



Bacteriological Analysis of Beef Production Chain in Birnin Kebbi Metropolis of Kebbi State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author FJA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IA and AYB managed the analyses of the study. Author FJA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The hygiene status of most abattoirs and retail outlets in Nigeria is very poor, and it contributes to the unacceptable level of bacteria load in beef, and poses a health risk to consumers. This study examined the bacteriological analysis of beef production chain in Birnin Kebbi metropolis. A total of 100 samples of meat, water and surface swab in Main Market, GRA, Badariya, Bayan Kara and Rafin Atiku, Birnin Kebbi Central abattoir and retail meat shops were collected aseptically, processed and analyzed. Meat from retail outlets were mostly contaminated by *Staphylococcus aureus* (24.4%), followed by *Escherichia coli* (17.3%), *Klebsiella pneumonia* (9.8%), *Salmonella typhimurium* (9.1%), *Enterobacter aerogenes* (8.5%), *Streptococcus pyogenes* (8.1%), *Proteus vulgaris* (7.8%), *Micrococcus luteus* (7.8%) and *Pseudomonas aeruginosa* (7.2%). In the abattoirs contact surfaces, contamination occurred mostly by *Staphylococcus aureus* (23.4%), *Salmonella typhimurium* (18.2%), *Escherichia coli* (16.9%), *Enterobacter aerogenes* (9.1%), *Streptococcus pyogenes* (7.8%), *Klebsiella pneumonia*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* (6.5%)

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each) and *Micrococcus luteus* (5.2%). The findings revealed a high bacterial load in both abattoir and retail meat outlets. In order to safeguard the health of the public against the risks of food borne infections, there is a need to educate and advocate good sanitation and meat handling practices in the abattoir and beef retail outlets.

Keywords: Abattoirs; bacterial load; beef; Birnin Kebbi; contamination.

1. INTRODUCTION

In recent years, there has been an increase in bacterial adaptation and evolution resulting in the emergence of a number of zoonotic microorganisms in food and water. Food-borne disease is a global public health concern [1]. There is an estimated 76 million food-borne illnesses, 325,000 hospitalizations and 5,000 deaths annually in United States. In the United Kingdom, an estimated 2.37 million cases of food-borne gastroenteritis occurred in 1995 [2]. Available data from United States Department of Agriculture Food Safety and Inspection Service indicated that 13 million Kilogram of ground beef were contaminated with *E. coli* O157:H7 on August 12, 1997 and 9.5 million Kilogram of beef trimmings and ground beef potentially contaminated with *E. coli* O157:H7 on July 19, 2002 [3].

Transmission of pathogens to humans may be from contaminated foods or water, or from infected persons, environments or animals [4]. Food animals, in particular mature cattle are usually asymptomatic carriers of *E. coli* O157, including STEC [5] can also serve as reservoirs of antimicrobial-resistant bacteria. Resistance to antibiotics is highly prevalent in bacterial isolates globally, particularly in developing countries such as Nigeria [6,7,8]. Carcass contamination from hides, skin and gut contents of animals can occur during bleeding, handling and processing of beef which are predominant in slaughtering, scalding, eviscerating and washing [9]. Unhygienic floor dressing of carcasses is a common practice in the developing nations of the world resulting in carcass contamination and isolation of pathogenic microorganisms from beef and slaughtering facilities in Nigeria [10].

Beef are perishable due to their chemical composition and characteristics. This explains why they provide the medium for growth of many harmful microorganisms which are the major causative agent of infections in humans. Beef contains varieties of nutrients required for the growth of bacteria, yeast and mold. Beef has high biologically valued nutrients that are needed

in proportionality for good health and vitality [11].

In spite of the increased consumer demand for beef in Nigeria, there are still poor hygienic and sanitary practices along the beef production chain which contribute to the unacceptable level of microbial load in beef. This poses a health risk to consumers. Although several studies have been conducted to assess the degree of beef losses due to contamination of carcasses [12] and detection of zoonotic conditions through post mortem inspection [13], limited studies have been conducted to assess microbial contamination of beef along the production chain from the abattoir to retail meat outlets [14].

The hygiene status of most abattoirs in Nigeria is very poor [15]. This may be linked to the level of poverty and the high rate of illiteracy among the beef handlers. It is on this basis that this study is embarked upon so as to carry out a bacteriological analysis of beef production chain in Birnin Kebbi metropolis. The information obtained from study will help to establish the public health implication of consumption of contaminated beef sold in Birnin Kebbi and further educate the public on the dangers involved in consumption of contaminated beef.

2. MATERIALS AND METHODS

2.1 Study Area

Birnin Kebbi is a city located in north-western Nigeria, and is the capital city of Kebbi State and headquarters of the Gwandu Emirate. Birnin Kebbi is situated at 12.45° North latitude, 4.2° East longitude and 235 meter elevation above the sea level. Kebbi is mostly inhabited by Hausa and Fulani ethnic groups with Islam as the main religion.

2.2 Sample Collection and Processing

Meat, water and surface swab samples from Birnin Kebbi Central abattoir and retail meat shops in Main Market, GRA, Badariya, Bayan Kara and Rafin Atiku were collected aseptically, processed and analyzed.

2.3 Surface Swabs from Abattoir and Retail Outlets Handling Equipment

Four samples from the surface swabs from Birnin Kebbi Central abattoir were collected aseptically using moistened cotton wool swab by rubbing firmly over the predetermined surface area using parallel stroke lines with slow rotation with respective chosen template surface area swabbed. In the abattoir, the moistened sterile cotton wool swabs were used to collect samples from tables, walls, floors, meat van floors and surface swabs from knives.

In the retail outlets, four samples were collected aseptically using moistened cotton wool swab by rubbing firmly over the predetermined surface area using parallel stroke lines with slow rotation with respective chosen template surface area. The moistened sterile cotton wool swabs were used to swab surface area in the template of 20 cm² by 20 cm² from weighing pans, butcher knives, meat chopping tables and meat wood cutting blocks and butchers hand.

The swabs were transferred to the respective capped sterile tubes containing 10 ml normal saline and labeled. The swabs were agitated up and down in the tubes to aid on rinsing the bacteria from the surface of the swabs. Samples were packed in cool box and transported to laboratory for microbiological analysis [16].

2.4 Waste Water Sample from Abattoir

Waste water sample from abattoir was collected directly with sterile bottle, labeled, placed in a cool box with ice pack and transported to laboratory for analysis [16].

2.5 Faecal Sample from Abattoir

A little quantity of the faecal sample was collected aseptically using a sterile container, labeled, place in a cool box with ice pack and then transported to the laboratory for analysis [16].

2.6 Preparation of MacConkey Agar

A total of 10.4 grams MacConkey agar (MAC) powder was weighed and suspended in 200 ml of distilled water and boiled for 10 minutes to dissolve and the agar was sterilized by autoclaving at 121°C. The sterilized medium was allowed to cool in a water bath and poured into

plates and allowed to solidify on a level laboratory bench. The prepared media was then incubated at 37°C to check for their sterility before use [17].

2.7 Preparation of Mueller-Hinton Agar

A total of 7.6 gm Mueller-Hinton (MH) agar powder was weighed and suspended in 200 ml of distilled water and boiled for 10 minutes to dissolve. The agar was sterilized by autoclaving at 121°C for 15 minutes. The agar was then allowed to cool in a water bath and thereafter poured into sterile petri dishes to solidify. The prepared media plates were incubated overnight at 37°C and checked for their sterility before been used [18].

2.8 Preparation of Nutrient Agar Slant

A total of 1.3 grams nutrient agar powder was dissolved in 100 ml distilled water. This was boiled to dissolved and sterilized by autoclaving at 121°C for 15 minutes after dispensing in bijou bottles. The medium was allowed to solidify in a slanting position and later packed and stored at 4°C in the refrigerator [19].

2.9 Preparation of Urea Agar

This was prepared by suspending 2.4 grams of urea base in 95 ml of distilled water. It was then dissolved by shaking and the suspension was sterilized by autoclaving at 121°C for 10 minutes.

Then 2 gm of urea was dissolved in 5 ml of distilled water and boiled for about 30 minutes. The two solutions were allowed to cool in a water bath set at 45°C. The urea was then added to the urea base and the mixture was then dispensed in 5 ml amounts into half ounce bottles. The media bottles were kept in a slant position to solidify [20].

2.10 Preparation of Triple Sugar Iron Agar (TSI)

A total of 6.5 gm TSI powder was suspended in 100 ml de-ionized water, soaked for 10 minutes, swirled and mixed then boiled. It was then be dispensed into tubes and sterilized by autoclaving for 15 minutes at 121°C. They were later placed in a slanting position to solidify, ensuring that the slant is over a butt at about 3 cm deep [21].

2.11 Preparation of Simmons Citrate Agar (SCA)

A total of 4.6 gm SCA powder was suspended in 200 ml of distilled water. This was boiled to dissolve completely. It was then dispensed into half ounce bottles (5 ml per bottle) and sterilized by autoclaving at 121°C for 10 minutes. They were then placed in a slanting position to solidify [22].

2.12 Normal Saline Preparation

In order to make a Phosphate buffered saline, 8.5 grams of sodium chloride was thoroughly mixed with distilled water. Several test tubes were filled with normal saline solution and sterilized in autoclave at 121°C for 15 minutes [23].

2.13 Sample Preparations

Ten grams (10 g) of each meat sample were weighed out and homogenized into 90 ml of sterile distilled de-ionized water using a sterile warring blender. Ten-fold dilutions of the homogenates were made using sterile pipettes as described by the methods of [24].

2.14 Surface Swabs

In the laboratory, each test tube and universal bottle with surface swabs and water samples were opened aseptically by flaming of the mouth part of test tubes and universal bottles. The samples were taken using sterile pipette and further diluted serially (6 folds dilution) into 6 test tubes. The diluents were mixed well and then one millilitre of diluted sample was poured into various sterile petri dishes and covered with 20 millilitres of sterile nutrient agar or MacConkey agar. Each plate was swirled gently taking care not to spill its contents and allowed to set. All samples inoculated with nutrient agar were incubated at 37°C for 24 hours in order to get TVC while samples inoculated in MacConkey agar was incubated at 37°C and 44°C for 24 hours for TCC and for TFC counts respectively [25].

2.15 Meat Samples

Meat sample weighing 20 g was grinded to fine particles using sterilized mortar and pestles and mixed with 180 ml of normal saline solution to make and diluted serially into six (6) folds. One millilitre of inoculum was taken from the test tube

using sterile pipette and poured into sterile petri dish. Then 20 millilitres of sterile nutrient agar or MacConkey agar was poured into each sterile petri dish, distributed and mixed evenly throughout. The petri dishes with molten inoculated media were allowed to solidify. All samples inoculated in nutrient agar were incubated at 37°C for 24 hours in order to get TVC while samples inoculated in MacConkey agar were incubated at 37°C and 44°C for 24 hours for TCC count [25].

2.16 Water Samples

A serial dilution of water sample was done into several test tubes. One millilitre of inoculum was taken from the test tube using a pipette and poured into sterile petri dish. Then 20 ml of sterile nutrient agar or MacConkey agar was added into sterile petri dish, distributed and mixed evenly throughout the petri dish and allowed to solidify. All samples inoculated in nutrient agar were incubated at 37°C for 24 hours in order to get TVC while samples inoculated in MacConkey agar were incubated at 37°C and 44°C for 24 hours for TCC counts [25].

2.17 Culturing of Isolates into Media

All the chemicals and reagents were of analytical grade, obtained from Sigma Chemical Co Ltd, England produced in December, 2016. Media used in this study include Nutrient Agar (NA) and Peptone Water (PW) as general and enriched media. Other media with selective and differential characteristics used were Mac Conkey Agar (MCA), Kligler Iron Agar (KIA), Citrate Agar (CA), Christensen's Urea Agar (CUA), and Mueller Hinton Agar. All media were prepared according to the manufacturer's specification and sterilized at 121°C for 15 min. From the 10-fold dilutions of the homogenates; 0.1 ml of 10⁻², 10⁻³ and 10⁻⁴ and 10⁻⁶ dilutions of the homogenate was plated in replicate on different media (in duplicates), using pour plate method. The plates were then incubated at 37°C for 24 – 48 hr. Mac Conkey Agar were used for coliform enumeration while Mannitol salt agar was used for the isolation of *Staphylococcus aureus*. Total viable aerobic bacteria count was performed on Nutrient Agar.

2.18 Enumeration and Isolation of Microbial Growth

The plates were then observed after 24 hours of incubation. At end of the incubation periods, colonies were counted using the illuminated

colony counter (Gallenkamp, England). The counts for each plate were expressed as colony forming unit of the suspension (cfu/g) [21].

2.19 Isolation of Microbial Growth

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 40°C [21].

2.20 Identification of Isolates

Colonies identifiable as discrete on the Mueller Hinton Agar were carefully examined macroscopically for cultural characteristics such as the shape, color, size and consistency. Bacterial isolates were characterized based on microscopic appearance, colonial morphology and Gram staining reactions as well as appropriate biochemical tests for example Kligler's Iron Agar (KIA) test, Indole production test, Triple sugar iron (TSI) test, Methyl Red (MR) test, Voges-Proscauer (VP) test, Citrate utilization test, Motility Indole Urea (MIU) test, Carbohydrate fermentation test and salt tolerance test as described by [26] and [27] were carried out. The isolates were identified by comparing their characteristics with those of known taxa, as described by Bergey's Manual for Determinative Bacteriology [28]. Data were analyzed using the Microsoft Excel Spread Sheet 2010.

3. RESULTS

3.1 Results of Mean Viable and Coliform Count for Retail Outlets

A total of one hundred (100) samples were collected and twenty (20) samples at each retail point. The butchers' knives (KNF), weighing pans (WP), tables (TB) and butcher's hand (BH) were swabbed with moist swab sticks. Table 1 shows the estimation of the total viable bacterial counts and total coliform counts in retail outlets using

Nutrient agar and MacConkey agar. The mean microbial load from the first retail outlet (Main market) ranged between 2.6×10^4 - 4.5×10^4 cfu/g and total coliform count between 2.2×10^4 - 3.7×10^4 cfu/g (Table 1), the load from the second retail outlet (GRA) ranged between 1.6×10^4 - 3.8×10^4 cfu/g and total coliform count between 1.6×10^4 - 3.4×10^4 cfu/g (Table 2), the microbial load from the third retail outlet (Badariya) ranged between 3.6×10^4 - 4.0×10^4 cfu/g and total coliform count between 2.6×10^4 - 4.7×10^4 (Table 3), the microbial load from the fourth retail outlet (Bayan Kara) ranged between 1.2×10^4 - 3.20×10^4 and total coliform count between 1.4×10^4 - 4.8×10^4 (Table 4) while the microbial load from the fifth retail outlet (Rafin Atiku) ranged between 1.3×10^4 - 4.4×10^4 cfu/g and total coliform count between 2.2×10^4 - 4.8×10^4 (Table 5).

3.2 Results of Mean Viable and Coliform Count for Abattoir

Table 6 shows the estimation of the total viable count and the total coliform count in abattoir using Nutrient agar and MacConkey agar. The mean total microbial count ranged from 2.8×10^4 - 4.8×10^4 while the mean total coliform count ranged from 2.8×10^4 - 4.6×10^4 .

3.3 Result of Identification of the Bacteria Isolates in Retail Outlets

The results in Tables 7 – 11 indicated that the micro organisms present in the swab butchers' knives, weighing pans, tables and butcher's hand were mostly *Staphylococcus aureus*. Out of the 305 bacteria isolated from the retail outlets in Birnin Kebbi metropolis, *Staphylococcus aureus* recorded 75 (24.4%) followed by *Escherichia coli* with 53 (17.3%), *Klebsiella pneumonia* 30 (9.8%), *Salmonella typhimurium* 28 (9.1%), *Enterobacter aerogenes* 26 (8.5%), *Streptococcus pyogenes* 25 (8.1%), *Proteus vulgaris* 24 (7.8%), *Micrococcus luteus* 24 (7.8%) and *Pseudomonas aeruginosa* 22 (7.2%).

Table 1. Mean viable and coliform count (cfu/g) from the retail outlet 1 (Main Market)

| Source | No of sample | Mean viable count (cfu/g) | Mean coliform count (cfu/g) |
|---------------|--------------|---------------------------|-----------------------------|
| Knife | 4 | 2.6×10^4 | 3.7×10^4 |
| Table | 4 | 2.8×10^4 | 3.2×10^4 |
| Weighing Pan | 4 | 3.8×10^4 | 3.4×10^4 |
| Cutting Board | 4 | 4.5×10^4 | 2.7×10^4 |
| Butcher Hand | 4 | 3.2×10^4 | 2.2×10^4 |
| Total | 20 | | |

Table 2. Mean viable and coliform count (cfu/g) from retail outlet 2 (GRA)

| Source | No of sample | Mean viable count (cfu/g) | Mean coliform count (cfu/g) |
|---------------|--------------|---------------------------|-----------------------------|
| Knife | 4 | 1.6×10^4 | 3.4×10^4 |
| Table | 4 | 3.1×10^4 | 1.6×10^4 |
| Weighing Pan | 4 | 3.8×10^4 | 3.3×10^4 |
| Cutting Board | 4 | 3.3×10^4 | 2.7×10^4 |
| Butcher Hand | 4 | 2.8×10^4 | 2.6×10^4 |
| Total | 20 | | |

Table 3. Mean viable and coliform count (cfu/g) from retail outlet 3 (Badariya)

| Source | No of sample | Mean viable count (cfu/g) | Mean coliform count (cfu/g) |
|---------------|--------------|---------------------------|-----------------------------|
| Knife | 4 | 4.0×10^4 | 2.6×10^4 |
| Table | 4 | 3.8×10^4 | 3.2×10^4 |
| Weighing Pan | 4 | 4.0×10^4 | 4.3×10^4 |
| Cutting Board | 4 | 3.7×10^4 | 3.0×10^4 |
| Butcher Hand | 4 | 3.6×10^4 | 4.7×10^4 |
| Total | 20 | | |

Table 4. Mean viable and coliform count (cfu/g) from retail outlet 4 (Bayan Kara)

| Source | No of sample | Mean viable count (cfu/g) | Mean coliform count (cfu/g) |
|---------------|--------------|---------------------------|-----------------------------|
| Knife | 4 | 3.2×10^4 | 4.2×10^4 |
| Table | 4 | 2.2×10^4 | 4.8×10^4 |
| Weighing Pan | 4 | 1.8×10^4 | 3.2×10^4 |
| Cutting Board | 4 | 1.7×10^4 | 2.4×10^4 |
| Butcher Hand | 4 | 1.2×10^4 | 1.4×10^4 |
| Total | 20 | | |

Table 5. Mean viable and coliform count (cfu/g) from retail outlet 5 (Rafin Atiku)

| Source | No of sample | Mean viable count (cfu/g) | Mean coliform count (cfu/g) |
|---------------|--------------|---------------------------|-----------------------------|
| Knife | 4 | 4.4×10^4 | 4.8×10^4 |
| Table | 4 | 4.1×10^4 | 4.2×10^4 |
| Weighing Pan | 4 | 3.2×10^4 | 3.0×10^4 |
| Cutting Board | 4 | 2.2×10^4 | 2.8×10^4 |
| Butcher Hand | 4 | 1.3×10^4 | 2.2×10^4 |
| Total | 20 | | |

Table 6. Mean viable and coliform count (cfu/g) from Birnin Kebbi Central Abattoir

| Source | No of sample | Mean viable count (cfu/g) | Mean coliform count (cfu/g) |
|---------------|--------------|---------------------------|-----------------------------|
| Knife | 4 | 3.3×10^4 | 3.2×10^4 |
| Floor | 4 | 4.2×10^4 | 3.6×10^4 |
| Van | 4 | 3.9×10^4 | 3.9×10^4 |
| Faecal Sample | 4 | 4.8×10^4 | 4.6×10^4 |
| Water Sample | 4 | 3.1×10^4 | 3.7×10^4 |
| Total | 20 | | |

3.4 Result of Identification of the Bacteria Isolate from Birnin Kebbi Central Abattoir

The results of identification of the bacteria isolates from the butcher's knives, floors, vans faecal and water samples include the following

nine genera; *Staphylococcus aureus* 18 (23.4%), *Salmonella typhimurium* 14 (18.2%), *Escherichia coli* 13 (16.9%), *Enterobacter aerogenes* 7 (9.1%), *Streptococcus pyogenes* 6 (7.8%), *Klebsiella pneumonia*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* 5 (6.5%) each and *Micrococcus luteus* 4 (5.2%) (Table 12).

Table 7. Identification of the bacteria isolate from (Retail Outlet 1) main market

| Bacteria identified | Samples | | | | Total |
|-------------------------------|---------|--------------|----------------|-------|-------|
| | Knife | Weighing pan | Butcher's hand | Table | |
| <i>Staphylococcus aureus</i> | 4 | 4 | 3 | 4 | 15 |
| <i>Escherichia coli</i> | 4 | 2 | 2 | 2 | 10 |
| <i>Micrococcus luteus</i> | 1 | 1 | 2 | 2 | 6 |
| <i>Streptococcus pyogenes</i> | - | 1 | 1 | 1 | 3 |
| <i>Klebsiella pneumonia</i> | 1 | 2 | 1 | 2 | 6 |
| <i>Salmonella typhimurium</i> | 2 | 1 | 1 | 1 | 5 |
| <i>Proteus vulgaris</i> | - | 2 | 1 | 1 | 4 |
| <i>Pseudomonas aeruginosa</i> | 2 | 1 | - | 2 | 5 |
| <i>Entrobater aerogenes</i> | 1 | 2 | 2 | 1 | 6 |
| Total | 15 | 16 | 13 | 16 | 60 |

Table 8. Identification of the bacteria isolate from (Retail Outlet 2) GRA

| Bacteria identified | Samples | | | | Total |
|-------------------------------|---------|--------------|----------------|-------|-------|
| | Knife | Weighing pan | Butcher's hand | Table | |
| <i>Staphylococcus aureus</i> | 4 | 4 | 4 | 3 | 15 |
| <i>Escherichia coli</i> | 3 | 4 | 2 | 2 | 11 |
| <i>Micrococcus luteus</i> | 1 | 1 | 1 | - | 3 |
| <i>Streptococcus pyogenes</i> | 2 | 2 | 2 | 1 | 7 |
| <i>Klebsiella pneumonia</i> | 2 | 1 | 1 | 1 | 5 |
| <i>Salmonella typhimurium</i> | 1 | 1 | 1 | 2 | 5 |
| <i>Proteus vulgaris</i> | 1 | 1 | - | 1 | 3 |
| <i>Pseudomonas aeruginosa</i> | 1 | 1 | 1 | 1 | 4 |
| <i>Entrobater aerogenes</i> | 1 | 1 | 2 | 2 | 6 |
| Total | 16 | 16 | 14 | 13 | 59 |

Table 9. Identification of the bacteria isolate from (Retail Outlet 3) Badariya

| Bacteria identified | Samples | | | | Total |
|-------------------------------|---------|--------------|----------------|-------|-------|
| | Knife | Weighing pan | Butcher's hand | Table | |
| <i>Staphylococcus aureus</i> | 3 | 4 | 4 | 4 | 15 |
| <i>Escherichia coli</i> | 4 | 3 | 2 | 2 | 11 |
| <i>Micrococcus luteus</i> | 1 | 2 | 1 | 1 | 5 |
| <i>Streptococcus pyogenes</i> | 1 | 1 | 1 | 1 | 4 |
| <i>Klebsiella pneumonia</i> | 2 | 2 | 2 | 1 | 7 |
| <i>Salmonella typhimurium</i> | 2 | 1 | 1 | 2 | 6 |
| <i>Proteus vulgaris</i> | 1 | 2 | 1 | 2 | 6 |
| <i>Pseudomonas aeruginosa</i> | 1 | 1 | 1 | 1 | 4 |
| <i>Entrobater aerogenes</i> | 1 | 2 | 1 | 1 | 5 |
| Total | 16 | 18 | 14 | 15 | 63 |

Table 10. Identification of the bacteria isolate from (Retail Outlet 4) Bayan Kara

| Bacteria identified | Samples | | | | Total |
|-------------------------------|---------|--------------|----------------|-------|-------|
| | Knife | Weighing pan | Butcher's hand | Table | |
| <i>Staphylococcus aureus</i> | 4 | 3 | 4 | 3 | 14 |
| <i>Escherichia coli</i> | 3 | 2 | 4 | 2 | 11 |
| <i>Micrococcus luteus</i> | - | 2 | 1 | 2 | 5 |
| <i>Streptococcus pyogenes</i> | 1 | 1 | 2 | 1 | 5 |
| <i>Klebsiella pneumonia</i> | 2 | 2 | 2 | 1 | 7 |
| <i>Salmonella typhimurium</i> | 2 | 1 | 2 | 1 | 6 |
| <i>Proteus vulgaris</i> | 1 | 2 | 1 | 1 | 5 |
| <i>Pseudomonas aeruginosa</i> | 1 | 1 | 1 | 2 | 5 |
| <i>Entrobater aerogenes</i> | 1 | - | 1 | 1 | 3 |
| Total | 15 | 14 | 18 | 14 | 61 |

Table 11. Identification of the bacteria isolate from (Retail Outlet 5) Rafin Atiku

| Bacteria identified | Samples | | | | Total |
|-------------------------------|---------|--------------|----------------|-------|-------|
| | Knife | Weighing pan | Butcher's hand | Table | |
| <i>Staphylococcus aureus</i> | 4 | 4 | 4 | 4 | 16 |
| <i>Escherichia coli</i> | 3 | 3 | 2 | 2 | 10 |
| <i>Micrococcus luteus</i> | 1 | 1 | 1 | 2 | 5 |
| <i>Streptococcus pyogenes</i> | 2 | 1 | 2 | 1 | 6 |
| <i>Klebsiella pneumonia</i> | 1 | 1 | 2 | 1 | 5 |
| <i>Salmonella typhimurium</i> | 2 | 2 | 1 | 1 | 6 |
| <i>Proteus vulgaris</i> | 1 | 1 | 2 | 2 | 6 |
| <i>Pseudomonas aeruginosa</i> | 1 | 1 | 1 | 1 | 4 |
| <i>Entrobater aerogenes</i> | 1 | 2 | 1 | 2 | 6 |
| Total | 16 | 16 | 16 | 16 | 64 |

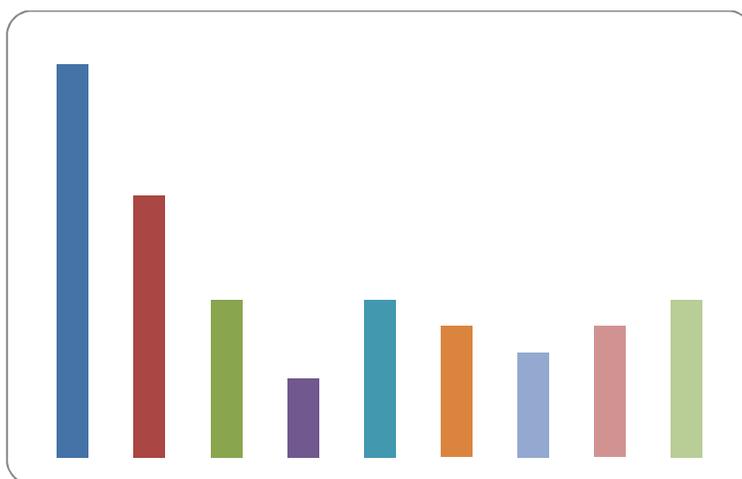
3.5 Frequency of Occurrence of Microbes in Retail Outlets

The frequency of occurrence of bacteria from the five retail outlets, Main Market, GRA, Badariya, Bayan Kara and Rafin Atiku is presented in

Figs. 1-5. The frequency of occurrence of *Staphylococcus aureus* were present in all the samples taken across the retail outlets. It recorded an average of 24.4% of the samples taken in all the retail outlets in Birnin Kebbi Metropolis.

Table 12. Identification of the bacteria isolate from Birnin Kebbi Central Abattoir

| Bacteria identified | Samples | | | | | Total |
|-------------------------------|---------|-------|-----|---------------|--------------|-------|
| | Knife | Floor | Van | Faecal sample | Water sample | |
| <i>Staphylococcus aureus</i> | 4 | 3 | 3 | 4 | 4 | 18 |
| <i>Escherichia coli</i> | 3 | 2 | 2 | 4 | 2 | 13 |
| <i>Micrococcus luteus</i> | 1 | 1 | 1 | - | 1 | 4 |
| <i>Streptococcus pyogenes</i> | 2 | 1 | 1 | 1 | 1 | 6 |
| <i>Klebsiella pneumonia</i> | 1 | 1 | 2 | 1 | - | 5 |
| <i>Salmonella typhimurium</i> | 2 | 3 | 2 | 3 | 4 | 14 |
| <i>Proteus vulgaris</i> | 1 | 1 | 1 | 2 | - | 5 |
| <i>Pseudomonas aeruginosa</i> | 1 | 2 | - | 1 | 1 | 5 |
| <i>Entrobater aerogenes</i> | 2 | 1 | 1 | 2 | 1 | 7 |
| Total | 17 | 15 | 13 | 18 | 14 | 77 |

**Fig. 1. Frequency of occurrence of isolated bacteria from Retail outlet 1(Main market)**

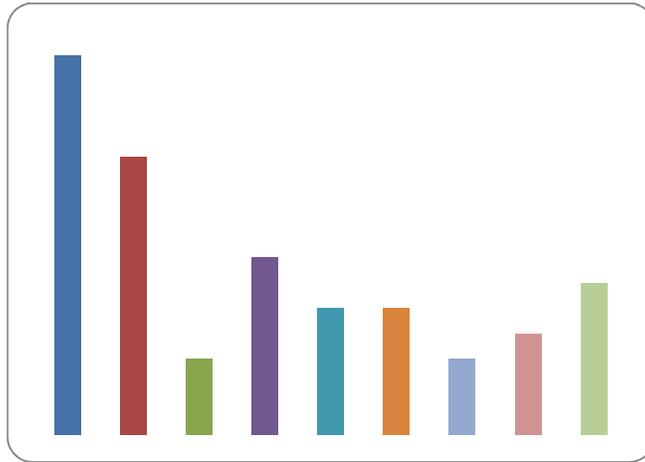


Fig. 2. Frequency of occurrence of isolated bacteria from Retail outlet 2 (GRA)

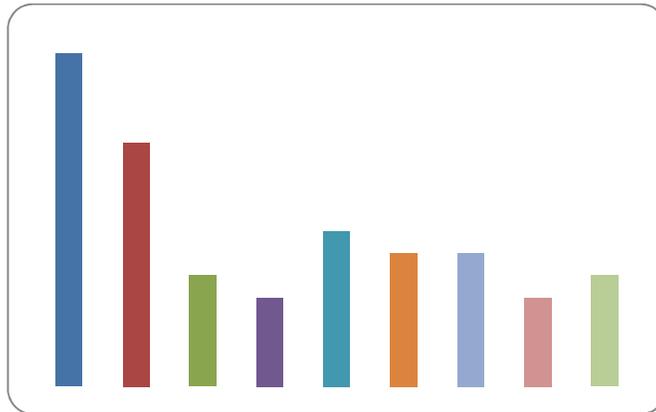


Fig. 3. Frequency of occurrence of isolated bacteria from Retail outlet 3 (Badariya)

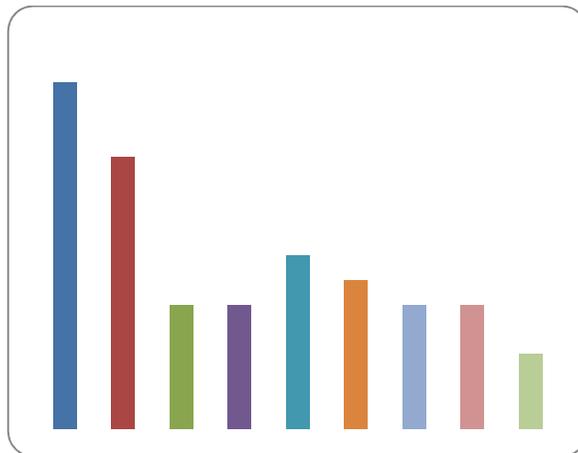


Fig. 4. Frequency of occurrence of isolated bacteria from Retail outlet 4 (Bayan Kara)

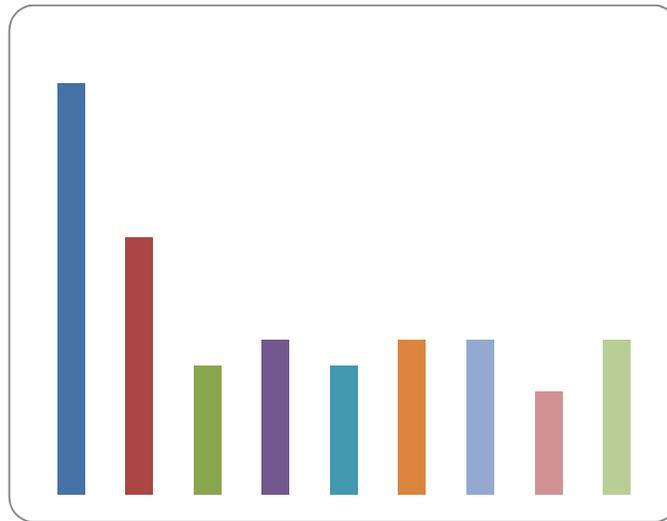


Fig. 5. Frequency of occurrence of isolated bacteria from Retail outlet 5 (Rafin Atiku)

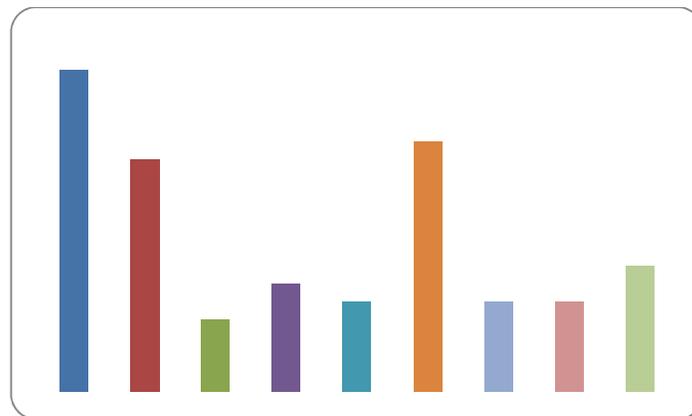


Fig. 6. Frequency of occurrence of isolated bacteria from Birnin Kebbi Central Abattoir

3.6 Frequency of Occurrence of Microbes in Abattoir

The frequency of occurrence of bacteria in the samples collected from Birnin Kebbi Central abattoir is presented in Fig. 6. The frequency of occurrence of bacteria indicated that *Staphylococcus aureus* were mostly present in all the samples collected and it was also observed that *Salmonella typhimurium* is high with 4 out of 20 samples collected, thus representing 20%.

4. DISCUSSION

The difference in the microbial load can be attributed to the way the meats were handled and unhygienic practices observed during data collection. It was observed that butchers handling

meat paid little or no attention to their personal hygiene and served the meat with dirty hands and clothing. Meat were put on tables which are not well cleaned before and after the day's work and also open, exposing the meat to houseflies and pathogenic agents. Poor sanitation was also observed in the immediate environment where meats are sold. It was observed that similar unhygienic practices in the handling of meat in the Yendi Municipality of the Northern Region of Ghana [29]. The aforementioned practices contributed to the high microbial load and the differences in the load observed. Mukhopadhyay (2009) reported that hot and humid climate areas contribute to increasing total bacterial count on meat; and that could have contributed to the high total bacterial counts of the meat in this study since Birnin Kebbi is a hot and humid area. Under poor processing conditions pathogenic

and non-pathogenic microorganisms are introduced during slaughtering of animals and processing of carcasses into meat [30,29].

The presence of different bacteria generated in the meat samples confirms the poor slaughtering, handling and environmental conditions under which animals, carcasses, and meats are handled, processed or sold in Birnin Kebbi metropolis of Kebbi State of Nigeria. [25] reported that the unhygienic practices of meat processing in developing countries results in these meats being contaminated with microorganisms.

The presence of these organisms in fresh meats depicts a deplorable state of poor hygienic and 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 *Staphylococcus aureus*, *Klebsiella pneumonia*, *Entrobater aerogenes*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, and *Proteus vulgaris*.

This agrees to previous reports by [31] who reported *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* in meat pie and [32] who reported *Entrobater aerogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhimurium*, *Micrococcus luteus*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* in seafood products. Actually, they consider the detectable presence of pathogens like *Salmonella typhimurium* as an indicator of adulteration [33].

Most of the organisms found in this study are those commonly found in soil and water. *Staphylococcus aureus* was the most isolated in this present study as reported in all previous work mentioned above, the presence of *Escherichia coli*, *Salmonella typhimurium* and *Entrobater aerogenes* in this fresh meat samples is an indication of faecal contamination of the meats. This might be due to possible contamination of fresh meats during sales or unhygienic handling of the meats right from the slaughtering, butchering plants and retailing or due to contamination from the skin, mouth, or nose of the handlers which can be introduced directly into foods by process line workers, with lesions caused by *Staphylococcus aureus* on hands and arms coming into contact with the food, or by coughing and sneezing [32].

The isolation of *Entrobater aerogenes* may be as a result of poor environmental conditions due to dust and contamination of the water used during slaughtering, because *Entrobater aerogenes* are also inhabitants of dairy products. *Salmonella typhimurium*, another organism found in the meats is also a pathogenic organism of public health significance and concerns [32]. The isolation of *Salmonella typhimurium* in this study is of practical impact. This organism might have contaminated the meats as a result of handling by meat sellers. This result agrees to previous reports by [34] that foods of animal origin (minced meat) either cooked or uncooked were predominantly contaminated with *Escherichia coli*. This is also in accordance to the assertion of [32] that improper handling and improper hygiene might lead to the contamination of ready-to-eat foods and this might eventually affect the health of the consumers. This was illustrated by the presence of the indicator organisms.

This study also reveals that fresh meats are often contaminated with bacteria. The presence of higher number of pathogenic *Klebsiella pneumoniae*, *Salmonella typhimurium* and *Escherichia coli* among others, encountered in fresh meat from conventional beef is alarming. The presence of these organisms in meat foods should receive particular attention, because their presence indicates public health hazard and give warning signal for the possible occurrence of food borne intoxication [35]. Since the control of faecal-orally transmitted pathogens is inadequate in many developing countries particularly in sub-Saharan Africa, acquired resistance to antimicrobial drugs is becoming more prevalent [7].

5. CONCLUSION

The results obtained from this study shows that there was higher microbial load in both abattoir and retail meat outlets. It suggests that meat from these contact surfaces are contaminated by these organisms *Staphylococcus aureus*, *Klebsiella pneumonia*, *Entrobater aerogenes*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, and *Proteus vulgaris* and may predispose the consumers to food poisoning. This may be due to the low level of Education of the abattoir and retail outlets workers because the carcasses were dressed on the floor and low level of hygiene and poor abattoir sanitation could also be responsible for the high TVC on the meat. The results above can be deduced that

contamination was present right from the abattoir to the retail meat outlets. However, some contaminants are added when meat was being transported to and at the beef retail outlets where they are sold. Thus the meat produced in the study area is contaminated before it gets into the hands of consumers. This development is of serious public health significance because the resistant isolates may be transferred to the consumers of such meat who will subsequently develop resistance to the therapeutic agents. The bacterial isolates are also enterotoxigenic elaborating heat-labile enterotoxins. Therefore, in order to safeguard the health of public against the risks of food borne infections, there is a need to educate and advocate good sanitation and meat handling practices in the abattoir and beef retail outlets.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tauxe RV. Emerging foodborne diseases: An evolving public health challenge. *Emerging Infectious Disease Journal*. 1997;3(4):14-25.
2. Adak GK, Longs SM, O'Briens SJ. Trends in indigenous foodborne disease and deaths. *England and Wales: 1999- 2000. Gut*. 2002;51:832-841.
3. Sofos JN. Challenges to meat safety in the 21st century. *Meat Sciences Journal*. 2008;78:3-13.
4. Schuster G. Transmission of diseases via animals and insects. *Environmental and Children's Health Training*. Texas A & M University – Kingsville; 2011.
5. Meng J, Zhao S, Doyle MP, Joseph SW. Antibiotic resistance of *Escherichia coli* O157: H7 and O157: NM from animals, food and humans. *Journal Food Protection*. 1998;61:1511-1514.
6. Hart CA, Kariuki S. Antimicrobial resistance in developing countries. *British Medical Journal*. 1998;317:647-650.
7. Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, Pablos-Mendez A, Klugman KP. Antimicrobial resistance in developing countries. Part I: Recent trends and current status. *The Lancet Infectious Diseases*. 2005;5(8):481-493.
8. Aibinu IE, Peters RF, Amisu KO, Adesida SA, Ojo MO, Odugbemi T. Multidrug resistance in *E. coli* O157 strains and public health implication. *Journal of American Scientist*. 2007;3(3):22-33.
9. Ikeme M. *Meat science and technology*. African Feb Publishers Ltd. 1990;370.
10. Umolu PI, Ohenhen ER, Okwu IG, Ogiehor IS. Multiple antibiotics resistant index and plasmid of *Escherichia coli* in Beef in Ekpoma. *Journal of American Science*. 2006;2(3):22-28.
11. Ikema HP, Hassen E. Moulds control in meat processing industry. *Fleischwirtschaft*. 1990;75: 52-59.
12. Mtenga LA, Lemma BE, Muhikambe VR, Maeda GK, Nnko SM, Makungu P. Assessment of bacterial contamination of meat, water and meat handling equipment at some abattoirs and butcher shops in Dar es Salaam City and its hygienic implication. *Sokoine University of Agriculture. SUA-NORAD PROJECT TAN -91, 28pp Market Poultry Meat in Bangalore, India. Journal of Animal and Veterinary Advance*. 2000;10(2):188-191.
13. Komba EVG, Komba EV, Mkupasi EM, Mbyuza AO, Mshamu S, Luwumba D, Busagwe Z, Mzula A. Sanitary practices and occurrence of Zoonotic conditions in cattle at slaughter in Morogoro Municipality, Tanzania: Implications for public health. *Tanzania Journal of Health Research*. 2012;14:21-29.
14. Ahmad MUD, Sarwar A, Najeeb MI, Nawaz M, Anjum AA, Ali MA, Mansur N. Assessment of microbial load of raw meat at abattoirs and retail outlets. *The Journal of Animal & Plant Sciences*. 2013;23(3):745-748.
15. Oluwafemi RA, Edugbo OM, Solanke EO, Akinyeye AJ. Meat quality, nutrition security and public health: A review of beef processing practices in Nigeria. *African Journal of Food Science and Technology*. 2013;4(5):96-99.
16. Doores S, Salfinger Y, Tortorello ML. *Compendium of methods for the microbiological examination of food*. American Public Health Association, Washington, DC; 2013.
17. Aryal S. *Mueller Hinton Agar (MHA) – Composition, principle, uses and preparation*; 2015b. Available:www.microbiologyinfo.com (Accessed 17th September, 2017)

18. Aryal S. MacConkey Agar- Composition, principle, uses, preparation and colony morphology; 2015a. Available:www.microbiologyinfo.com (Accessed 17th September, 2017)
19. Jones N. Five steps to prepare agar slants; 2017. Available:www.sciencing.com/five-steps-prepare-agar-slants (Accessed 17th September, 2017)
20. MacFaddin JF. Biochemical tests for identification of medical bacteria, 3rd Ed. Williams and Wilkins, Baltimore. Md.; 2000.
21. Acharya T. Triple Sugar Iron Agar (TSI): Principle, procedure and interpretation; 2013. Available:www.microbeonline.com/triple-sugar-iron-agar-tsi (Accessed 17th September, 2017)
22. Aryal S. Simmons Citrate Agar - Composition, principle, uses, preparation and result interpretation; 2015c. Available:www.microbiologyinfo.com (Accessed 17th September, 2017)
23. Bozic N. Normal saline: How to prepare at home; 2011. Available:www.aboutkidshealth.ca (Accessed 17th September, 2017)
24. Fawole MO, Oso BA. Laboratory manual of microbiology. Revised Edition, Spectrum Books, Ibadan, Nigeria; 2001.
25. Bhandare SG, Paturkar AM, Waskar VS, Zende RJ. Bacteriological screening of environmental sources of contamination in an abattoir and the meat shops in Mumbai, India. Asian Journal Food Agro-Industry. 2009;2(3):280-290.
26. Cheesbrough M. Medical laboratory manual. Tropical Health Technology, Low Priced Edition. Doddington, Cambridgeshire, England. 2003;20-35.
27. Oyeleke SB, Manga SB. Essentials of laboratory practical in microbiology, 1st Edition. Tobest Publishers, Niger State, Nigeria. 2008;20-33.
28. Buchanan RE, Gibbons NE. Bergey's manual of determinative bacteriology (8th Edition). Williams and Wilkins Company, Baltimore, USA; 1974.
29. Adzitey F, Teye GA, Dinko MM. Pre and post-slaughter animal handling by butchers in the Banku Municipality of the Upper East Region of Ghana. Livestock Research for Rural Development. 2011;23(39).3 Available:www.lrrd.org/lrrd23/2/adzi23039.htm (Accessed 2/5/2017)
30. Norrung B, Andersen JK, Schlundt J. Incidence and control of *Listeria monocytogenes* in foods in Denmark. International Journal of Food Microbiology. 1999;53:195–203.
31. Clarence SY, Obinna CN, Shalom NC. Assessment of bacteriological quality of ready to eat food (Meat pie) in Benin City metropolis, Nigeria. African Journal Microbiology Research. 2009;3(6):390-395.
32. Okonko IO, Donbraye E, Ogun AA. Microbiological quality of seafood processors and water used in two different sea processing plants in Nigeria. Electronic Journal of Environmental, Agricultural and Food Chemistry. 2009;9(1):239-247.
33. Yousuf AHM, Ahmed MK, Yeasmin S, Ahsan N, Rahman MM, Islam MM. Prevalence of microbial load in shrimp, penaeus monodon and prawn, *Macrobrachium rosenbergii* from Bangladesh. World Journal of Agricultural Sciences. 2008;4(5):852-855.
34. El-Gohany AH. Sausage and minced meat as a source of food poisoning microorganisms to man. Asian Veterinary Medicine Journal. 1994;30:146-215.
35. Kabir SML. The role of probiotics in the poultry industry. International Journal of Molecular Science. 2009;10:3531-3546.

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