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Table of Contents: Volume 10   Number 4   April   2018

ARTICLES

Full Length Research Paper

A cross sectional study on prevalence of cattle fasciolosis and associated economical losses in cattle slaughtered at Gondar Elfora Abattoir, northwest Ethiopia

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Fasciolosis is a parasitic disease caused by either *Fasciola hepatica* or *Faciola gigantica*. These parasitic infections are of global significance causing diseases in different mammalian species including humans. In this study, the prevalence and economic significance of Fasciolosis in cattle slaughtered at Gondar Elfora abattoirs was assessed. A total of 400 cattle were examined and 85 cattle (21.2%) were affected by fasciolosis. This findings indicated that, the prevalence of cattle fasiolosis is significantly affected by the age of the animals (P < 0.05), where young animals (27.7%) were more affected than the adult ones (17.1%). Body conditions disclosed a significant relation with *Fasciola* infection. Poor body conditioned animals showed the highest prevalence (30.8%) followed by medium (19.5%) and good body conditioned animals (17%). There were statistical significant differences between the different geographical locations. Highest prevalence of fasciolosis was exhibited in animals originated from Dembiya (50%) followed by Debarq (31.6%), Wogera (15%), Gondar zuria (13.5%), Belesa (12.9%), Dansha (11.9%) and Metema (4.7%). As recorded, due to cattle fasciolosis livers were condemned for human consumption. Thus, based on retail value of cattle liver, the direct economic loss from fasciolosis in Gondar Elfora abattoir was estimated to be 63,600 Ethiopian Birr (2316.948 USD) annually. In conclusion, cattle fasciolosis is one of the major parasitic diseases in the study area. Therefore appropriate control measures should be designed and implemented so as to reduce financial losses that may occur from organ condemnation and loss of animals from the disease.

Key words: Cattle, economy, Elfora abattoir, fasciolosis, prevalence.

INTRODUCTION

Ethiopia is rich in livestock and believed to have the largest livestock population in Africa. The central statistical agency report indicated the total cattle population of the country which is estimated to be about

59.5 million, female (55.5%) and male (44.5%). The sector has been subsidizing a significant portion to the country economy and still promising to rally round the economic development of the country (CSA, 2017). Despite the presence of this huge livestock population, Ethiopia is not exploiting its livestock resources as expected due to a number of factors such as animal diseases, recurrent drought, infrastructures problem, rampant animal diseases, poor nutrition, poor husbandry practices, shortage of trained man power and lack of government policies for disease prevention and control (ILRI, 2009).

Among the animal diseases that affect animal health, parasitic infections have a great economic impact particularly in developing countries. Fasciolosis is of the parasitic diseases of domestic livestock caused by *Fasciola hepatica* and *Faciola gigantica,* commonly called liver flukes that are the most important trematodes afflicting the global agricultural community (Cwiklinski et al, 2016; Deepak and Singla, 2016; Andrews, 1999). Fasciolosis is a neglected tropical disease having both economy and zoonotic importance usually affects poor people from developing countries (Mas-Coma et al., 2014).

It has been estimated that, at least 2.6 million people are infected with fasciolosis worldwide (Fürst et al., 2012).

In Ethiopia, this disease is endemic in most part of the country long time ago as reported by several workers such as Graber, (1978), Goll and Scott (1978), Fufa et al. (2010), Yilma and Mesfin (2000), and Tolosa and Tigre (2007). Although many surveys were conducted, the case is still economic and public health issue. This study assesses the current status of fasciolosis, economical loss due to liver condemnation and identifies associated risk factors for the occurrence of faciolosis in cattle slaughtered at Elfora abattoir enterprise, Gondar, Ethiopia.

MATERIALS AND METHODS

Description of the study area

This study was carried out on 400 slaughtered cattle at Elfora abattoir; Amhara regional state, Northwest Ethiopia, from November 2016 to May 2017. Gondar town is located 739 Km away from Addis Ababa at an elevation of 2,220 m above sea level. The town is aligned on latitude of 12°36'N 37°28'E and longitude of 12.6°N37.467°E. Rain fall varies from 880 to1172 mm with the average annual temperature of 20.3°C (Shewangzaw and Addis, 2016).

Study animals

This study was conducted on 400 slaughtered male cattle brought from different areas nearby Gondar town. The cattle come mainly from Dembiya, Metema, Debarq, Belesa, Dansha, Gondar zuria and wogera.

Study design and sample size determination

A cross-sectional resarch was done to conduct this study and systematic random sampling technique was used to select appropriate samples. The sample size was determined according to the formula given by Thrusfield (2005). Previous study conducted by Mulat et al. (2012) shown the prevalence rate of 29.75% cattle fasiolosis in the same abattoir.

Hence, using 29.75% as expected prevalence and 5% absolute precision at 95% confidence level, the number of sampled animals needed in the study was 320. However, to increase the level of precision and accuracy of the data, the study was carried out on 400 cattle.

Ante-mortem examination

Ante-mortem examination was conducted in lairage, before slaughtering of animals according to Gracy et al., (1999) recommendation. Risk factors such as age, origin and body condition of individual animal were identified and recorded. Body condition for each cattle was estimated based on Nicholson and Butterworth (1986) ranging from score 1 (emaciated) to 5 (obese).

Therefore, in this study three classes of scoring which include poor (Score 2), medium (Score 3 and 4) and good (score 5) were used. No animals were slaughtered at score 1. The age of the animal was estimated on the basis of dentitions (Cringoli et al., 2002).

Post mortem examination

The liver of each study animal was carefully examined externally for the presence of lesions suggestive of *Fasciola* infection and incised for further confirmation. Liver flukes were detected by cutting the infected liver into fine, approximately 1 cm slices with a sharp knife. Investigation and identification of *Fasciola* species was done according to their distinct morphological characteristics following the standard guidelines given by Urquhart et al. (1996).

Direct economic loss assessment

All fasciola infected livers were considered to be unfit for human consumption and if any liver was infected by *Fasciola* at the Gondar Elfora abattoir, it was totally condemned. Therefore it was analyzed by considering the average number of annually slaughtered cattle in the abattoir from retrospective recorded data, the mean selling price of one liver at Gondar town and the prevalence of fasciolosis in the present study (21.2%).

The average market price of one liver at Gondar town was taken as 50 Ethiopian birr. The mean number of cattle slaughtered in this municipal abattoir was 6000 per year which depends on two years recorded data economic losses, calculated based on condemned livers due to fasciolosis. The estimated annual loss from condemned liver was calculated according to mathematical computation using the formula set by Ogunrinade and Adegoke (1982).

$$ALC = CSR \times LC \times P$$

$$=6000*50*21.2\%=63,600 \text{ Ethiopian birr}$$

Where:
ALC = Annual loss from liver condemnation,
CSR = mean annual cattle slaughtered at Gondar Elfora abattoir,
LC = mean cost of one liver in Gondar town and,
P = Prevalence of bovine fasciolosis at Gondar Elfora abattoir.

Table 1. Total number of animal examined and expected prevalence from November 2016 to May 2017.

Total samples	Infected animals	Prevalence (%)
400	85	21.25%

Table 2. Prevalence of cattle fasiolosis based on origin of animals from November 2016 to May 2017.

Origin	Prevalence (%)	X^2 (P-value)
Metema (n=64)	4.7	
Dembiya (n=72)	50	
Debarq (n=57)	31.6	57.218 (0.000)
Dansha (n=42)	11.9	
Belesa (n=31)	12.9	
G/zuria (n=74)	13.5	
Wogera (n=60)	15	

Table 3. Prevalence based on body condition from November 2016 to May 2017   .

Body condition	Prevalence (%)	X^2	P- value
Good (n=135)	17		
Medium (n=174)	19.5	6.663	0.036
Poor (n=91)	30.8		

Data management and analysis

All data collected was stored in Microsoft excel spreadsheet for statistical analysis and was analyzed using statically package of social science (SPSS) software version (20.0), to determine the prevalence of cattle fasiolosis and significance of associated risk factors. Association between the variable and the distribution of observed lesion in slaughtered cattle was determined using Chi-square test at critical probability value of $p<0.05$.

RESULTS

Overall prevalence of fasciolosis

As shown in Table 1, A total of 400 cattle were examined for the occurrence of fasciolosis out of which, 85 (21.25%) were found infected with facsiola.

Prevalence cattle fasciolosis based on origin of cattle

Statistical significant differences were recorded among animal origins (X^2 = 57.218 P= 0.000). As denoted by Table 2, the highest prevalence of fasciolosis was obtained from Dembiya (50%) followed by Debarq (31.6%), Wogera (15%), Gondar zuria (13.5%), Belesa (12.9%), Dansha (11.9%) and Metema (4.7%).

Prevalence based on body condition

As indicated by Table 3, poor body conditioned animals were mostly affected by cattle fasiolosis compared to medium and good body conditioned animals and shown a high statistical significant differences (P=0.036).

Prevalence based on age

Young cattle were highly affected (27.7%) by cattle fasciolosis. As presented in Table 4, there was a statistical differences between young and adult cattle (P=0.012).

DISCUSSION

Fasciolosis is an important zoonotic disease that is responsible for a significant loss in food resource and animal productivity (Jaja et al., 2017). Cattle are less

Table 4. Prevalence based on age groups.

Age	Prevalence (%)	X^2	P-value
Adult (n=245)	42(17.1)		
Young (n=155)	43(27.7)	6.373	0.012

susceptible to showing clinical signs of fasciolosis as compared to small ruminants (Stella et al., 2017). Therefore, cattle fasciolosis mainly exhibits as a subclinical chronic disease, associated with hepatic damage and blood loss caused by parasites in the bile ducts (Kaplan, 2001). Hence, cattle fasciolosis is of significant economic importance as the resultant liver condemnations need serious consideration in abattoir industries (Abunna, 2010).

The results of the present study publicized that; origin, body condition and age of the animals have significant effect on the prevalence of cattle fasciolosis. The overall prevalence of bovine fasciolosis (21.25%) in the current study was supported by other abattoir-based studies conducted in different parts of Ethiopia such as Alemu and Mekonnen (2013) (22.14%) from Dangila municipal abattoir, Asressa (2011) (24%) from Andassa livestock research center and Berhe et al. (2009) (24.3%) from Mekelle. In contrast to studies such as Mulat Nega et al. (2012), 29.75% was reported from Gondar Elfora abattoir and Yilma and Mesfin (2000) (90.7%) was conducted in Gondar Municipal abattoir; the overall prevalence record in this study was lower.

In the current study, the variations in prevalence rate which based on the origin of animals were probably due to epidemiological factors such as snail population, as a result of favorable conditions. For instance, the occurrence of bovine fasciolosis in Dembiya was the highest and this might be due to the availability of more appropriate environmental conditions such as watershed areas, slowly flowing waterways and lakes like Lake Tana. These factors will create ideal conditions for the occurrence of fasciola infection.

The statistical significance difference between the age groups in this study might be the fact that, young animals are more susceptible to different disease because of poor immunity development and lack of adaptation (Gebremeskel et al., 2017). In this study, the prevalence rate was higher in poor body conditioned cattle than other body condition scores. Different studies revealed the relationship between body conditions and fasciolosis has shown that there is a positive association between fasciolosis and cattle weight loss (Jaja et al., 2017). It is known that animals in good intensive management systems and with adequate veterinary care should be in better body condition than cattle extensively managed with little veterinary services (Jaja et al., 2017). Therefore, types of management system and veterinary services correlate with cattle fasciolosis.

The direct economic loss due to liver condemnation in Gondar Elfora abattoir was closely related with the earlier records of Bekele et al. (2010) (57,960.00 Ethiopian birr) (ETB)) from Adwa and Bekele et al. (2014) (88,806.85 ETB) from Hosanna. These variations in financial loss due to liver condemnation might be as a result of difference in the prevalence of fasciolosis among different study site, period and price of a liver.

Conclusion

Fasciolosis is one of major problem for livestock development in the study area by inflicting direct economic losses and its occurrence closely linked to the presence of environment suitable to the development of snail intermediate host. As reported by the current study, there was a high cattle fasciolosis in the study area. Statistical significant differences were recorded between the risk factors investigated.

Therefore, based on the findings we recommend integrated approach with a combination of chemotherapy. Vector control should be considered more practically and economically, control strategies targeted on the parasite and the intermediate hosts as well as implementation of appropriate grazing management in the study area are warranted due to, the reduction in the risk of infection by planned grazing management especially during high outbreak months by the application of zero grazing (Cut and carry). Farmers who rear cattle should be aware of how to improve feeds to their animals so that the animal can have good body condition that confers some level of resistance against fasciolosis.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw cow milk and fresh fruit juice in Mekelle, Tigray, Ethiopia

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This study was conducted to evaluate drug resistance profile of *Staphylococcus aureus* in raw milk, fresh fruit juice and dairy farms settings of Mekelle, Tigray. A cross-sectional study was conducted on the total 258 samples of raw cow milk and fresh fruit juice. Antimicrobial resistance status was also checked for identified *S. aureus* using various commonly used antimicrobial discs. The overall viable staphylococcal count mean and standard deviation of samples from milk shop, fruit juice and dairy milk were found to be 8.86±107, 7.2 × 107, 8.65±107 cfu/ml, 33.87±106, 6.68±106 and 22.0±106, respectively. Among the total 258 samples, 75 (29.07%) samples were found positive for *S. aureus*. Proportion of the isolation from milk shop, fruit juice and dairy milk samples were 20 (23.26%), 32 (37.21%) and 23 (26.74%), respectively. Antimicrobial test of the high resistance revealed vancomycin (100%), ampicillin (90.9%). ciprofloxacin (90.9%), ceftaroline (63.6%), penicillin-G (81.8%) and clindamycin (72.7%) whereas they are highly susceptible to some antibiotics like gentamicin (100%), streptomycin (81.8%), norfloxacin (63.6%), chloramphenicol (81.8%), sulfamethoxazole (96%), kanamycin (72.7%), polymixin B (72.7%), erythromycin (72.7%) and tetracycline (81.8); also, some *S. aureus* also showed multi-drug resistance pattern. The present study, we isolated and determined the drug Resistance profile of S. aruesu in Mekelle city, Northern Ethiopia alarmingly, the S. aureus isolates circulating in the raw cow milk, fresh fruit juice and dairy milk. High level of S. aureus isolation from personnel and equipment besides food samples reveals that the hygiene practice is substandard. Prudent drug use and improved hygienic practice is recommended in the raw cow milk of dairy farms and fresh fruit juice to safeguard the public from the risk of acquiring infections and multiple drug resistance (MDR) pathogenic *S. aureus.*

Key words: Antibiogram, bacterial load, multiple drug resistance (MDR), Mekelle, Milk, *Staphylococcus aureus.*

INTRODUCTION

Foods borne illness are public health problem in developed and developing countries. *Staphylococcus aureus* is among the most significant pathogens causing a wide spectrum of diseases in both humans and animals. In humans, nosocomial and community acquired infections are common (Klotz et al., 2003). Pathogenic

strains are usuallycoagulase-positive and cause disease in their hosts throughout the world. *S. aureus* is one of the most significant food-borne pathogens (Le Loir et al., 2003). Raw unpasteurized milk may become contaminated with enterotoxigenic coagulase-positive *S. aureus* either through contact with the cow's udder during milking or by cross contamination during processing (Normanno et al., 2005, Ekici et al., 2004).

Food-borne pathogens are recognized as a major health hazard associated with street foods, the risk being dependent primarily on the type of food and the method of preparation and conservation (CDC, 2011; FAO/WHO, 2005). *S. aureus* is a gram positive, catalase and coagulase positive microorganism responsible for various foodborne outbreaks. Contamination of food with entero-toxigenic *S. aureus* causes staphylococcal enterotoxins (SEs) intoxication hence the associated symptoms like vomiting and diarrhea (Veras et al., 2008). In countries where food borne illness were investigated and documented, the relative importance of pathogens like *S. aureus*, Campylobacter, *Escherichia coli* and Salmonella species were recorded as a major cause (WHO, 2004).

The ability of these microorganisms to survive under adverse conditions and to grow in the presence of low levels of nutrients and at suboptimal temperatures and pH values presents a formidable challenge to the agricultural and food processing industries. The continued prominence of raw meats, eggs, dairy products, vegetable sprouts, fresh fruits and fruit juices as the principal vehicles of human foodborne diseases poses a major challenge to coordinate sectorial control efforts within each industry (Knife and Abera, 2007). Studies conducted in different parts of Ethiopia also showed the poor sanitary conditions of catering establishments and presence of pathogenic organisms (Knife and Abera 2007; Mekonnen et al., 2012).

In addition to toxic effect of food borne microbial pathogens, antibiotic resistance remains a major challenge in human and animal health (Shekh et al., 2013). Food contamination with antibiotic-resistant bacteria can therefore be a major threat to public health, as the antibiotic resistance determinants can be transferred to other bacteria of human and animals, thereby has significant public health implications by increasing the number of food-borne illnesses and the potential for treatment failure (Adesiji et al., 2011). Studying antimicrobial resistance in humans and animals is important for detecting changing patterns of resistance, implementing control measures on the use of antimicrobial agents and preventing the spread of multidrug-resistant strains of bacteria. Up to now, many researchers have focused on the spread of resistant *S. aureus* in clinical settings (Ateba et al., 2010; Addo et al., 2011). However, limited number of investigations has been studied about the presence of antimicrobial resistance in food animals in Ethiopia (Mekonnen et al., 2005; Hundera et al., 2005). So far, there are no studies conducted on the burden and drug sensitivity profile of *S. aureus* in Mekelle city, Northern Ethiopia. In this study, S. *aureus* was isolated and the drug Resistance profile was determined.

MATERIALS AND METHODS

Study area

The study was conducted from October 2016 to June 2017 in Mekelle city. Mekelle is the capital city of Tigray Regional State located at about 783 km away north of Addis Ababa, Capital city of the Federal Democratic Republic of Ethiopia at geographical coordination of 39°28` East longitude and 13°32` North latitude. The average altitude of the city is 2300 m.a.s.l. with a mean annual rainfall and average annual temperature of 629 mm and 22°C, respectively (TBA, 2017). The population of the city is 406,338 (195, 605 male and 210,733 female) (TBA, 2017). The city has seven sub-cities and 33 Kebelles where over 139 juice houses, 48 dairy farms and 123 milk shops (street vender or retailer shops) are inhabited. Besides, the city possesses an extensive public transport network and active urban-rural exchange of goods with about 30,000 micro and small enterprises (Bryant, 2017).

Study design

A cross-sectional survey was conducted from October 2016 to June 2017 on raw cow milk and fresh fruit juice samples collected from different sources of raw milk shops and dairy milk supply centers, and juice houses in Mekelle. Purposive sampling technique was employed.

Sampling technique and collection

A total of 258 food samples were collected among which 172 are milk samples (86 from milk shops, 86 from dairy farms) and the remaining 86 are fresh juice samples (from 86 juice houses) in the seven sub-cities of Mekelle city. Samples were collected according to standards described by Oyeleke and Manga (2008). After aseptic collection, samples were labeled and packed with sterile bottles and transported with an ice box to Mekelle University, College of Veterinary Medicine, Microbiology and Public health laboratories for bacterial isolation and characterization. Samples were processed immediately for bacterial identification to species level using culture media and then isolates were kept in refrigerator at 4°C until microbial characterization with regular sub-culturing described (Oyeleke and Manga, 2008).

Enumeration of total viable

One milliliter and gram of raw milk and fruit juice samples respectively were homogenized into 9 ml of serial peptone water/NSS and 10 g/1 g of each food item was weighed out and homogenized into 90 ml/9 ml of sterile distilled deionized water. Then, serial dilutions were prepared. From the 10-fold dilutions of the homogenates; 1 ml of 10-4, 10-5 and 10-6 dilutions were cultured in replicate on standard plate count agar (Hi Media, India), using the pour plate method. The plates were then incubated at 37°C for 24 – 48 h. At the end of the incubation period, colonies were counted using the illuminated colony counter. The counts for each plate were expressed as colony forming unit of the suspension (cfu/g) (Fawole and Oso, 2001). In order to determine the presence of *S. aureus*, 0.1 ml of samples from each dilution

Table 1. Antibiotics used, their concentration and drug sensitivity interpretive zone of inhibition diameters

Antibiotic	Disc code	Potency (µg)	Zone diameter		
			S	M	R
Erythromycin	ERY	15	≥23	14-22	≤13
Penicillin-G	P	10	≥29		≤28
Norflaxon	f	50	≥17	13-16	≤12
Sulphoxazole-trimethoprim	SXT-TMP	300	≥16	11-15	≤10
Streptomycin	S	10	≥15	12-14	≤11
Kanamycin	KAN	30	≥18	14-17	≤13
Chloramphenicol	CHL	30	≥18		≤18
Tetracycline	TE	30	≥22	19-21	≤19
Gentamicin	GM	10	≥18	-	≤18
Ampicillin	AMP	10	≥15	12-14	≤11
Ciprofloxacin	CIP	5	≥20		≤20
Ciftriaxone	CRO	30	≥23	20-22	≤19
Vancomycin	VA	30	≥12	10-11	≤19
Clindamycin	CC	10	≥21	15-20	≤14
Polymixin B	P	10	≥12	12-14	≤11

R= Resistant, I= intermediate, S = sensitive.
Source: CLSI (2008).

was introduced onto the Baird Parker agar (Oxorid). The plates were incubated at 37°C for 24 h. All the isolates were subjected to morphological and biochemical confirmation according to the prescribed method (Fawole and Oso, 2001).

Isolation and identification of *S. aureus*

About 10 ml of each raw cow milk and fresh fruit juice sample was suspended in 90 ml of Brain heart infusion broth supplemented with NaCl (6.5%) and incubated at 37°C for 24 h. A sterile loopful of the broth inoculum was streaked onto Baird Parker agar and incubated at 37°C for 24 h. Colonies that appeared black or greyish-black were Gram-stained and subjected to biochemical tests. Gram positive cocci that occurred singly and in pairs, tetrads, short chains and irregular grape-like clusters were suggestive of *S. aureus*. Conventional biochemical tests carried out for presumptive identification of *S. aureus* isolates included gram staining, catalase, coagulase, 5 % sheep blood agar, pigmentation (Mannitol salt agar) and DNase activity (McFaddin, 2000).

Antimicrobial susceptibility test

Antimicrobial susceptibility test, through Kirby diffusion test, was performed for all *S. aureus* isolates following the protocol of CLSI (2008). For susceptibility test, a pure culture of all *S. aureus* were taken and transferred to a tube containing 5 ml of sterile normal saline and mixed gently to make homogenous suspension which was adjusted to a turbidity equivalent to a 0.5 Mc Far land standard as measured by turbidity meter. The bacterial suspension was inoculated onto Muller–Hinton agar (Oxorid, UK) with the sterile swab to cover the whole surface of the agar. The inoculated plates were left at room temperature to dry. Before using the antimicrobial disks, they were kept at room temperature for 1 h and then

dispended on the surface of media. Following this, the plates were incubated aerobically at 37°C for 24 h (CLIS, 2012).

The diameters of the zone of inhibition around the disks were measured to the nearest millimeter using calibrated rulers, and the isolates were classified as susceptible, intermediate and resistant according to the interpretative accordance with the guidelines of (CLSI, 2008) as indicated in Table 1.

Data management and analysis

All data were checked against the standards and methods were used to perform the study. Data was entered in Microsoft Excel spreadsheet and analyzed using STATA Version 12. Descriptive statistics such as means, percentage and frequencies were computed to report desired outputs. The antimicrobial resistance test was analyzed using WHONET software version 5 statistical package (http://www.who.int/medicines/areas/rational_use/AMR_WHONET_SOFTWARE/en/). Analysis of Variance (ANOVA) was used to test the significant difference at $p < 0.05$.

RESULTS

Enumeration of total viable

The overall mean viable bacterial count recorded was 8.24 x 107. The individual sample type mean viable count and standard deviation of milk shop, fruit juice and dairy milk is indicated in Table 2.

Isolation and Identification of organism

Among the total 258 raw cow milk and fruit juice samples

Table 2. Total viable *S. aureus* count for different sample types.

Sample type	Mean bacterial count	±SD	Minimum bacterial count	Maximum bacterial count
Milk shop	8.86 ± 10^7	33.87 ± 10^6	1.5 ± 10^7	1.25 ± 10^8
Fruit juice	7.2 ± 10^7	6.68 ± 10^6	6.37 ± 10^7	8.5 ± 10^7
Dairy milk	8.65 ± 10^7	22.0 ± 10^6	6.4 ± 10^7	1.23 ± 10^8
Total	8.24 ± 10^7	23.8 ± 10^6	1.5 ± 10^7	1.25 ± 10^8

SD= standard deviation.

Table 3. Rate of isolation of *S. aureus* from raw cow milk and fruit juice samples.

Sample type	Number positive (%)	X^2	p-value
Milk shop (n=86)	20 (23.26)		
Fruit juice (n=86)	32 (37.21)	4.3987	0.111
Dairy milk (n=86)	23 (26.74)		
Overall (n=258)	75 (29.07)		

collected from different sources of Mekelle sub city, 75 (29.07%) samples were found positive for *S. aureus*. Proportion of the isolation from milk shop, fruit juice and dairy milk samples is described (Table 3). A statistically significant difference (χ2=4.3987, P-value =0.111) was recorded among samples from the three sites (Table 3 and Figure 1).

Antimicrobial susceptibility profile of *S. aureus*

The antimicrobial resistance profile of the bacterial isolates from raw cow milk and fruit juice samples are summarized in Table 3. *S. aureus* showed high resistance to antibiotics like, vancomycin (100%), ampicillin (90.9%). ciprofloxacin (90.9%), ceftaroline (63.6%), pencillin-G (81.8%) and clindamycin (72.7%) whereas it was highly susceptible to some antibiotics like, gentamicin (100%), streptomycin (81.8%), norfloxacin (63.6%), chloramphenicol (81.8%), sulfamethoxazole (96%), kanamycin (72.7%), polymixin B (72.7%), erythromycin (72.7%) and tetracycline (81.8) (Table 4 and Figure 2).

The multi-drug resistance pattern of the bacterial *S. aureus* isolates is presented in Table 5. In general, antimicrobial susceptibility test revealed that gentamicin, streptomycin, norfloxacin, chloramphenicol, sulfamethoxazole, kanamycin, polymixin B, erythromycin, and tetracyclin were the antimicrobials indicated as active against *S. aureus* isolated from this study.

A total of 10 multiple drug resistance patterns were observed. The highest multiple drug resistance (MDR) noted was PEN, VAN, CIP and AMP (45.5%). The maximum multiple drug resistance registered was resistant to three and four antibiotics with the combination PEN, VAN, CIP and AMP.

DISCUSSION

The current finding indicates that samples from milk shop, fruit juice and dairy milk were found with a viable staphylococcal count load of 8.86 × 107, 7.2 × 107 and 8.65 × 107 cfu/ml respectively with an overall mean viable bacterial count of 8.24 × 107cfu/ml. The highest mean value of microbial load (8.86 × 107 cfu/ml) was found from milk shop samples.

The current study was found with higher viable staphylococcal count than previous reports such as viable bacterial count from raw cow milk and fresh fruit juice samples in 6.0 × 103 cfu/ml to 2.5 × 105 cfu/ml of (Lucky et al., 2016) in Bangladesh, 1.4 × 104 cfu/ ml (Tasnim, 2016) in Dhaka city; Staphylococcal count was from fresh vended fruit juices.

In the present study, out of 258 samples, 75 (29.07%) samples were found to be positive for *S. aureus* of which 20 (23.26%) were from milk shop, 32 (37.21%) from fruit juice and 23 (26.74%) from dairy milk. The result shows a high contamination rate, which might be attributed to poor hygeinic sanitation and handling improper. Statistically significant difference (P< 0.05) among the sample types in the prevalence of *S. aureus* was recorded. A similar report was also made by previous researchers (Abebe et al., 2013; Reta et al., 2016) that *S. aureus* was 15.5 and 24.2% in raw milk samples respectively in Ethiopia and in the other parts of the world which is contrary to this; different literatures revealed a very significant isolation rate of *S. aureus* from raw milk samples (Olatunji et al., 2009; Pourhassan and Taravat-Najafabadi, 2011; Mohanty et al., 2013; Sanaa et al., 2005).

The present overall isolation rate in raw cow milk products was 29.07% which seems to be higher than the findings of 6.25% by Thaker et al. (2013), 17% in Egypt (El-Gedawy et al., 2014), 18.2% in Turkey (Ekici et al.,
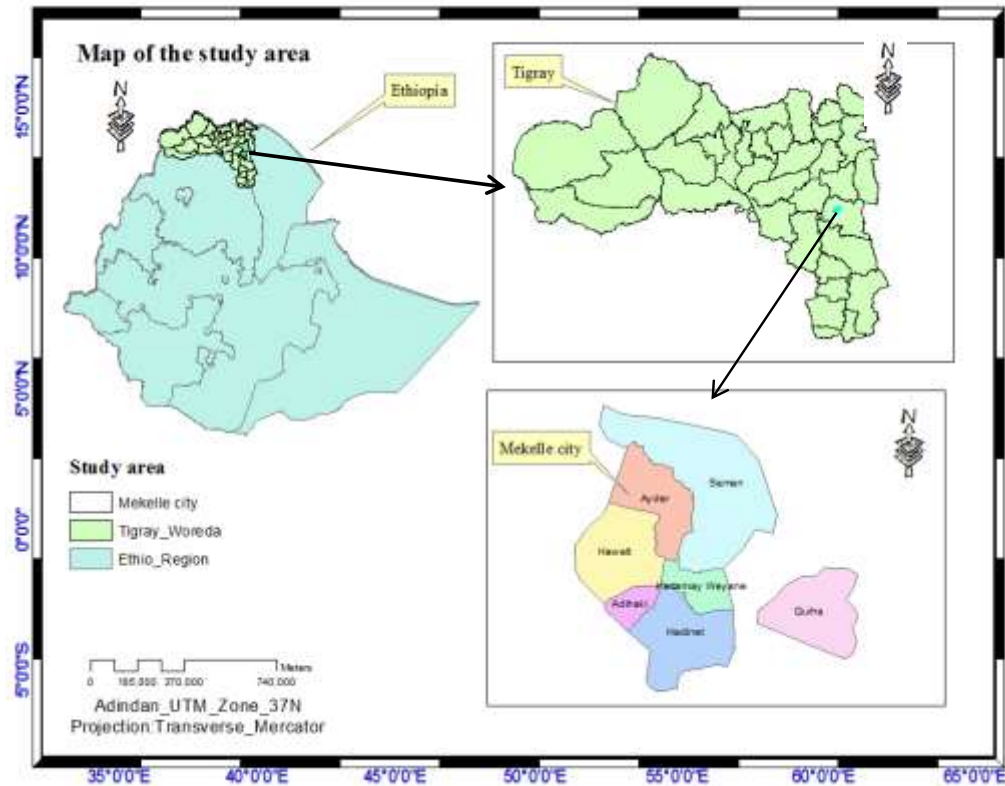
Figure 1. Map of the study area.

2004), 20.8% in Turkey (Aydin et al., 2011) and 21% in Iran (Ahmadi et al., 2009); were previously reported in milk samples collected from dairy farms. The present finding was much higher compared to the finding reported previously (Santana et al., 2010; Ekici et al., 2004) which found 17.39 and 18.18%, respectively; 4 (8%) of pasteurized milk samples, 9 (18%) of traditional butter samples and 12 (24%) of traditional cheese samples (Mirzaei et al., 2011) in Sarab whereas the new finding was much lower compared to the finding reported; 40% (Zakary et al., 2011), 22.5% (Hamid et al., 2017), 61.70% (Lingathurai and Vellathurai, 2011) and 44% (Mirzaei et al., 2011) from raw milk samples in Sarab.

The variation could be due to the reason that even when drawn under aseptic condition, milk always contains microorganisms which are derived from the milk ducts in the udder. In addition, contaminants coming from milking utensils, human handlers, unclean environmental conditions and poor udder preparation might expose raw milk to bacterial contamination.

Moreover, the present study showed the resistance of *S. aureus* to vancomycin (100%), ampicillin (90.9%), ciprofloxacin (90.9%), ceftaroline (63.6%), pencillin-G (81.8%) and clindamycin (72.7%) whereas was highly susceptible to some antibiotics like, gentamicin (100%), streptomycin (81.8%), norfloxacin (63.6%),

chloramphenicol (81.8%), sulfamethoxazole (96%), kanamycin (72.7%), polymixin B (72.7%), erythromycin (72.7%) and tetracycline (81.8) (Table 4). Similarly, the present investigation indicated that the resistance pattern of penicillin was found to be 93.1% by Melese et al. (2016) which is similar to the finding (87.2 %) of Tariku et al. (2011) in Ethiopia, 80% in Sweden (Landin, 2006), 57% in Iran (Gooraninejad et al., 2007) and 50% in Finland (Myllys et al., 1998). This is in contrast to findings observed by (Adesiyun, 1994) who reported 23% of resistance to penicillin-G in West India.

The highest drug resistance recorded in the current study migh be due to high antimicrobial use in dairy farms and individual cows, to treat various diseases affecting the dairy sector. Similarly, several studies have indicated that *S. aureus* isolated from raw milk showed high resistance to vancomycin, ampicillin, ciprofloxacin, penicillin-G followed by ciprofloxacin and clindamycin thus indicating the safety of food products. However, few numbers of isolates exhibited resistance towards ampicillin (Thaker et al., 2013).

Antibiotic resistance development among the bacteria poses a problem of concern. Effectiveness of current treatments and ability to control infectious diseases in both animals and humans may become hazardous.

Different researchers reported antimicrobial resistant *S.*

Table 4. Antimicrobial resistant of *S. aureus* isolated from raw cow milk and fruit juice of sample.

Antibiotic	%R	%I	%S	%R 95%C.I.
Penicillin G	81.8	0	18.2	47.7-96.8
Ampicillin	90.9	0	9.1	57.1-99.5
Cefoxitin	63.6	0	36.4	31.6-87.6
Gentamicin	0	0	100	0.0-32.1
Kanamycin	18.2	9.1	72.7	3.2-52.3
Streptomycin	18.2	0	81.8	3.2-52.3
Ciprofloxacin	90.9	0	9.1	57.1-99.5
Norfloxacin	0	0	100	0.0-32.1
Sulfamethoxazole	27.3	9.1	63.6	7.3-60.7
Clindamycin	72.7	0	27.3	39.3-92.7
Polymixin B	27.3	0	72.7	7.3-60.7
Erythromycin	9.1	18.2	72.7	0.5-42.9
Vancomycin	100	0	0	67.9-100
Chloramphenicol	0	18.2	81.8	0.0-32.1
Tetracycline	18.2	0	81.8	3.2-52.3

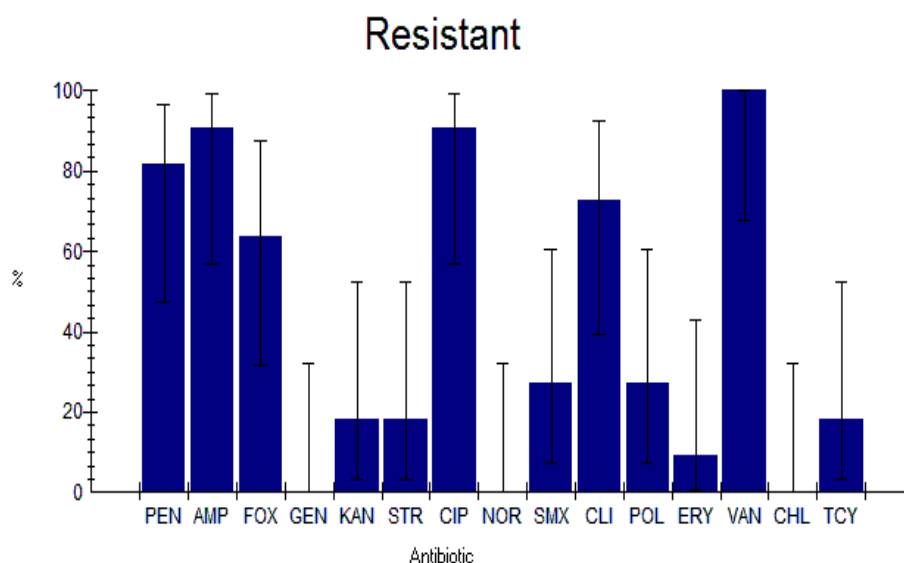R= resistance, I= intermediate, S= susceptibility.

Figure 2. Antibiotic sensitivity pattern of *S. aureus* isolated from different samples.

aureus isolates of raw milk in their previous studies from Ethiopia. Reports from other researchers had also indicated *S. aureus* isolates resistance to vancomycin (100%), ampicillin (90.9%), ciprofloxacin (90.9%), ceftaroline (63.6%), penicillin-G (81.8%) and clindamycin (72.7%). Similar result was most frequently observed for penicillin (100%), ampicillin (100%), followed by erythromycin (95.7%) (Wang et al., 2014) in China, penicillin (100%), ampicillin (96%), amoxicillin (92%), and trimethoprim- sulphamethoxazole (88%) (Beyene, 2016) in Ethiopia, whereas the percent were found higher compared to what was reported by Hamid et al. (2017) of

94.4, 83.3 and 50% resistance for penicillin, ampicillin and ceftriaxone, respectively of *S. aureus* isolates with particular emphasis on penicillin G. The present observation agrees with preliminary finding conducted by Reta et al. (2016) of 93.1%. This is in accordance with the findings of Tariku et al. (2011) who reported resistance of *S. aureus* to chloramphenicol (16%), vancomycin (3%) it lower with compare the present finding while as (Khakpoor et al., 2011) in other study reported that all isolate of *S. aureus* from mastitis cattle were resistant to penicillin and 83.3% resistance to ampicillin in this study is much higher than that of 41.44%

Table 5. Multi-drug resistance of *S. aureus* isolated from raw cow milk and fruit Juices sample.

Number of AMR	MDR profile	% Isolates
1	VAN CIP	9.1
	VAN CIP AMP	9.1
2	PEN VAN AMP	9.1
1	PEN VAN CIP AMP	45.5
	PEN VAN CIP AMP STR	9.1
2	CHL PEN VAN CIP AMP	9.1
1	CHLPEN VAN CIP AMP STR	9.1

VAN= Vacomycin; PEN= penicillin G; AMP= ampicillin; STR= Streptomycin; CHL = chloramphenicol; CIP = ciprofloxacin.

as reported by (Mubarack et al ., 2012) and lower as compared to 100% resistance reported by (Khakpoor et al., 2011).The current study was highly susceptible to some antibiotics like, gentamicin (100%), streptomycin (81.8%), norfloxacin (63.6%), chloramphenicol (81.8%), sulfamethoxazole (96%), kanamycin (72.7%), polymixin B (72.7%), erythromycin (72.7%) and tetracycline (81.8); similar results were found for chloramphenicol, streptomycin and gentamycin (100, 94, and 90%), respectively by Beyene (2016) in Ethiopia but disagree with the observation made by Kassaify et al. (2013) in the case of streptomycin (95%), but it disagree with the observation made by Tariku et al. (2011) in the case of tetracycline (0%) and clindamycin (4%) in dairy farms in Jimma town, cefoxitin (100%) and clindamycin (each 100%) (Wang et al., 2014) in China. The probable explanation could be that *S. aureus* strains have the capacity to change their resistance behavior to the exposed antimicrobials.

The emergence degree of resistance to many drugs represents public health hazard due to the fact that food borne outbreaks would be difficult to treat and this pool of MDR S. aureus in food supply represents a reservoir for communicable resistant genes. The reason for the existence of antimicrobial resistant salmonella isolates could be due to the indiscriminate use of antimicrobials, self-medication, administration of sub therapeutic dose of antimicrobials to livestock for prophylactic purpose (Szyfers and Acha, 2001) and limited updating of the long used drug groups. Hence, due to the relatively limited access and high price to get the newly developed cephalosporin and quinolone drugs, the reports of prevalence of antimicrobial-resistant to relatively low-priced and regularly available antibiotics are alarming for a low-income society living in most developing countries, like Ethiopia.

Conclusion

The current study showed insights into the magnitude and incidence of *S. aureus* from raw cow milk and fresh fruit juice samples. The study revealed the development of antibiotic resistant against *S. aureus* which could pose serious threat for consumers as well as for health professionals in the study area. Hence, attention should be given to proper handling of the food items, and using recent antibiotics in the treatment of diseases both in humans and animals.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

Abbreviations: AMP, Ampicillin; **ANOVA,** analysis of variance; **CHL,** Chloramphenicol; **CIP,** Ciprofloxacin; **ERY,** Erythromycin; **I,** Intermediate; **MDR,** multiple drug resistance; **NSS,** normal saline solution; **R,** resistant; **S,** Sensitive; **SD,** standard deviation, **O,** Observation; **STR,** Streptomycin; **TCY,** Tetracycline.

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