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# Evaluation of Microbial Load of Beef of Arsi Cattle in Adama Town, Oromia, Ethiopia

## Arse Gebeyehu<sup>1\*</sup>, Mohammed Yousuf<sup>2</sup> and Ameha Sebsibe<sup>3</sup>

<sup>1</sup>Adami Tulu Agricultural Research Center, P.o.box 35, Batu, East Shoa, Ethiopia <sup>2</sup>Haramaya University, P.o.box 138, Dire Dawa, Ethiopia <sup>3</sup>Food and Agricultural Organization, Ethiopia, P.o.box 5536, Addis Ababa, Ethiopia

#### Abstract

The study was conducted in Adama town, on carcass samples collected from the Arsi breed of cattle with the objective of evaluating beef microbiological qualities with standard procedures. Carcass samples were randomly chosen at abattoir using systematic random sampling techniques. On the night carcass samples were aseptically excised and collected from all parts of the exposed body of carcasses. The methods described by the Nordic Committee on Food Analysis (NMKL) were adopted to analyze each of the parameters considered. Aerobic plate count, total coliform count and fecal coliform counts were significantly different among different sampling days and batches of samples (P<0.05). The mean AP, Total coliform, Fecal coliform, E. coli and staphylococci counts were 1.62×10<sup>5</sup>, 5.29×10<sup>1</sup>, 9.05×10<sup>1</sup>, 8.97×10<sup>1</sup> and 5.54×10<sup>5</sup>, respectively. Salmonella and Shigella bacteria were not isolated per 25 g samples. In Adama, carcasses are normally transported to the butchers' shop either in vans, minibus, taxi, three wheel motor cycle and horse-cart. This exposes the meat to a number of pathogens some of which may be pathogenic. Therefore, since the general hygienic condition of the abattoir and carcass harvesting process was poor, beef consumers in Adama are advised to eat properly cooked beef to avoid intoxication due microbes.

**Keywords:** Aerobic plate count; Total coliform count; Fecal coliform count; E. coli and Staphylococci count

#### Introduction

Ethiopia is one of the top possessors of livestock in Africa with an estimated population of 52.13 million, 24.22 million, and 22.62 million heads of cattle, sheep and goats, respectively (CSA, 2012). This livestock is playing an important role in the national economy, contributing to both agricultural products and national GDP [1-3]. But the contribution of livestock and livestock products export to earn foreign exchange is not too large due to the fact that the country is not fully exploiting this resource. Several studies reveal that the production and productivity of the animal is low and products being produced are of low quality [1].

According to MoFED [4] annual report, the national economy is growing in not less than double digits. This economic growth is likely to stimulate people's interest to high quality and value added products [5]. Education and economic prosperity makes people more sensitive and selective to matters they use particularly healthier consumption of foods [6]. In virtue of this, meat commercialization has a great potential to become a key source of income to smallholders of Ethiopia.

As a result, in recent years feedlot firms are flourishing and getting engaged in the export of processed meat to the Middle East and North African (MENA) and absorbing foreign currency from international markets. Standards, such as acceptable microbial load level are being adopted by almost all international markets [7,8]. According to the World Bank [9] report, Ethiopian meat production and marketing has been plagued by lack of quality and sanitation, prevalence of disease and unqualified meat production process.

Several scientists have indicated the importance of continuous assessment on meat microbial load qualities. Kirton, Herenda and von-Braun [7,10-11] recommend the continuous investigation and inspection of meat to provide safe and wholesome meat for human consumption. As far as microbial load level of Ethiopian meat is concerned there is limited information on the microbial quality of Ethiopian beef that is being retailed in different outlets. Plus, the need for assuring safety cannot be underestimated in this modern world. Therefore, this study was conducted to assess the sanitary conditions of beef cuts being retailed in various butcheries (from slaughterhouse to butcheries) of Adama town.

## Materials and Methods

## Study area

This study was conducted in Adama town, Ethiopia. Adama town is situated on the high way from Addis Ababa city to Harar. Adama has an altitude of 1666 m above sea level and located at 100 km east of Addis Ababa. According to CSA (2012) population census report, Adama town has 271,562 residents. Adama is situated in the center of the country and more frequently visited by national and international tourists. The municipality of Adama town has its own abattoir that gives service to towns' community. The abattoir is semi-modern that operates with services featuring cattle, sheep and goat slaughtering. The annual average minimum and maximum temperatures of Adama town are 18°C and 32°C [12,13]. The specific geographical location of the towns' abattoir is on the geographic coordinates of: 8° 33' 05.79'' N and 39° 15' 34.83'' E. The altitude of the abattoir is 1639 meter above sea level [14].

\*Corresponding author: Arse Gebeyehu, Adami Tulu Agricultural Research Center, P.o.box 35, Batu, East Shoa, Ethiopia, E-mail: arse.gebeyehu@yahoo.com

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## Sample collection and preparations

A total of 12 carcass samples were collected from 12 cattle slaughtered in the slaughterhouse of Adama town. The carcass samples were collected in two non-consecutive sampling days. The samples were collected by systematic random sampling techniques. In the first day of sampling a total of 63 cattle were slaughtered and the carcass samples were taken on every 10 counts thus 6 designate beef cattle were tagged in the abattoir and the samples of beef were collected from the beef owners (butcher) on the other day. In the second day of sampling a total of 62 cattle were slaughtered and the carcass samples were taken on every 10 counts thus 6 designate beef cattle were tagged in the abattoir and the samples of beef were collected from the beef owners (butcher) on the other day. Each sample weighs a kilogram. The samples were collected by the classical method of excision from all portions of the carcass [15]. The excision method was preferred over wool swab as the numbers of bacteria recovered by excision sampling exceed between 50% and 90% of those recovered by cotton wool swabs [16]. The samples were collected aseptically into sterile polyethylene bags and transferred immediately to the laboratory. The samples were collected in two non-consecutive days of sampling. The abattoir personnel were not informed about the sampling of carcass so that they continue as normal [17].

The methods described by the Nordic Committee on Food Analysis (NMKL) were adopted to analyze each of the parameters considered. Ten grams of each beef sample and 90ml of normal sterile saline water was homogenized in a stomacher bag blender for 1-3 minutes. Appropriate dilutions (0.1 and 0.01 ml) were made and plated on the following media for microbial count.

### Detection of aerobic plates

One millilitre of each dilution was poured into a sterile Petri dish and about 15 ml of molten plate count agar (PCA) was added. The inoculum was evenly spread by gently shaking and left to solidify for 10-15 minutes. Then the plates were incubated at 30°C for 72 hours. Colonies on selected plates were counted using a colony counter [18].

### Detection of Staphylococcus spp

One millilitre of each dilution was pipetted into a Petri dish that contains Baird-parker agar plates. The inoculum was evenly spread by shaking and incubated in incubators for 24 and 48 hours at  $37.0 \pm 1.0^{\circ}$ C. Typical colonies of *Staphylococcus spp* (black or grey, shining and convex), diameter 1.0-1.5 mm after 24 hours incubation and 1.5-2.5 mm after 48 hours incubation and with each colony surrounded by a clear zone were isolated and tested for coagulase positive as a confirmatory test and finally recorded [18].

#### Detection of total coliform and fecal coliform

Total *coliform* and *fecal coliform* were detected by transferring 1.0 ml of sample from each dilution into sterile Petri dish and 15 ml of molten and cooled Violet Red Bile agar were added. The inoculum was evenly mixed by shaking and left to solidify and incubated the plates at  $37^{\circ}$ C and  $44.5^{\circ}$ C for  $24 \pm 2$  hours. The red colonies were counted and then confirmed by transferring the selected five colonies to BGB and E.C Broth by their gas production [18].

#### Detection of Escherichia coli

Suspected colonies of the fecal *coliform* were confirmed in E.C Broth at 44°C for 24 hours with the production of gas, after which one loop of the positive tube were transferred into Tryptone water and incubated at 44°C for 48 hours 3 drops of Kovac's reagent was added to the test culture and observed for any reaction. Formation of red colour indicated a positive reaction, thereby confirms the presence of *Escherichia coli*, and recorded those organisms producing red ring as indole positive [19].

#### Detection of Salmonella and Shigella spp

The NMKL method No 71 was used to isolate and enumerate the *salmonella* and *shigella* spp. (Figure 1).

#### Experimental design, model and statistical analysis

The experimental design of the study was Completely Randomized Design. The General Linear Model procedure of SAS [20] was employed to analyse the variation between days and batch samples. Mean separation was done by Duncan Multiple Range Test. The following model was used:  $Y_{ijk} = \mu + \alpha_i + e_{ijk}$ 

Where;  $Y_{ii}$  = the response variable,

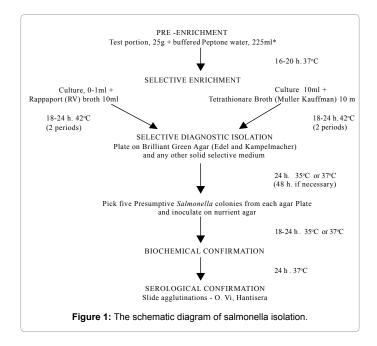
 $\mu$  = Overall mean common to all observation,

 $\alpha_i = \text{treatment effect},$ 

e<sub>iik =</sub> Random error

## **Results and Discussion**

The overall F-test of model for *Aerobic plate* (AP), Total *Coliform*, Fecal *coliform*, *E. coli* and *staphylococci* counts variation across days and batches are given in table 1 and were significantly varied between days of sampling and batches of same day sample (P<0.05). The variation of these microorganisms in days and batches of same day may indicate the existence of non-consistent unhygienic condition attributed to lack of standard operation procedure that help to control processing systems in the abattoir. Variations in microbial load on fresh carcasses between sampling days are also reported by several researchers. This report is in good agreement with the report of Charlebois et al. [21] that indicated significant differences in mean fecal *coliform* counts between the days and sampling location such as front and hind quarters. Significantly higher variation of AP, fecal *coliform* and *E. coli* load between days of



The present report is also in agreement with Koffi-Nevry et al. [23] who reported a statistically significant difference (P<0.05) in AP, Total *coliform*, Fecal *coliform*, *E. coli* and *Staphylococci* counts were found between days and samples. The present findings were also comparable with a report of Bosilevac et al. [24] that the prevalence of *E. coli* O157 and Aerobic bacteria varied in days. Cason et al. [25] also reported a significant difference in *E. coli* counts between the left and right halves. This means that to some extent the degree of microbial load on carcass depend on the care and skill of personnel handling the carcasses.

The Least Square Means of microbial load between sampling days and batches are presented in table 1. In all considered microbes, day 2 had significantly exceeded day 1, this imply that microbial load varies from day to day indicating inconsistently unhygienic condition that prevail in the abattoirs. A inconsistently unhygienic condition may be attributed to continuously firing and hiring new unskilled labor forces and lack of training on carcass handling to minimize contamination in addition to lack of stringent quality control system such as GHP and HACCP procedure.

## Implication of microbial load

The mean microbial load counts on beef are given in table 2. The mean AP, Total *coliform*, Fecal *coliform*, *E. coli* and *staphylococci* counts were  $1.62 \times 10^5$ ,  $5.29 \times 10^1$ ,  $9.05 \times 10^1$ ,  $8.97 \times 10^1$  and  $5.54 \times 10^5$  respectively. All counts exceeded the established standard microbiological contents of beef set by different countries. *Salmonella* and *shigella* bacteria were not isolated per 25 g samples. This report on AP is comparable with Mezgebu and Mogessie [26] that reported an aerobic mesophilic counts that ranged from  $2 \times 10^7$  to  $2 \times 10^8$  cfu/g in the traditional minced beef locally called 'kitfo'. But AP in current report is higher than that of reported by Badrie et al. and Clarence et al. [27, 28] reported AP ranging from  $10^2 - 10^5$  cfu/g and  $3.02 \times 10^3 - 5.01 \times 10^3$ cfu/g in beef investigated in Trinidad and Tobago and Benin City of Nigerian, respectively. This difference may be due to lack of quality control system in the abattoirs. But as high as  $8.5 \times 10^5 - 8.7 \times 10^{10}$  cfu/g AP on beef was also reported by Mukhopadhyay et al. [29] in India.

Aerobic plate count is a general measure of the microbiological status of meat [8].

According to FAO [30] microbiological standard, the safe level of aerobic plate count is less than  $1\times10^4$  cfu/g and the critical condition lies between  $1\times10^4$  -  $1\times10^5$  cfu/g and more than  $1\times10^5$  cfu/g is not acceptable. According to Anon [8] to confirm beef carcass as safe the AP in all five samples sampled must be less than  $5\times10^6$  cfu/g and

three samples must be less than  $5 \times 10^5$  cfu/g. AP below  $\times 10^5$  cfu/g is acceptable [8]. In the republic of Trinidad and Tobago the acceptable limits of AP is less than  $1 \times 10^5$  cfu/g [27].

The mean *Staphylococcus spp* count in the studied beef sample is about  $5.5 \times 10^5$  cfu/g. This finding is comparable with  $3.3 \times 10^5$  -  $9.12 \times 10^6$ cfu/g reported by Mezgebu and Mogessie [26] in Addis Ababa beef serving restaurants. This report is not agreement with the claim of Adams and Moss [31] that the presences of *Staphylococcus aureus* are usually in small number in normal condition. *Staphylococcus spp* count is also higher than that of reported by Clarence et al. [28]  $3 \times 10^3$  - $1.8 \times 10^4$  cfu/g of beef investigated in Benin City metropolis of Nigerian. According to NACMCF [32] *Staphylococcus spp* level about  $1 \times 10^5$  cfu/g is regarded as product is clearly unsafe for human consumption. In Trinidad and Tobago republic, the acceptable limit of *Staphylococcus spp* is less than  $1 \times 10^2$  cfu/g [27].

The mean *E. coli* in beef sample studied was  $8.97 \times 10^{1}$  cfu/g. This is found to be lower when compared with Badrie et al. and Clarence et al. [27,28] that reported  $3.1 \times 10^{2}$  and  $3 \times 10^{3} - 5 \times 10^{3}$  in meats sold in street and kiosks respectively. It is in good agreement with Eisel et al. [33] that reported *E. coli* of  $1 \times 10^{1} - 1 \times 10^{2}$  cfu/g. Biswas et al. [34] claim that *E. coli* are significantly high in beef than in other type of meat. *E. coli* and *Staphylococcus spp* are disease causing organisms [17]. The acceptable limit of *E. coli* in meat is less than  $1 \times 10^{2}$  cfu/g in Republic of Trinidad and Tobago [27]. According to Anon [8] to confirm beef carcass as safe *E. coli* prevalence in all five samples taken at a time must be less than  $5 \times 10^{3}$  cfu/g or three samples must be less than  $5 \times 10^{2}$  cfu/g. *E. coli* and *Coliform* counts indicate processing hygiene [35]. Sources of meat contamination during slaughter are from animal, processing practices, abattoir facilities and employees [35,16].

The mean total *coliform* count in fresh carcass sample was 5.3×10<sup>1</sup> cfu/g. This finding is in agreement with Eisel et al. and Stopforth et al. [33,36] that reported 2.5×101 - 1.58×103 cfu/g and 1.2×101 - 6.3×101 cfu/g for total coliform count in fresh beef samples, respectively. The present finding's figure is much lower than  $4.4 \times 10^3$  -  $4.8 \times 10^5$  cfu/g reported by Mezgebu and Mogessie [26]. Kornacki [37] claims that coliforms are often best indicator of the hygienic conditions of carcass production processing and handling systems. Therefore, high total coliform obtained in this study might be attributed to poor hygienic condition and product handling practices leading to contamination [38]. The prevalence of fecal *coliform* count in the fresh beef carcass was 9.05×10<sup>1</sup> cfu/g. Yalcin et al. [39] reported mean fecal coliform counts of the different sites of the carcasses were 3.16 - 8.9 cfu/cm. All the counts exceeded the standard microbiological criteria set by NACMCF (National Advisory Committee on Microbiological Criteria for Foods) for beef meat.

Variables	APC(cfu in log <sub>10</sub> )	TCC(cfu in log <sub>10</sub> )	FCC(cfu in log <sub>10</sub> )	ECC(cfu in log <sub>10</sub> )	StC(cfu in log <sub>10</sub> )
Days	*	***	Ns	ns	*
Day 1	4.945 <sup>b</sup>	1.126⁵	1.653	1.649	5.481 <sup>b</sup>
Day 2	5.474ª	2.320ª	2.260	2.256	6.006ª
Batches	***	***	***	***	***
Sample 1	4.977 <sup>b</sup>	1.255 <sup>b</sup>	2.312ª	2.299ª	4.977 <sup>b</sup>
Sample 7	4.913℃	0.998°	0.994 <sup>b</sup>	0.998 <sup>b</sup>	5.984ª
Sample 8	5.984ª	2.299ª	2.221ª	2.221ª	5.998ª
Sample 9	4.963 <sup>b</sup>	2.341ª	2.301ª	2.292ª	6.013ª
CV (%)	0.288	2.239	2.927	2.097	0.492

Ns = Nonsignifican at P > 0.05, \* = P < 0.05, cfu = colony forming unit,  $\log_{10}$  = to common logarithm, APC = Aerobic plate count, TCC = Total *coliform* count, FCC = Fecal *coliform* count, StC = *Staphylococci* counts. Means with the same letter in the same column are not significantly different at P > 0.05.

 Table 1: Effect of days and batches on microbial load of carcasses (LS means).

According to this study Salmonella spp and Shigella spp were not isolated in meat. Biswas et al. [34] claimed that Salmonella spp is the primary microbial challenge for poultry. But Amenu [40] reported 30% of beef produced in Arba Minch town's abattoir and beef retailing outlets (in Ethiopia) has salmonella strains. This high prevalence rate could be due to the fact that most of the Ethiopian carcass dressers in the rural areas and retailing butchers in rural town have home reared poultry and may have carried the Salmonella strains and transferred it to carcass. But the Adama case could be different because it is mega town with no or less number of home reared poultry in the town. To judge meat as satisfactory or acceptable regarding Salmonella the prevalence should be less than 4% or less that 2/50 sample [8]. According to Badrie et al. [27] in Trinidad and Tobago republic, the acceptable limit of Salmonella is zero per 25g sample. Haimanot et al. [41] report indicates that 60% of Ethiopian butchers and 100% of Ethiopian abattoir were found to be positive for pathogenic micro organisms.

#### Indicator micro-organisms

Table 3 shows the correlation degrees between the microorganisms. There was no significant correlation between AP and total coliform counts (P>0.2). This report is not in agreement with Cason et al. [25] that reported a correlation coefficient of 0.69 between AP and total *coliform* (P<0.0001) implying further study is needed to prove by increasing sample size and colony counting accuracy. But is in good agreement with Cason et al. [25] report of a correlation coefficients of 0.69 and 0.39 between E. coli and coliform and between AP and E. coli (P<0.0001) respectively. Significant correlation between E.coli and AP is also reported by Kornacki [37] that E.coli were highly correlated with AP in bovine carcasses. This means, if there is significant correlation between the microbes one of the other could be examined which could indicate the likely existence of the correlated microbe. Examining the indicator microbe will definitely minimize costs and laborious laboratory work. Thus the one used to examine about the other is called indicator. For example, E. coli is used as an indicator organism for the possible presence of pathogens like coliforms and fecal-coliforms. E. coli are particularly useful as indicator of contamination [42].

The routine monitoring for all the possibilities is either impossible or impractical [43]. The solution to the problem has been the use of indicator bacteria that would be present when potential pathogen

Variables	N	Mean count cfu/g	Log Mean	SD
Aerobic Plate count	12	1.62x10⁵	5.210	0.468
Total coliform count	12	5.29x10 <sup>1</sup>	1.723	0.631
Fecal coliform count	12	9.05x10 <sup>1</sup>	1.957	0.583
E-coli count	12	8.97x10 <sup>1</sup>	1.953	0.577
Staphylococcus count	12	5.54x10⁵	5.744	0.463

Salmonella spp and shigella spp are not isolated per 25g samples of beef, SD = standard deviation.

Table 2: Mean microbial load of beef in colony forming unit and at common log.

Variables	APC	T coliform	F coliform	E-coli	Staphylococ.spp
APC	1	0.57	0.32	0.32	0.29
Total coliform		1	0.66	0.66	0.46
Fecal coliform			1	0.99	-0.34
E-coli				1	-0.34
Staphylococ.spp					1

ns = non-significant correlation, APC= aerobic plate count, *E-coli* = Escherichia coli, staphylococc = staphylococci, F *coliform* = fecal *coliform*, T *coliform* = Total *coliform* 

Table 3: Correlation matrix between the common micro-organisms in beef.

containing material was present. Fecal *coliform* bacteria are used as indicators of fecal contamination and of the potential presence of pathogens [44]. In addition, the use of indicators is attractive because it reduces the complexity and cost of analyzing samples for individual pathogens [44]. Total *coliform*, fecal *coliform*, and *E. coli* are all indicators of wholesomeness of products [45]. *Coliform* bacteria will not always likely cause illness [45]. But the presence of *coliform* bacteria in product indicates that other disease-causing organisms (pathogens) may be present in the production system. The presence of fecal *coliforms* is indicative of fecal contamination and of the potential presence of enteric pathogens especially bacterial pathogens [44]. Thus, the above microbial load table 2 indicates the presence of health threatful microbe on the meat produced in Adama town. To avoid such threat meat products should be fried with optimum cooking temperature.

### Summary

This study was conducted to explore the overall microbiological quality of beef in Adama town, Oromia region, Ethiopia. The study entails the specific objectives of investigating microbiological qualities of beef from Arsi cattle. Aerobic Plate, Total Coliform, Fecal coliform, E. coli and Staphylococci counts were significantly varying between days and batches (P<0.05). The variation of these microorganisms between days and batches of same day may indicate the existence of inconsistent unhygienic carcass harvesting practices and this condition is attributed to lack of the basic Good Hygienic Practices (GHP) procedure in the abattoir. In addition to this, abattoir staffs are not given any training on carcass handling to prevent excessive contamination. The mean AP, total coliform count, fecal coliform count, E. coli count and staphylococci count were 1.62×105, 5.29×101, 9.05×101, 8.97×101 and 5.54×105 respectively. All counts exceeded the already established standard microbiological contents of beef set by different countries. Salmonella and shigella bacteria were not isolated per 25 g samples.

The abattoirs have weak supervision and inspection by higher officials. Municipality abattoirs are operating in poor hygienic and traditional system. Generally, the studied abattoirs had poor meat producing and processing facilities. Meats are normally transported to the butchers' shop either in vans, minibus, taxi, three wheel motor cycle and horse-cart. This exposes the meat to a number of pathogens some of which may be pathogenic. The high load of microorganisms could also be the result of unhygienic handling and processing using (in butchers' shop) unclean knives, cutting boards, and storage ladder added to the poor hygienic status of food handlers. The high number of staphylococci, which is usually related to human skin and clothing is indicative of this situation.

## Recommendation

Based on the findings of the study, the following recommendations are forwarded.

Obviously, in Ethiopian big towns' a large number of beef consumers prefer to eat raw beef but since general hygienic conditions of the abattoirs and carcass production, the process of poor beef is not microbiologically wholesome. Consumers in Adama are advised to cook beef appropriately to avoid intoxication due to microbes.

The study of microbial load demonstrated the importance of sanitation of retail beef cuts. The abattoirs have to put due attention on the ways that minimize contamination during carcass harvesting by following modern sanitation procedures and implementing stricter operation laws. The GHP/HACCP procedure can help to prevent or minimize biological contamination. Further research work is needed to characterize the microbiological quality of beef being produced from other Ethiopian breed cattle.

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