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Assessment of Bacteriological Quality of Sold Meat in the Butcher Shops of Adigrat, Tigray, Ethiopia

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Abstract: The study was carried out from November 2011 to April 2012, through which purposive sampling methodology was used to assess the bacteriological quality of sold meat in abattoir and butcher shop of Adigrat. On a total of 80 meat samples (20 samples from each stage; abattoir and butcher house of hanging meat, minced meat and refrigerated meat). The mean values of bacterial load of hanging meat, minced meat, abattoir meatand refrigerated meat were $5.5x10^7$, $6.5x10^7$, $3.2x10^7$and $3x10^7$CFU/g respectively indicating high mean values of microbial load in the hanging meat and minced meat sales with statistically significant difference ($P<0.05$).Theresult also indicated that 13.75, 46.25 and 40% of the meat are acceptable,marginally acceptable and unacceptable respectively. The major bacterial pathogens isolated were *Escherichia coli*; *Staphylococcus aurous Streptococcus* species and non-lactose fermenting bacteria. Most of the isolated bacteria were susceptible for the commonly used antimicrobials.Careless handling of meat at the slaughtering places and butcher shops affect the quality of meat which indicated as the presence of contamination. Therefore, particular attention to meat hygienic should be strengthened in both butcher houses and abattoirs workers.

Key words: Abattoir · Bacterial load · Butchery shops · Coli forms · *Escherichia coli* · *Staphylococcus aureus*

INTRODUCTION

Meat refers to animal tissue used as food, mostly skeletal muscles and associated fat but it may also refer to organs including lungs, livers, skin, brains, bone marrow, kidney and a variety of other internal organs as well as blood [1]. It is the major source of protein and valuable qualities of vitamins for most people in many parts of the world, thus they are essential for the growth, repair and maintenance of body cells and necessary for our everyday activities. The chief constituents of meat are water, protein and fat, phosphorus, iron and vitamins. Meat has high water content corresponding to the water activity approximately 0.99 which is suitable for microbial growth [2]. Due to the chemical composition and biological characteristics, meat is highly perishable food which provide excellent source for growth of many hazardous microorganisms that can cause infection in humans and spoilage of meat and economic loss [3]. The preservation of meat as a perishable food usually is accomplished by a combination of preservation methods which greatly lengthen the meat keeping quality. So, to increase meat quality assurance in accordance with microbial load assessment is deemed necessary [4]. It has been reported that Gram negative bacteria account for approximately 69% of the cases of bacterial food-borne disease [5]. Turtura [6] reported that the most frequently coliform identified on meat were *Citrobacter freundii*, *Escherichia coli* and less frequently strains are of the genera *Klebsiella, Shigella sonnie* and *Proteus. E. coli and Staphylococcus aureus* are normal flora in human and animals, their presence in foods are indications of excessive human handling [5]. Members of the Gram negative bacteria e.g. *E. coli* are widely distributed in the environment are the major source for food contamination. The possible sources of these bacteria are skin of the animal the equipment used for each operation clothes and hands of personnel and the physical facilities themselves.

At present the occurrence of antibiotic resistant strains are great problems worldwide even though the use of antibiotics has been proven to be an effective means for the prevention and control of bacterial infection [7].

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In developing countries like Ethiopia Food-borne diseases occur commonly 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 [8]. Even if data regarding meat borne diseases in Ethiopia are extremely scarce, a few studies conducted in different parts of the country showed that pathogenic organisms like *Campylobacters*, *Salmonella*, *Taenia, Toxoplasma*, *Mycobacteria*, *Brucella*, *Escherichia coli*, *Echinococcos/hydatid*cysts were reported [9-17].

The demand for animal and animal products in the study site has been increasing rapidly while the available food centers especially the meat produced from butchery shops are not properly handled in such way that all measures necessary to ensure the safety, soundness and wholesomeness and processed in a hygienic manner. Previously there was no study conducted toassess food safety practice and food borne pathogens in butchery shops of Adigrat town, which hinders governments' to accurately apply measures on the impact of food contamination problems on public health. Therefore, to develop an effective data regarding the assessment of food safety practice and food borne pathogens in the butcher shops of the town, such studies could provide useful information. Therefore, the present study was designed:

To assess the quality of sold meat in abattoir and butcher shops of Adigrat and to investigate the antimicrobial susceptibility patterns of bacterial species recovered from meat of the study site.

MATERIALS AND METHODS

Study Area: The study was carried out from November 2011 to April 2012 in Adigrat butchery shops and municipality abattoir where thousands of cattle are brought from different woreda and kebele for slaughter which is located 115 Kms far on North East of Mekelle and 898 Kms North of Addis Ababa. Geographically Adigrat is located at latitude of 14° 16' North and 39° 27' East and it has an altitude of 2497 meters above sea level (m.a.s.l). The mean minimum and maximum annual rain fall is 400mm and 600 mm, respectively and the mean annual temperature have minimum and maximum values of 9.28 and 21.94 °C respectively. The rain fall pattern of the area is bimodal, with short rainy period from February to April and long rainy season from mid-June to end of August. Agro climatically Adigrat 80% middle land and 20% high land.

Study Design: A purposive sampling methodology design was employed to assess the meat quality and the source of contamination, in butcher shop of Adigrat. For this study meat from different sources namely abattoir, minced meat, hanged meat and refrigerated meat samples in butcher shops were taken for bacteriological analysis of meat with the intention of viable colony count, identifying pathogenic bacteria and investigating their antimicrobial susceptibility profiles.

Sample Collection: All the 20 butcher shops in the study area were selected purposively based on availability. 80 meat samples from different sources; abattoir, butcher shops hanging in the wall, minced meat in the table and refrigerated meat samples, 20 each were collected. The samples were collected aseptically in a clean polyethylene bag and transported immediately in icebox to Mekelle University, College of Veterinary. Medicine, for bacteriological analysis [18].

Enumeration of Total Viable Count: One gram of collected meat sample was weighted and transferred to sterile flasks containing 9 mL of normal saline solution (NSS) and the samples were homogenized using a meat grinder under aseptic conditions and was stored for further analysis.

Further 10 –fold dilution were prepared using 9mL NSS and 1mLfrom the homogenized meat samples. From the 10-fold dilutions of the homogenates; 1mLof 10^{-6}, $10^{-7}$and 10^{-8} dilutions of the homogenates were plated in on standard plate count agar, using pour plate method. The plates were then incubated at 37°C for 24 - 48h. At the end of the incubation period colonies were counted using the Quebec colony counter. The counts for each plate were expressed as colony forming unit of the suspension (CFU/g) [19].

Bacteriological Culture of Meat Samples: Bacteriological examination was done with some modification according to [20] and [21]. Pure bacterial colony was taken from PCA and streaked on tryptose blood agar base enriched with 7% defibrinated sheep blood (Oxoid, UK) and Mac Conkey agar (Oxoid, UK) plates. Both agar plates were incubated aerobically at 37°C for 24-48 h. Similarly; Mac

Conkey agar plates were examined for gross colony morphology and presence or absence of lactose fermentation. Lactose fermenter organisms were differentiated on the basis of their morphology and colour change in the medium. Pure culture colonies(from blood agar) were selected and sub cultured on nutrient agar (Oxoid, UK) and incubated aerobically at 37°C for 24 - 48 h for further biochemical identification.

Identificationof Gram Positive and Gram Negative Bacteria: The bacteria were identified using nutrient agar (NA) and peptone water (PW) as general and enriched media and other media with respective selective and differential characteristics. All media were prepared according to the manufacturer's specification and suspected. The samples were inoculated on Mac Conkey agar (MCA), Eosin Methylene blue agar (EMB) and Salmonella Shigella agar. The plates were incubated at 37°C for 24 - 48h. Discrete colonies were sub cultured into fresh agar plates aseptically to obtain pure cultures of the isolates. Pure isolates of resulting growth were then stored at 4°C and used for further identification using microbiological/biochemical methods [21]. Generally the identification bacteriaas Gram negative organisms include, Gram staining, growth on selective media (SS agar, & EMB agar) oxidase, citrate, indole, MR, VP,Citrate and Triple sugar iron test (TSI).

Antimicrobial Sensitivity Test: The disk diffusion method was used for antimicrobial sensitivity test. Susceptibility patterns of the isolated organisms were tested against a wide range of antibiotics and the test was conducted on the isolated or identified bacteria of *S. aureus*, *Staphylococcus* species other than *S. aureus,*Coliforms and non-coliform recovered during the study period. The isolates were tested for six antimicrobials using the Kirby-Bauer disk diffusion method [20, 22]. The following antimicrobial disks (Oxoid, UK) with their corresponding concentration were used: amoxicillin (AML, 2µg), tetracycline (TE, 30µg), streptomycin (S, 10µg), penicillin G (P, 10µg), polymyxinB (PB, 300µg) and gentamicin (CN, 10µg). The inhibition zone was reported as the diameter of the zone inhibition surrounding the individual disk in which bacterial growth was absent and the interpretation was made as per the zone inhibition size interpretation chart provided by CLSI [21].

Data Analysis: The date were entered in to Microsoft excel spread sheet and coded properly. Following coded the data was analyzed using Statistical Package for Social Sciences (SPSS 16). For data analysis descriptive statistics were used to present the findings. Mean of total viable count of microbial load in Adigrat, butchery shops were compared with one way ANOVA.P < 0.05 and 95% confidences interval (CI) were used to determine the statistical significance of explanatory variables (categories).

RESULT

Viable Bacterial Colony Count: The highest mean of total viable count of microbial load was observed in the meat sources that were sampled from hanged meat and minced meat. However, less microbial load were observed among the samples collected from abattoir and refrigerated meat (Table1).

Table 1: Mean of total viable count of microbial load in abattoir and butchery shops of Adigrat (in log 10).

Source of sample	No of sample	Mean ±SD	Minimum bacterial count	Maximum bacterial count	p-value
Abattoir	20	6.24±0.32	6.19	7.21	<0.001
Hanging in the wall	20	7.98±0.25	7.65	8.99	<0.001
Minced meat in the table	20	8.32±0.06	8.14	8.41	0.01
Refrigerator	20	6.02±0.03	6.00	6.12	< 0.001
Total	80	7.06±1.03	6.19	8.99	< 0.001

Table 2: Mean difference among the meat sample groups

Sample source	Sample source Groups	Mean difference among groups	p-value
Abattoir	Minced meat	-1.72	0.000
	Hanging meat	-2.06	0.000
	Refrigerated meat	-	0.001
Minced meat	Hanging meat	-3.35	0.000
	Refrigerated meat	1.9	0.000
Hanging meat	Refrigerated meat	2.06	0.000

The mean difference is significant at 0.05 levels

Table 3: Mean difference among the meat sample groups

		Meat Quality grade, No (%)		
Site of collection	Sample size	Acceptable	Marginally acceptable	Un acceptable
Abattoir	20	3(15%)	10(50%)	7(35%)
Meat hanging in the table	20	1(5%)	9(45%)	10(50%)
Minced meat in the table	20	2(10%)	6(30%)	12(60%)
Meat from refrigerator	20	5(25%)	12(60%)	3(15%)
Total	80	11(13.75%)	37(46.25%)	32(53.3%)

Table 4: Proportion of bacteria isolated from the different meat sources

	No(%) of isolated bacteria			
Isolated bacteria	Abattoir meat	Hanging meat	Minced meat	Refrigerated meat
E.coli	3(15%)	6(30%)	6(30%)	3(15%)
Other Coli form bacteria	0	1(5%)	1(5%)	2(10%)
Non-Lactose fermenters	7(35%)	5(25%)	5(25%)	0
S.aureus	0	6(30%)	6(30%)	3(15%)
Streptococcus species	3(15%)	1(5%)	1(5%)	0
Total No (%)	16(25.8%)	19(30.6%)	19(30.6%)	8(12.9%)

Table 5: Antimicrobial resistance profiles for *S. aureus* and *Streptococcus* spp.

Antimicrobials	Resistant	Intermediate	Susceptible
	No (%)	No (%)	No (%)
Ampicillin	6(100)	0	0
Streptomycin	0	0	6(100)
Gentamicin	0	0	6(100)
PolymyxinB	0	6(100)	0
Tetracycline	0	6(100)	0
PenicillinG	4(66.6)	2(33.3)	0

Table 6: Antimicrobial resistance profiles for *E.coli*, other coliform and nonoliform bacteria

Antimicrobial	Resistant	Intermediate	Susceptible
Ampicillin	No (%)	No (%)	No (%)
	14(100)	0	0
Streptomycin	0	1(7.1)	13(92.9)
Gentamicin	0	0	14(100)
Polymyxin B	10(72)	4(28)	0
Tetracycline	0(100)	11(78.5)	3(21.5)
Penicillin G	14(100)	0	0

The level of bacterial contaminations in the meat processing was highest when the meat was hanged and minced but lower bacterial contamination value on abattoir and refrigerated meats were observed.

Based up on the Indian raw meat bacteriological standard, the quality of meat samples collected from the abattoir, hanged meat, minced meat and from refrigerated meat were summarized in Table 3.

Major Bacterial Species Isolated: The non-lactose fermenting bacteria were the predominant isolate in abattoir with rates of 7(35%) followed by *S. aureus*, *Streptococci* and *E. coli*with similar rates each i.e. 3(15%) In addition, the predominant bacteria isolated from butcher shops hanged meat and minced meat were *S. aureus* and *E. coli* with the rate 6(30%) each, followed by the non-lactose fermenters (25%) and the least bacteria were *Streptococci*. Butin case of refrigerator meat *S. aureus* and *E.coli* 3(15%) and other coliform bacteria 2(10%) were isolated.

Antimicrobial Susceptibility Test: Tables 5 and 6 summarize the antimicrobial resistance profiles of the different bacterial isolates recovered in the present study. Out of the 62 bacterial isolates recovered in the present study antimicrobial susceptibility tests were performed on a total of 20 bacterial isolates (3 *Staphylococcus, 3 Streptococcus* species and 14 *Enterobacteriacae)*

DISCUSSION

The study revealed that bacterial contaminants were grown in all meat samples collected from butchery shops (abattoir, hanging meat, minced meat and refrigerated meat) with the highest rate of isolation being from hanging meatand minced meat. The presence of these organisms on meat parts could be attributed to the fact that meat contains an abundance of all nutrients required for the growth of bacteria in adequate quantity.High mean values of microbial load ($5.5x10^7$) were found in hanging meat and ($6.5x10^7$) in the minced meat. This might be due to high exposure to dusts from the environment. However lower mean values of microbial load (3×10^6) were found in meat from refrigerated. There is significance difference in the bacterial loads of meat sourced from different sites where the highest bacterial load were found in minced meat ($p<0.05$). [4] and [24] reported that the highest contamination was observed in minced meat as compared to the other sources of meat. The highest mean value of the microbial loads in minced meat exceeds the FAO/WHO standard limit for food products and water. The current findings were also in agreement with that of. [25] who reported the presence of high mean values of microbial load of table scrapings from meat stalls in Ibadan metropolis, Nigeria.

Similarly, A total of 62 isolates comprising of 8 different genera of Gram negative and Gram positive bacteria wereisolated in this study with an average rate of 25.8% in abattoir, 30.6%, in both hangingmeatand minced meat and 12.9% in refrigerated meat. This showed that all the meat in the butcher shops and abattoir were contributed differently to the microbial diversity reported in this study. The bacteria isolates were identified as *E.coli*, other coliform, *S.aureus*, *Streptococcus* spp.andnon-lactose fermenter bacteria which were similar with the finding of [26] and [27].

The presence of these organisms in fresh meats depicts a deplorable state of poor hygienicand sanitary practices employed in the slaughtering, processing and packaging of fresh meats. From the results obtained, fresh meats sample were contaminated with high level of *E.coli*, other coliformbacteria, *S.aureus*, *Streptococcus*, other *staphylococcus* species andnon-lactose fermenters. This agrees with previous reports of.[5] and [28] which stated that these organisms are the main sources of contamination.

The isolation of non-lactose fermenter bacteria may be as a result of poor environmental conditions due to dust and contamination of the water used during slaughtering, because some of the non-lactose fermenter bacteria are also inhabitants of dairy products, as reported by [29] and also a pathogenic organism of public health significance and concerns. Similarly, the current finding is in agreement with [30] who reported that foods of animal origin (minced meat) either cooked or uncooked were predominantly contaminated with *E. coli*.

The current finding indicated that some of the isolated bacteria are resistant to some of the antibiotics. The problem may be due to the natural resistance of bacterial species to certain antibiotics [31], possible transfer of antibiotic resistance among species and the use of sub-therapeutic doses of antibiotics in animal feeds to improve animal productivity, which could also select for resistant strains [32].

CONCLUSION

From the current finding we can conclude that there is high level of bacterial contamination from the different meat sources due to poor personal hygiene and environmental contamination. The highest load of bacterial was found in butcher houses compared to other settings. The place where these meats are kept, use of open housing during selling might be the possible source for the occurrence of contamination and most of the Gram positive and Gram negative bacteria were susceptible to the commonly used antibiotics whereas only few of them were resistant. If measures are not put in place, there may be a possible outbreak of food poisoning and or food borne infections due to consumption of the contaminated meat. This may lead to serious economic and public health problem.Hence based on these findings the following points are recommended

- Awareness creation to butcher shop workers regarding meat hygiene is essential.
- Currently used antibiotics should be checked for their efficacy and species specific.
- Meat inspection should be strengthened by veterinary professionals in the town before and after slaughtering and before the meat is distributed to the general public;
- Good meat handling practices should be adhered strictly by butchers and those selling the meat

- Water used in washing the meat should be sterile and also the equipment must be washed properly before use

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