Microbiol 2017; 17(1): 141.
[http://dx.doi.org/10.1186/s12866-017-1048-9] [PMID: 28655298]

[57] Mueena J, Marzia R. Md. Shafiullah P, Shah Md ZHC, Md. Enamul H, Md. Abdul KT, Sultan A. Isolation and characterization of *Staphylococcus aureus* from raw cow milk in Bangladesh. J Adv Vet Anim Res 2015; 2(1): 49-55. [http://dx.doi.org/10.5455/javar.2015.b47]

[58] Abera M, Demie B, Aragaw K, Regassa F, Regassa A. Isolation and identification of *Staphylococcus aureus* from bovine mastitic milk and their drug resistance patterns in Adama town, Ethiopia. AJDFMP 2012; 1(2): 019-23.

[59] Zeryehun T, Aya T, Bayecha R. Study on prevalence, bacterial pathogens and associated risk factors of bovine mastitis in small holder dairy farms in and around Addis Ababa, Ethiopia. J Anim Plant Sci 2013; 23(1): 50-5.

[60] Joshi LR, Tiwari A, Devkota SP, Khatiwada S, Paudyal S, Pande KR. Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in dairy farms of Pokhara, Nepal. Int J Vet Sci 2014; 3(2): 87-90.

[61] Suleiman AB, Umoh VJ, Kwaga JKP, Shaibu SJ. Prevalence and antibiotic resistance profiles of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from bovine mastitic milk in Plateau State, Nigeria. Int Res J Microbiol 2012; 2(8): 264-70.

[62] Sant PT, Rajesh KJ, Namita J. Characterization and antimicrobial sensitivity of *Staphylococcus aureus* isolates from subclinical bovine mastitis. TROP ANIM HEALTH PRO 2013; 1(2): 20-3.

[63] Fitsum T. Prevalence and drug resistance patterns of *Staphylococcus aureus* in lactating dairy cow's milk in Wolayta Sodo, Ethiopia. ECVE 2016; 2(5): 226-30.

[64] Akindolire MA, Babalola OO, Ateba CN. Detection of antibiotic resistant *Staphylococcus aureus* from milk: A public health implication. Int J Environ Res Public Health 2015; 12(9): 10254-75. [http://dx.doi.org/10.3390/ijerph120910254] [PMID: 26308035]

[65] Pati BK, Reena M. Characterization of Staphylococcus aureus isolates of bovine mastitis origin and antibiotic sensitivity pattern from Northern Plains of India. J Vet Res Ani Husb 2016; p. 105.

[66] Ananya M, Pranab BM. Detection of staphylococci in raw milk and milk products and evaluation of their antibiotic sensitivity: a report from Southern Assam, India. IOSR J Environ Sci Toxicol Food Technol 2015; 9(1): 17-22.

[67] Deresse D, Solomon G, Dawit Y. Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia. ANN CLIN MICROB ANTI 2012; 11(26): 1-6. [http://dx.doi.org/10.5897/AJMR11.1615]

[68] Shimels T, Bruk T, Tesfaye S. Isolation and antimicrobial susceptibility of Staphylococcus aureus and occurrence of methicillin resistant Staphylococcus aureus (MRSA) in mastitic dairy cows in the Selale/Fitche Area, North Showa, Ethiopia. MSc Thesis, College of Veterinary Medicine and Agriculture, . Addis Ababa University, Debre-Zeit, Ethiopia 2014; 1-80.

[69] Bekele M, Admasu M, Mesele A, Alemayehu R, Fufa A. Mastitis in lactating cows at Hawassa town: Prevalence, risk factors, major bacterial causes and treatment response to routinely used antibiotics. AEJSR 2012; 7(2): 86-91.

[70] Asmelash T, Mesfin N, Addisu D, Aklilu F, Biruk T, Tesfaye S. Isolation, identification and drug resistance patterns of methicillin resistant *Staphylococcus aureus* from mastitic cow's milk from selected dairy farms in and around Kombolcha, Ethiopia. J Vet Med Anim Health 2016; 8(1): 1-10. [http://dx.doi.org/10.5897/JVMAH2015.0422]

[71] Abo-Shama UH. Prevalence and antimicrobial susceptibility of *Staphylococcus aureus* isolated from cattle, buffalo, sheep and goat's raws milk in Sohag Governorate, Egypt. Assiut Vet Med J 2014; 60(141): 63-72.

[72] Tilahun KA, Mamo YA, Mulatu RA. Isolation and identification of methicillin resistant *S. aureus* (MRSA) from mastitic cow's milk. Int J Vet Wildl Sci 2016; 1(3): 014-23.

[73] Zutic M, Cirkovic I, Pavlovic L, *et al*. Occurrence of methicillin-resistant *Staphylococcus aureus* in milk samples from Serbian cows with subclinical mastitis. Afr J Microbiol Res 2012; 6(29): 5887-9.

[74] Anand KB, Agrawal P, Kumar S, Kapila K. Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for *mec*A gene for detection of MRSA. Indian J Med Microbiol 2009; 27(1): 27-9. [PMID: 19172055]

[75] Green M, Bradely A. Clinical forum: *Staphylococcus aureus* mastitis in cattle. UK VET 2004; 9: 1-9.