

Bacteriological Quality of Raw Beef Collected from Municipality Slaughterhouse and Local Markets in and around Wolaita Soddo Town, Southern Ethiopia

Research Article

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Abstract

A cross sectional study was conducted from October, 2011 to May 2012 in Wolaita Soddo town and suburbs to determine the microbiological quality, source of contamination and sanitary and hygienic condition of meat selling environment. A total of 260 raw beef samples were randomly collected out these 55 from Soddo Municipality Slaughterhouse, 55 from restaurants/hotels, 50 from Boditte local retail market, 50 from Humbo local retail market and 50 from Baqullo Sagno market. 250 g of each meat sample was collected in sterile polythene bags, packed in a box embedded with ice packs. The samples were analyzed for the microbiological quality; standard plate count and isolation and confirmation of *Staphylococcus aureus* by selective plating, microscopic examination and biochemical characterization. The mean aerobic plate count in beef was slightly higher in butchers shop (restaurants/hotels) (2.82×10^6) than slaughterhouse in sodo (2.28×10^6). Similarly the coliform count in meat sample from butchers shop (restaurants/hotels) (3.73×10^4 cfu/g) were to some extent larger than slaughterhouse in Sodo (3.36×10^4 cfu/g). The highest aerobic plate count in meat samples from local retail market were in Baqullo Sagno market (7.45×10^6 cfu/g). The mean coliform count from raw beef sample in Humbo local retail market (6.13×10^4 cfu/g) were found considerably higher than other places. There were no significant ($p > 0.05$) variation in the means of aerobic plate and coliform count found in raw beef from different locations. The prevalence of *Staphylococcus aureus* in was highest Humbo local retail market 28 % followed by Baqullo sagno market 22 % and raw beef from butchers shop (restaurants/hotels) 21.8 %. The results indicated that the microbiological quality of the raw beef samples analyzed was unsatisfactory, and could be an important cause of food poisoning. Good manufacturing practices (GMP) for slaughtering and processing of raw beef should be accepted as strategies to control pathogenic microbes which pose health risks to humans or which pose public health risks.

Keywords: Aerobic Plate Count; Coliform Counts; Raw Beef; *Staphylococcus Aureus*; Wolaita Soddo.

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Background

Food borne infections and illnesses is a major international health problem with consequent economic reduction. It is a major cause of illness and death worldwide [1]. According to Clarence *et al.* (2009), food borne diseases are diseases resulting from ingestion of bacteria, toxins and cells produced by microorganisms present

in food. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individuals to the toxin [10].

The meat is potentially subjected to contamination from a range of sources within and outside animal during the slaughter of animal and during its sale. In fact, tissue from healthy animal are sterile however, it has been pointed that during slaughter, dressing and cutting, microorganisms came chiefly from the exterior of the animal mainly from the hide of the animal and the faeces and its intestinal tract but that more added from knives, cloths, air, carts and equipment in general [18]. The place of slaughter, the environment of the slaughter house [23], the floor of the retail outlet, the air in the outlet and the vehicle used for the transport of the meat from the slaughter house to the retail outlet act as the external sources for the contamination of the meat [26].

Food borne microbiologic hazards may be responsible for as many cases of illness as possible each year and are thus an important food safety challenge. To lower the incidence of food borne disease, many experts and stakeholders urge the development of a science-and risk-based food safety system, in which decision makers prioritize hazards and interventions using the best available data on the distribution and reduction of risks [6]. Such a system requires an understanding of the many risk factors between the point of production and the point of consumption and the ability

to systematically target intervention efforts along this "farm-to-fork" continuum [6].

In Ethiopia, the wide spread habit of raw beef consumption is a potential cause for food borne illnesses besides, the common factors such as overcrowding, poverty, inadequate sanitary conditions, and poor general hygiene. Raw meat is available in open-air local retail shops without appropriate temperature control and this is purchased by households; and also minced meat (Kitfo) is served at restaurants as raw, slightly-cooked or well-cooked. In addition, outbreaks of infections somehow related with poor hygiene and consumption of contaminated food were reported in Ethiopia [15]. In spite of huge production of meat in Ethiopia, quality control is either lacking or poorly developed [4]. Most of the abattoirs are traditionally operated without quality control systems.

Meat processing units are also generally not equipped with quality control system in the country. People of Wolaita sodo and its surrounding are well known for their raw meat consumption either in the form of minced meat (Kitfo) or big slices of meat (Kurt). However, there is no or limited information available on microbiological quality of raw meat and the health challenges from food borne diseases in these highly populous community. Therefore, the study was conducted to assess the bacteriological quality of raw beef at the study area by using total aerobic viable bacteria count, coliform count and by identification of *Staphylococcus aureus* in fresh ready to eat raw bovine meat. And to determine hygienic situation and condition of beef marketing, handling facilities, the selling environment and to identify the possible sources of contamination.

Materials and Methods

Study design

A cross sectional survey was conducted at Wolaita Soddo town and surrounding area of the southern nation's nationalities regional state from October, 2011 to May 2012. The microbial quality raw beef and the general hygienic and sanitation of the beef selling environments were evaluated through observation. The microbial evaluation of the raw beef sample was conducted at Diagnostic Veterinary Laboratory which is located at Wolaita Soddo town, South Ethiopia.

Sampling methods

According to Thrusfield (2005), a sampling frame is a list of all the units within the study area from which samples are taken. In this study slaughterhouse, restaurants/hotels and open local retail raw beef markets were sampling frames. Raw beef from municipality slaughterhouse, from randomly selected restaurants and hotels and from randomly selected popular open local retail markets were collected. Restaurants/hotels which sell ready to eat raw beef in Wolaita Soddo town were selected by simple random sampling. And three popular open retail markets were also purposively selected based on accessibility and popularity which includes Boditte local retail market, Humbo local retail market and Bqullo sagno local retail market.

Collection and transportation of raw beef samples

A total of 260 raw beef samples which included 55 raw beef sam-

ple from Wolaita Soddo Municipal Abattoir, 55 raw beef samples from restaurants/hotels of Wolaita Soddo town which sell raw beef (ready to be eaten), 50 raw beef samples from Boditte retail market, 50 raw beef samples from Humbo retail market and 50 raw beef samples from Bqullo sagno retail market were collected. The samples from local retail markets were obtained from the surrounding areas of Wolaita Soddo in local market days which were practiced by the local peoples. This is because in these local market days known as "yegebeya keni" the raw beef was displayed in open markets for sell and raw beef in some rural areas originated from back yard slaughtering operation. The samples from slaughterhouse, restaurants/hotels and from local markets were obtained in the morning as much as possible within few hours of post-slaughter in order to minimize the microbial changes due to environmental temperatures and post-slaughter timings. 250 g of each meat sample was collected in sterile polythene bags, packed in a box embedded with ice packs and were transported to the Microbiology Laboratory of Diagnostic Veterinary Laboratory of Wolaita Soddo. The samples were processed within 24 hours after bringing to the laboratory. Each raw beef sample was chopped by the butcher and treated as any food purchased by a consumer. Due to resource, financial and laboratory facility limitations the samples are analyzed using aerobic plate count, coliform count and evaluated for the presence of *staphylococcus aureus* only.

Sample preparation for bacteriological examination

At the laboratory from each beef samples in polyethylene bag 10 gram of each sample was weighed into a mortar (that had been previously sterilized) and ground with a sterile pestle until it became smooth and 90 ml of sterile distilled water was poured into the mortar. After maceration, appropriate serial dilutions was made, which was transferred to a test-tube followed by serial dilution up to 10^{-7} dilution. Serial dilutions will be prepared in the usual way to a dilution of 10^{-7} using 9 ml aliquots of sterile distilled water. This dilution was made to express microbial counts as CFU/g or \log_{10} CFU/g. For bacteriological examination of raw beef samples, the methods used by Uzeh and co-workers (2006) and Rindhe and co-workers (2008) were used.

Enumeration of aerobic plate count

To determine total viable counts, 1 ml of prepared test sample of each of 10^{-5} and 10^{-7} dilutions were pipetted and plated on nutrient agar plates in triplicates using the spread plate method. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile spreader. Spreader was always flamed and cooled in between different dilutions when spreading. The plates were incubated at 37°C for 24 hours. Following incubation, plates exhibiting 25-250 colonies were counted. The result was calculated using the following formula:

$$N = \sum c / (n_1 + 0.1 * n_2) d$$

$\sum c$: Sum of colonies counted on all the dishes retained.

n_1 : Number of dishes retained in the first dilution.

n_2 : Number of dishes retained in the second dilution.

d: Dilution factor corresponding to the first dilution.

Enumeration of coliform counts

To determine coliform counts, 1 ml of pre prepared test sample of each of 10^{-3} and 10^{-5} dilutions were pipetted and plated on

MacConkey agar plates in triplicates using the spread plate method. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile spreader. Spreader was always flamed and cooled in between different dilutions when spreading. The inoculated plates were kept at 37°C for 24 hours and plates exhibiting 20-200 colonies were counted. The result was calculated using formula as mentioned in the above.

Isolation and identification of *Staphylococcus aureus*

Staphylococcus aureus, which is potentially food poisoning pathogen, were isolated by using selective media. The specific bacteria were identified and confirmed by the colony characters on the selective media, microscopic examination [7] and biochemical characterization.

Observation survey

To determine the general hygiene, sanitation and possible source of contamination observations were made. Practices such as meat handling by the salesmen, the meat selling and marketing environments situation (sanitation) and the hygienic condition where the beef was sold and also the personal hygiene of the men who sold the raw beef in local markets and restaurants/hotels and the location of the markets were observed and recorded through an observation checklist (Annex 1).

Statistical analysis

Data was evaluated using descriptive statistics and all counts were converted to log values to enable statistical analysis and to express count to log₁₀ CFU/g, and to compare the results. The data were analyzed using SPSS software (Statistical Package for the Social Sciences, version 16, SSPS). One way ANOVA was performed to compare the mean of bacterial counts obtained from beef sample based on their location. Statistical significance was set at a P value of < 0.05.

Results

Aerobic plate count

Raw beef samples obtained from different locations were ana-

lyzed and the results were summarized in Table 1. The overall aerobic plate count (APC) in raw beef computed ranged between 1.04×10⁵ and 3.20×10⁷ cfu/g (mean, 5.91×10⁶). Roughly all meat samples analyzed are go beyond the limit of 10,000 CFU/gram of total viable count, the limit set by Food Safety and Standards (FSS) Regulations, India, 2011. Furthermore, the results showed that there were no significant differences (p > 0.05) in means of aerobic plate counts detected from raw beef samples of different sampling location.

Coliform count: The coliform counts of beef sample from Soddo municipality slaughterhouse, Soddo town restaurants/hotels, Boditte local retail market, Humbo local retail market and Baquillo Sagno local retail market were analyzed and the results are presented in Table 2. The overall mean of coliform count in raw beef computed in ranged between 1.01 × 10³ and 2.11× 10⁵ cfu/g (mean, 4.88 × 10⁴). Additionally, the results showed that there were no significant (p > 0.05) variation in the means of coliform count found in raw beef collected from different locations and market.

Identification of *Staphylococcus aureus*

Examination of 260 raw beef samples collected from municipality slaughterhouses, different restaurants/hotels and local retail market for identification of *Staphylococcus aureus* revealed an overall prevalence of 20.3 % (n = 53). The specific prevalence rate of *Staphylococcus aureus* based on the origin and the location of the raw beef is shown in Figure 1. Soddo municipality slaughterhouse, Soddo town restaurants and hotels, Boditte local retail market, Humbo local retail market and Baquillo Sagno local retail market revealed the prevalence of 12.7% (n = 7), 21.8% (n = 12), 18% (n = 9), 28 % (n = 14) and 22% (n = 11), respectively.

Result of observations survey

According to the observations made, the raw beef handling and selling environment both in Soddo town restaurants/hotels and local market were very poor. The raw beef which was sold in restaurants/hotels came from the Soddo town municipality slaughterhouse and was transported with meat transporting van. The raw beef was kept in separate room which was only used for hanging of raw beef and displayed to the consumer which

Table 1. Total aerobic bacteria counts from raw beef in different locations.

Beef Sample location	No of Sample	Mean bacterial count (cfu/g)	Range		Mean log value cfu/g)
			Min (cfu/g)	Max (cfu/g)	
Municipality slaughterhouse	55	2.28×10 ⁶	1.27 × 10 ⁵	1.05 × 10 ⁷	6.35
Restaurants/hotels	55	2.82×10 ⁶	1.08 × 10 ⁵	2.00 × 10 ⁷	6.45
Boditte local retail market	50	6.35 × 10 ⁶	1.62 × 10 ⁵	3.00 × 10 ⁷	6.8
Humbo local retail market	50	6.12 × 10 ⁶	1.04 × 10 ⁵	3.20 × 10 ⁷	6.78
Banquillo sagno local market	50	7.45 × 10 ⁶	2.35 × 10 ⁶	3.20 × 10 ⁷	6.77
Total	260	5.91 × 10 ⁶	1.04 × 10 ⁵	2.11 × 10 ⁷	4.68

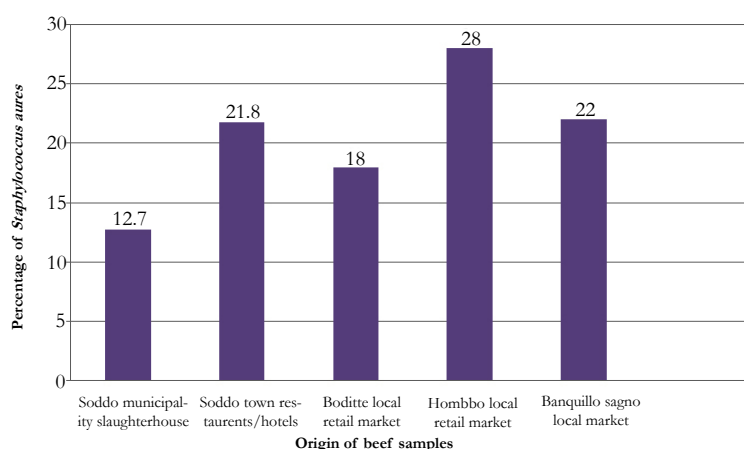
Min. minimum and Max maximum (p > 0.05)

Table 2. Coliform counts from various raw beef in different locations.

Beef Sample location	No of Sample	Mean bacterial count (cfu/g)	Range		Mean log value (cfu/g)
			Min (cfu/g)	Max (cfu/g)	
Municipality slaughterhouse	55	3.36×10^4	1.26×10^3	2.10×10^5	4.52
Restaurants/hotels	55	3.73×10^4	1.01×10^3	1.18×10^5	4.57
Boditte local retail market	50	5.80×10^4	2.30×10^3	1.19×10^5	4.76
Humbo local retail market	50	6.13×10^4	1.22×10^3	1.19×10^5	4.78
Banquillo sagno local market	50	5.64×10^4	1.22×10^3	2.11×10^5	4.72
Total	260	4.88×10^4	1.01×10^3	2.11×10^5	4.68

Min. minimum and Max maximum (p> 0.05)

Figure 1. Prevalence of *Staphylococcus aureus* in raw beef of different location.



was in some hotels/restaurants covered with glass window. Even though the meat selling conditions was poor relatively, the meat selling environments was clean compared to the local open markets (Boditte, Humbo and Baquillo sagno markets). Regarding the personal hygiene of the peoples who were selling raw beef was also poor according to the observation made even though in some restaurants and hotels the peoples used white gowns (coats) and a hat.

In local retail open markets, the sanitation and hygienic conditions of both the beef selling environments and personal hygiene of the people was very poor as in Figure 2 and 3. The raw beef sold in Baquillo sagno came from open air slaughtering (back yard) which was parallel to the livestock market near a big market and transported to selling area with human shoulder using stick which was also inside the market. In Boditte and Humbo local markets, the raw beef salesmen had receipt which was an evidence that there animals were slaughtered in slaughterhouse. But the personal hygiene and the sanitation in selling environment were very poor. The means of transportation of meat to the market was carts which were pulled by humans and donkeys.

Discussion

Raw beef samples from all locations yielded marked growth of bacteria. 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 [18]. The high total viable counts recorded in this study showed the microbial diversity (differences in form or species) in these locations and markets, condition of the market and the hygienic practice employed by meat sellers and butchers. This determined the variation of bacterial contamination.

The highest mean aerobic plate count (APC) was observed from beef of Baquillo sagno local retail market which was 7.45×10^6 cfu/g. Even though the highest number of aerobic bacteria was enumerated from this market, the count was below 10^7 - 10^8 for which spoilage of meat was apparent [31, 27]. The result was drastically higher than Kumar et al. (2010) found a high total aerobic plate (APC) count of 75.91 % in beefs produced and marketed in some parts of Tigray region (ranged 2.5×10^5 cfu/g to 1.63×10^8 cfu/g), In Ethiopia, and Adzitey et.al. (2011) (1.67×10^6 cfu/cm²) in beef from different market In Ghana, and by half higher than the result In Oja-gboro, Nigeria, Raji (2006) of 3.5×10^4 cfu/g. Nevertheless raw beef sold on Baquillo sagno local retail market can be said to be near spoilage.

It was observed that in Baquillo sagno local retail market, the meat originated from back yard slaughtering process which was in an open air that only had fence with woods and the process of slaughtering took place without any infrastructure and without any inspection with qualified veterinary inspector. It was observed that in Baquillo Sagno local retail market, the meat was transported

Figure 2. The raw beef selling environment in local markets.



Figure 3. Salesmen and their raw beef in rural retail market.



by human shoulder hanging with a wood stick to the market and the meat was sold inside huge market and the meat sellers displayed and advertised their meats on wooden tables which were not clean, and the environment was littered with dirt and the meat sellers (butchers) themselves appeared dirty. This could be the reason for high aerobic plate count detected in this market.

Boditte local retail market and Humbo local retail market had roughly similar in mean of aerobic plate count (APC) 6.35×10^6 cfu/g and 6.12×10^6 cfu/g, respectively which was also second highest aerobic plate count recorded in the study area. The result was more or less comparable with In Ukut et.al. (2010) Nigeria recorded 5.01×10^5 cfu/g APC from fresh meat sold in Calabar metropolis where as relatively higher than Rose et al (2011) \log_{10} 4.93 cfu/g for retail sale in Cote. The present study areas had also big markets where butchers sold their meat.

Even though the cattle's were slaughtered in slaughterhouses the transportation and meat holding environment condition is on the worst side. The raw fresh meats were displayed on wooden table which was covered with banana leaf and advertised by the butchers. The local market of Boditte is located near the main road. The hygienic conditions of the meat selling environment in both markets were poor and contaminated with dirt and dust which was not appropriate environment for handling of meat.

Poor personal hygiene of the butchers and retailers, possible cross contamination of between adjacent raw meat through unclean hands of the handlers and or/flies, contamination with the dust, handling of the carcass and the money with the same unwashed hands and careless sneezing and coughing among butchers can lead to a contamination of the raw beef. This could be the reason for high aerobic plate count detected in all of the local retail mar-

kets. Biswas et al., (2011) indicates that Unhygienic handling of meats can affect the ultimate quality of fresh product.

Raw beef which was collected from Soddo municipality slaughterhouse had mean aerobic plate count (APC) 2.28×10^6 cfu/g and Soddo town restaurants/hotels 2.82×10^6 cfu/g. The raw beef in Soddo town restaurants was placed in building (room) separate from the other rooms of the restaurants and the meat was hanged over on metal structure and covered with glass which was like windows. This seems to reduce the number of flies within the meat holding area. The meats sold here were originated from Soddo municipality slaughterhouse. The main source of contamination may be the tables and handling of meat with unsterilized instruments such as knives. The beef collected from Soddo municipality slaughterhouse had the least mean aerobic plate (APC) (2.28×10^6 cfu/g). Even though their numbers were below the spoilage causing limit, the meat was unwholesome.

The higher incidence of microbial load in raw beef obtained from raw beef of slaughterhouse and hotels/restaurants in this study might be attributed to unhygienic and improper handling of animals during slaughter, dressing, evisceration, transportation and marketing. The usual practice of washing the carcass with the same water in which intestines and offal had been washed was considered as one of the predominant reasons for increased microbial counts of the carcasses. A complete ignorance on the part of the meat handlers/butchers in hygienic handling of carcasses during slaughter and retailing processes might be the main factors for producing meat with high microbial load. Hot and humid climate of this study area would have contributed in increasing the microbial load. A high APC usually related to poorer quality and reduced shelf life [11]. High APC can be dangerous for the consumers, as some of the microorganisms could be pathogenic [8].

There are no available regulatory standards for the microbiological safety criteria for locally (nonindustrial) prepared ready to eat foods to compare with this study findings. As per the recommendations of ICMSF (1985), viable count of fresh meat tissue generally should be less than $\log 6.0$ per gram. Microbiological specifications for fresh meat in developing countries like India is 1×10^6 and the values greater than 1×10^6 are not considered as good [5]. Considering these specifications, 205 (78.8%) of 260 beef samples were not of good quality. The results demonstrated that the sanitary conditions under which the beef was produced and sold in the study areas were not of acceptable standards. The undesirable level of bacterial load might have originated from sources like abattoir or meat processing, meat selling environment, contaminated instruments, from meat handlers, from meat salesmen and from transportation methods used.

The current study also revealed a high coliform count from raw beef samples of different sources. In alignment with the current study high coliform count also has been reported from different developing countries. In Nigeria, 3.72×10^5 cfu/g coliform count from fresh meat sold in Calabar metropolis was recorded by Ukut et al. (2010). Rose and co-investigators (2011) reported fecal coliform count which ranged between 1.83 to 4.73 \log_{10} cfu/g from Cote d'Ivoire beef offered for retail sale. Higher concentration of coliform count in the raw beef meat is assumed to be an indicator of fecal contamination and fresh meats sold to the public in open markets are grossly contaminated with coliform bacteria. According to Pace (1975) and Solberg et al. (1986), coliform count higher than 10^2 /g in delicatessen food products are indicative of dangerous contamination.

Evaluation of 260 raw beef sample collected from different restaurants and market for identification of *Staphylococcus aureus* revealed an overall prevalence of 20.3% ($n=53/260$). The presence of high percentage of *S. aureus* was also reported from Ethiopia and other developing country. Haimanot and co-workers (2010) reported 12% of *S. aureus* in unprocessed slices of meat and abattoir from Jimma town of Ethiopia. Raw beef sample originated from Osogbo, Nigeria, yielded 28% *S. aureus* [2]. The high isolation rate of *S. aureus* in this study could be due to poor personal hygiene of the workers, working practices of the meat handlers during the processing stage as well as lack of sterilization of utensils, and working surfaces and the technique used for opening the abdomen, and the presence of cross contamination, which is usually related to human skin and clothing. Such a high level of contamination with *S. aureus* has been associated with increased risk of staphylococcal food poisoning [19, 17].

It is concluded that the high aerobic bacteria count, coliform count and isolation of *Staphylococcus aureus* from the raw beef samples is an indication of its low bacteriological quality, and this can make it a potential source of food infection. Fresh meats sold to the public in open markets are grossly contaminated with high count of bacteria. The finding of this study revealed that fresh beef meat sold at different restaurants and local markets around Wolaita Soddo area are contaminated by various bacterial species including *S. aureus*.

The possible source of contaminants are due to the unhygienic manner of handling meat from the slaughters to the markets and contamination during slaughtering in slaughterhouses and also during backyard slaughtering which includes unhygienic environ-

ment for marketing of meat. The general sanitary conditions at the meat shops and local markets in addition to poor hygienic practices by the butchers and person who sell beef are probable contributors to the microbial contamination on the beef. This also implies that these meats are viable source of various diseases. Some diseases could spread and acquire epidemic status which poses serious public health hazards. Since improper handling and hygiene might lead to the contamination of fresh meats and this might eventually affect the health of the consumers.

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