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Bacteriological Assessment of Fresh Beef Sold in Birnin Kebbi Central Market, Kebbi State, Nigeria

Abbas Bazata Yusuf *, Bashar Haruna Gulumbe, Basiru Aliyu and Zaharaddin Muhammad Kalgo

Department of Microbiology, Federal University Birnin Kebbi, Kebbi State, Nigeria

**Corresponding e-mail: aybazata91@yahoo.com*

ABSTRACT

Introduction: *Microbiological quality of meat and meat products is of great public health significance since the consumption of contaminated meat has been reported as one of the major causes of food-related diseases.* ***Aim:*** *The aim of this study was to assess the bacteriological quality of fresh beef sold in Birnin Kebbi Central Market. This was with a view to determining its safety for human consumption.* ***Materials and methods:*** *Beef samples were collected in triplicate from 10 different meat outlets from the market and were analysed using standard procedures.* ***Results:*** *The mean mesophilic aerobic bacterial counts from the 10 locations ranged from 3.2×10^5 to 3.9×10^8 cfu/g whereas a total of 49 isolates belonging to 7 genera including Bacillus subtilis 2 (4.1%), Proteus vulgaris 3 (6.1%), Enterobacter spp. 12 (24.5%), Pseudomonas aeruginosa 7 (14.3%), Escherichia coli 14 (28.6%), Salmonella spp. 3 (6.1%) and Staphylococcus aureus 8 (16.3%) were identified. The difference in the mean bacterial load among the 10 sampling location was statistically insignificant ($p>0.05$).* ***Conclusion:*** *High mesophilic aerobic bacterial counts and the presence of potentially pathogenic bacteria such as Salmonella, Escherichia coli and Pseudomonas aeruginosa in beef pose a serious potential health hazard. Authorities and stakeholders should, therefore, intensify efforts to ensure that quality control and hygiene measures strictly adhere during meat handing.*

Keywords: Bacteria, Fresh meat, Beef, Bacterial contamination, Meat hygiene's

INTRODUCTION

With its considerable contributions to human dietary needs, meat is an excellent source of proteins and essential amino acids [1]. Microbiological quality of meat and meat products is of significance as consumption of contaminated meat have been linked to not only outbreaks of a number of human health problems, but economic losses to producers due to recalls from the market and sometimes death [2]. It is believed that meat from healthy animals is free of microorganisms, but methods of processing and retailing make it vulnerable to microbial contamination [3].

Potential sources of contamination of meat include sources that are directly linked to the animal itself such as its skin or fecal material [2]. External sources of meat contamination include the slaughter house environment, the retail outlet environment; the vehicle used for the transport of the meat from the slaughter house and of course the meat handlers [2]. The crude meat processing tools due to lack of required tools could also serve as a source of meat contamination [4].

Bacteria associated with fresh meat have been reported to include *Pseudomonas spp., Acinetobacter spp., Brochothrix thermosphacta, Lactobacillus spp., Salmonella spp., Listeria monocytogenes, Staphylococcus aureus, Yersinia enterocolitica, Escherichia coli, Clostridium perfringens* and occasionally *Clostridium botulinum*. Most of these organisms can cause not only the deterioration of meat but foodborne infections [5]. The genera *Pseudomonas, Acinetobacter*, *Moraxella* and *Flavobacterium* are the most commonly reported cause of deterioration of meat stored under aerobic condition whereas the predominant flora associated with meat stored under anaerobic condition include Gram-positive bacteria, particularly lactic acid bacteria (LAB) and *B. thermosphacta* [5,6].

Meat is regarded as spoiled when it is unfit for human consumption. Meat may be subjected to spoilage by its own enzyme, microbial action and due to fat oxidation causing textural or organoleptic change when microorganisms release metabolites [7]. These changes result in unpleasant odour and/or unusual taste.

Retail meat is considered both a significant source for food-borne infections and a potential carrier for the dissemination of resistant bacteria in the community [8]. Lack of modern slaughter house facility, the existence of small retail outlets and non-compliance with the hygienic production protocols have been reported as the major challenges hampering hygienic meat production [2,9]. Additionally, lack of quality control inspections and enforcement can be regarded as a reason for the nonchalant attitude of meat handlers to hygienic handing of meat. In order to mitigate the incidence of foodborne diseases, experts advocate for proper and frequent risk assessment [8]. Despite the high rate of consumption of meat, studies are lacking on the microbial evolution of beef quality in the study area hence the need for the present study. The aim of this study was to assess the bacteriological quality of fresh beef sold in Birnin Kebbi central Market with a view to determining its safety for human consumption.

MATERIALS AND METHODS

Sample Collection

Total 30 fresh beef samples were collected from 10 different retail meat outlets in Birnin Kebbi Central Market, Kebbi, Nigeria. About 3 samples were collected from each meat retail meat outlets. Meat samples were collected in a sterile polythene bags, packed in a container embedded with ice packs and transported to the laboratory. The samples were processed within 24 hours after bringing to the laboratory [2].

Sample Preparations

Total 10 grams of meat sample was taken and homogenized into 90 ml of sterile normal saline using a meat grinder under sterile conditions. Ten-fold dilutions of the homogenates up to 10^{-5} in normal saline were made using sterile pipettes [10].

Total Mesophilic Aerobic Counts

Total mesophilic aerobic counts were assessed in accordance with the method described by Salihu, et al., and Jansen, et al., [4,11]. After incubation at 37°C for 24 h, plates containing 30 and above colonies were enumerated. The mean number of colonies was multiplied by the inverse of the dilution, the bacterial counts were obtained. Results were reported as colony-forming units per gram of samples (CFU/g).

Isolation and Identification of Bacteria

To obtain pure bacterial isolates, a distinct colony from mixed culture was picked using a sterile wire loop and placed on a fresh nutrient agar medium. After streaking, the petri dish was incubated at 37°C for 24 hours. All isolates from this pure culture were maintained in an agar slant for further analyses. All bacterial isolates were identified according to their physical (colonial) characteristics (shape, colour, odour, pigmentation) and biochemical tests such as Gram's staining, Coagulase, Catalase, Indole, Urea, Citrate, Bacterial Spore stain, Motility test, Voges Proskauer test, Methyl red test and Oxidase test. Additional selective/differential plating was employed to further identify the isolates [12-14].

Presumptive *Staphylococcus aureus* isolates were inoculated on mannitol salt agar and incubated at 37°C for 48 hours. Colonies characterized by yellowish pigmentation were considered as *S. aureus*. Presumptive *Pseudomonas aeruginosa* isolates were inoculated on cetrimide selective agar at 37°C for 24 hours. Colonies characterised by blue-green and yellow-green were considered as *P. aeruginosa*.

Presumptive *E. coli* and *Salmonella spp*. colonies based on biochemical reactions were further inoculated on Eosin methylene blue (EMB) and incubated at 37°C for 24 hours. Colonies characterized by metallic green sheen were considered as *E. coli* whereas grey colonies were regarded as *Salmonella spp*. In addition, bacterial isolates were further confirmed using a combination of Analytical Properties Index API 20E, API STAPH test system and API 50CHB (BioMerieux, Marcy l'Etoile, France).

Statistical Analysis

All samples were collected in triplicates. The statistically significant difference in the bacterial counts among sampling sites was determined by one-way ANOVA test. Data were analysed using SPSS version 20 and $p<0.05$, was considered statistically significant.

RESULTS

The mean mesophilic aerobic bacterial counts from the 10 meat retail outlets ranged from 3.2×10^5 to 3.9×10^8 cfu/g and no significant difference between the 10 sampling sites were observed (Table 1).

Table 1 Mean mesophilic aerobic bacterial counts from beef sold in Birnin Kebbi central market

SN	Sampling points	Mesophilic Aerobic Bacterial Count (cfu/g) (mean ± SD)
1	MRA	$4.01 \times 10^6 \pm 2.5^a$
2	MRB	$3.2 \times 10^5 \pm 3.0^a$
3	MRC	$3.4 \times 10^6 \pm 4.0^a$
4	MRD	$4.10 \times 10^7 \pm 3.0^a$
5	MRE	$3.9 \times 10^5 \pm 2.1^a$
6	MRF	$3.6 \times 10^7 \pm 1.6^a$
7	MRG	$3.9 \times 10^8 \pm 2.2^a$
8	MRH	$2.4 \times 10^7 \pm 3.3^a$
9	MRI	$3.3 \times 10^6 \pm 1.9^a$
10	MRJ	$4.0 \times 10^5 \pm 0.8^a$
Key: MRA to MRJ= Meat Retail A to J; a: indicate no statistical significance difference		

A total of 49 isolates belonging to 7 genera including *Enterobacter spp*. 12 (24.5%), *Pseudomonas aeruginosa* 7 (14.3%), *Escherichia coli* 14 (28.6%) and *Staphylococcus aureus* 8 (16.3%) were observed as predominant (Table 2).

Table 2 Prevalence of the bacterial isolates from fresh beef sold in Birnin Kebbi central market

SN	Organism	Frequency of Occurrence (%)
1	*Pseudomonas aeruginosa*	7 (14.3%)
2	*Escherichia coli*	14 (28.6%)
3	*Salmonella spp.*	3 (6.1%)
4	*Proteus vulgaris*	3 (6.1%)
5	*Staphylococcus aureus*	8 (16.3%)
6	*Bacillus subtilis*	2 (4.1%)
7	*Enterobacter spp.*	12 (24.5%)
	Total	49 (100.0%)

DISCUSSION

The result of the present study indicated that fresh meat sold in Birnin Kebbi central market was contaminated with bacteria. The high mesophilic aerobic counts recorded in this study is an indication of unhygienic handling of meat in the market. The abundance of nutrient in meat, unhygienic handling of meat and lack of hygienic environment have been attributed to microbial contamination of meat [7]. The mean bacterial counts in the current study ranged between 3.2×10^5 to 3.9×10^8 cfu/g. Similar to the results of the present study, Brashears reported mesophilic aerobic bacterial counts of 7.0×10^5 to 5.8×10^6 cfu/g in fresh refrigerated meat [15]. Relatively high bacterial concentration in meat maybe due to the improper handling of meat during slaughtering, transportation, and non-compliance with the hygiene procedures [9]. Since it represents the total amount (cfu) of mesophilic microorganisms, the mesophilic bacterial counts is a general microbiological indicator for food quality [11]. In fact, total aerobic mesophilic bacterial counts provide information on the overall degree of meat contamination and constitute a criterion to classify slaughter-houses according to their hygienic quality [9]. Bacterial load of 107 cfu/g in meat have been documented to cause noticeable changes such as odors and slime [15].

Bacillus subtilis, Proteus vulgaris, Enterobacter spp., Pseudomonas aeruginosa, Escherichia coli, Salmonella spp. and *Staphylococcus aureus* were the bacterial agents isolated from fresh beef in the present study. *Pseudomonas spp., Acinetobacter spp., Brochotrix thermosphacta, and Lactobacillus spp., Salmonella spp., Listeria monocytogenes, Staphylococcus aureus, Yersinia enterocolitica, Escherichia coli, Clostridium perfringens* and occasionally *Clostridium botulinum* have been reported as common meat bacterial contaminants [5,9]. Depending on their number and species present, they can cause meat deterioration and in some cases cause food poisoning or food intoxication or both [5].

The identification of isolates from different bacterial species revealed the level of contamination dominated by Gram

negative bacteria. This corroborates the findings of Ruiz-moyano, et al., who reported Gram negative bacteria as predominant bacteria isolated from fresh goat meat [5].

In the present study, *E. coli* had the highest frequency of occurrence of 14 (28.6%). *E.coli* is widely used as indicator organism, its presence in meat or water generally indicates direct and indirect fecal contamination [7]. Salihu, et al., linked high prevalence of enteric bacteria in meat to fecal contamination [4]. The potentially high death rate associated with *E. coli* and *E. coli* 0157: H7 strain infection makes the presence of *E. coli* in any food material a serious source of public health concern as many of the outbreaks recorded have been traced to consumption of contaminated beef [16].

Enterobacter spp. was the second most frequently isolated bacteria in the present study. This could be as a result of poor environmental conditions due to dust and contamination of the water used during slaughtering and post slaughtering activities. This corroborates with the findings of Ukut, et al., who reported that poor environmental conditions could be responsible for the contamination of meat with *Enterobacter spp* [7].

Salmonella spp. was isolated from fresh beef in the present study, a finding in agreement with previous studies on poultry meat, which asserted *Salmonella* as a common contaminant of meat [11,17]. The sources of *Salmonella spp*. in meat can range from the production and processing or cross-contamination during meat processing [18]. Isolation of these potential pathogens in beef is of public health significance. *Salmonella spp.* is one of the pathogen frequently reported cause of illness [18]. It continued to be a major etiologic agent of gastrointestinal bacterial infections [11]. Additionally, reports of rejection of assorted meats including beef due to the presence of *E. coli* and *Salmonella* have been documented [11]. The main reservoir of zoonotic enterobacteria including *Salmonella* is food animals, and the main sources of infections in industrialized countries are animal-derived products, notably fresh meat products, and eggs.

Staphylococcus aureus had a frequency of occurrence of 8 (16.3%). The presence *S. aureus* on meat may indicate possible cross-contamination with human body discharges. Similarly, improper personal hygiene during handling and processing have been attributed to high contamination of food with *S. aureus* [11]. The fact that bacterial toxins, including those from *Staphylococcus spp*., have been regarded as one of the leading cause for foodborne outbreaks, made its presence in meat even more worrisome.

The level of contamination observed in the present study pose a serious public health concern since most of the isolates have been reported to cause various health problems including foodborne diseases and intoxication. High level of contamination recorded may be linked to the deplorable state of poor hygienic and sanitary practices employed in the slaughtering, processing, and packaging of fresh meats. There is, therefore, the need to enlighten meat retailers of the significance of environmental and personal hygiene. Consumption of fresh or undercooked beef should be discouraged. Strict compliance with veterinary clearance before slaughtering of animals should be enforced.

CONCLUSION

The results of the present study have shown a high level of bacterial contamination of beef sold in the study area. Some potentially pathogenic bacteria such as *Bacillus subtilis, Proteus vulgaris*, *Enterobacter spp*., *Pseudomonas aeruginosa*, *Escherichia coli, Salmonella spp*. and *Staphylococcus aureus* was identified, while the mean bacterial counts, which is higher than prescribed limits, ranged between 3.2×10^5 to 3.9×10^8 cfu/g. In conclusion, we believe that the level of contamination of fresh beef observed could be traced due to the lack of hygienic handling, which we attribute to both lack of knowledge of basic hygiene practice of meat handler and lack of enforcement of hygiene regulations. Therefore, if lack of enforcement continues to linger, it will continue to impede improvement of hygiene and safety of meat.

DECLARATIONS

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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