



Microbial Loads of Beef and Hygienic Practice of Butchers in Jos Municipal Abattoir

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The microbial loads of processed beef in Jos abattoir were investigated in this study, as well as the hygiene practices of butchers. Fresh beef samples were randomly collected from Jos-south abattoir, Plateau State, Nigeria. A total of twenty samples were collected in a separate sample bag. They were kept in cool box filled with ice and transported to the microbiology laboratory of Federal College of Animal Health and Production Technology (FCAH&PT), National Veterinary Research Institute, Vom, Nigeria for analyses. Meat samples weighing one gram each were grinded to fine

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particles using mortar and pestles and mixed with the normal saline solution to make 10 milliliters and diluted serially into ten test tubes. One milliliter of inoculum was taken from the test tube using sterile pipette and poured into sterile Petri dish. 20 milliliters of sterile nutrient agar was poured into each sterile Petri dish, distributed and mixed evenly. 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 the viable bacterial counts. Petri dishes containing 30 - 300 colonies on nutrient agar plate were selected. These were counted using colony counter, enumerated and expressed in CFU/g/ml of meat samples from every location respectively. Incubated nutrient agar plates were examined for yellow and amber growth. Isolates were examined microscopically using Gram staining procedure for Gram- positive cocci in clusters. These suggested the presence of *Staphylococcus species*. The incubated Eosin Methylene blue agar (EMB Agar) plates were examined for characteristic dark centered greenish and translucent colorless amber growth which suggest the presence of *Escherichia coli*, and translucent, amber colored or colorless growth which suggest the presence of *Salmonella* or *Shigella species*. The isolates were examined microscopically using Gram-staining procedure for Gram-negative straight short rods which suggested the presence of *Escherichia coli* and Gram-negative long rods which suggests the presence of *Salmonella* and *Shigella species*. The growth on Mac-Conkey plates were examined for pink colonies for *Escherichia coli* which is a lactose fermenter and amber colonies for *Salmonella* or *Shigella species*. The incubated Salmonella-Shigella agar plates were examined for colourless colony with black centered growth which suggest the presence of *Salmonella species* (hydrogen sulphite producing bacteria), pink growth which suggests the presence of *Escherichia coli* (lactose fermenter) and colorless colony which suggests the presence of *Shigella species* (non-lactose fermenter and non-hydrogen sulphite producing bacteria). The biochemical characteristics were carried out according to the method described by [14,15]. *Escherichia coli* had the highest percentage load of 34.1%, *Salmonella spp.* with 31.8%, *Staphylococcus aureus* had 20.5% and *Shigella spp.* had 13.6%. To ascertain the hygiene level of butchers in the abattoir, a total of 50 copies of the questionnaire were distributed to abattoir attendants/butchers in Jos-south Local Government and 43 out of this were retrieved. The response of 86% was considered sufficient for analysis in this study. This study captured the general information on the respondents such as gender, age and educational status. In this case, all the respondents (100%) were males with age range 18-44 years.

Keywords: Microbial load; butchers; hygiene; Jos; Abattoir.

1. INTRODUCTION

Food safety is an issue of paramount concern and of public health importance particularly when the environment in which the food is handled is heavily contaminated [1]. Raw meat remains an essential and possibly the chief source of pathogenic bacteria in human food-borne infections. There has been difficulty in obtaining food animals free of pathogenic bacteria in spite of decades of control [2]. Most fresh food, particularly those from animals are highly vulnerable to microbial contamination and food poisoning since meat is an ideal medium for growth of a good number of microorganisms due to its nutritive value [1]. The primary unit of meat is carcass which usually represents the ideal meat after discarding the head, intestines, hides and blood. The edible portions of carcass would include lean flesh, fat flesh and edible glands or organs which include the heart, kidney, liver, brain and tongue [3]. Healthy animal tissues are

normally sterile, but can be contaminated by microorganisms from the exterior of the animal and its intestinal tract during slaughter, dressing and cutting [3]. Microbial contamination of meat and meat products must not surpass levels which could have negative impacts on the shelf life of meat products and render it unhealthy for human consumption. Food of animal origin tends to deteriorate more rapidly under tropical conditions, thereby becoming an important medium for gastrointestinal infections, inadvertently jeopardizing consumers' health [4]. Probable sources of contaminations arising from the meat handlers, hides, cutting knives, intestinal contents, chopping boards, containers, meat selling environment and vehicle for transporting carcasses have been reported [5]. Also weighing scales and wooden boards from meat retail outlets are sources of bacterial contamination, especially *Staphylococcus aureus* and *Shigella species* [6]. In Nigeria, a good number of abattoirs and slaughter houses are

substandard; their practices unsupervised and far from hygienic, raising concerns about health safety of consumers as meat is an important regular in the Nigerian dish. Food security is a complex issue in Africa generally, where animal proteins such as meats, meat products, fish and fishery products are often regarded as a high risk commodity to infection and toxicities [7]. These food borne infections and the consequent illnesses are some of the major international challenges that lead to high mortality and economic loss [8]. In the industrialized world, food borne infection cause considerable illnesses that heavily affect healthcare systems [8]. Food borne diseases are diseases resulting from ingestion of bacteria, toxins and also cells produced by microorganisms present in food [9]. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individuals to the toxin. Meat and meat products are sometimes contaminated with germs after leaving the manufacture plant and during handling [10]. Microbial contamination of meat and products of meat must not extend beyond levels which could seriously affect the shelf life of the product; if it does it renders the meat unwholesome and not fit for human consumption [11]. Reduction of risk for human illness associated with raw produce can be better achieved through controlling points of potential contamination in the field, during harvesting, during processing or distribution, or in retail markets, food-service facilities, or the home [12].

2. METHODOLOGY

2.1 Materials

- Questionnaire
- Incubator
- Colony counter
- Fresh meat
- Light microscope
- Culture media
- Cotton wool
- Refrigerator
- Wire loop

2.2 Sample Collection

A total of twenty fresh beef samples were collected in separate sample bag. They were kept in cool box filled with ice which was transported immediately to Microbiology laboratory of FCAH&PT, National Veterinary Research Institute, Vom for analysis.

2.3 Preparation of Meat Samples and Bacteriological Counts

Meat sample weighing one gram weighed in a Kern 572-33 (Kern and Sohn GmbH, Germany) weighing scale was grinded to fine particles using mortar and pestles and mixed with the normal saline solution to make 10 milliliters and diluted serially into ten test tubes. One milliliter of inoculum was taken from the test tube using sterile pipette and poured into each sterile petri dish. Then 20 milliliters of sterile nutrient agar was poured into each sterile petri dish, distributed and mixed evenly throughout. All samples inoculated in nutrient agar (Oxoid, USA) were incubated in an incubator (Mettler incubator, Becking Schwabach, Germany) at 37°C for 24 hours in order to get the viable bacteria count.

2.4 Interpretation of Microbial Growth

Petri dishes containing 30 - 300 colonies on nutrient agar plate were selected. These were counted using colony counter (Stuart, Bibby Scientific, UK), enumerated and expressed in CFU/g/ml of meat, samples from every location respectively.

2.5 Isolation of Pathogenic Bacteria from Meat

The pure colonies were obtained according to the method described by Clinical and laboratory standards institutes (CLSI (2005)). One nutrient agar plate, Mac-Conkey agar plate, Salmonella Shigella agar plate and Eosin Methylene Blue Agar plates (Oxoid, USA) for each were streaked using a wire loop with inoculum gotten from the prepared meat samples and incubated in an incubator (Mettler incubator, Becking Schwabach, Germany) at 37°C for 24 hours. The isolates obtained were observed for morphological and cultural characteristics in each of the cultured media for all the samples and biochemical tests.

2.6 Identification of Isolate

The identification of the isolates *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp. and *Escherichia coli*, was done following the procedure described by [13] and [14] as follows:

2.7 Morphological and Cultural Characteristics

Incubated nutrient agar plates were examined for yellow and amber growth. Isolates were

examined microscopically (Olympus Microscope, Zeiss 080600 Zeiss, Germany) using Gram staining procedure for Gram- positive Cocci in clusters. These suggested the presence of *Staphylococcus* species. The incubated EMB Agar plates (Oxoid,USA) were examined for characteristic dark centered greenish and translucent amber growth which suggest the presence of *Escherichia coli*, and translucent, amber colored or colorless growth which suggest the presence of *Salmonella* or *Shigella* species. The isolates were examined microscopically (Olympus Microscope, Zeiss 080600 Zeiss, Germany) using Gram-staining procedure for Gram-negative straight short rods which suggested the presence of *Escherichia coli* and Gram-negative long rods which suggest the presence of *Salmonella* and *Shigella* species. The growth on Mac-Conkey plates (Oxoid, USA) were examined for pink colonies for *Escherichia coli* which is a lactose fermenter and amber colonies for *Salmonella* or *Shigella* species. The incubated Salmonella Shigella Agar plates (Oxoid, USA) were examined for colorless colony with black centered growth which suggest the presence of *Salmonella* species (hydrogen sulphite producing bacteria), pink growth which suggests the presence of *Escherichia coli* (lactose fermenter) and colorless colony which suggests the presence of *Shigella* species (non-lactose fermenter and non-hydrogen sulphite producing bacteria).

2.8 Biochemical Characteristics

The biochemical activities were carried out according to the method described by [14] and [15].

2.9 Catalase Production Test

A wire loop (sterile) was used to take a speck of growth from each plate of 24 hours growth and a suspension was made with sterile distilled water on a clean microscope slide. Few drops of hydrogen peroxide were added using a pipette. Positive result showed the evolution of gas bubbles (effervescence) while negative result produced no bubbles.

2.10 Motility Test

The isolates were cultured in peptone water for 24 hours before the motility test was carried out. A drop of the suspension was placed on a glass slide and covered with a cover slip. The whole

preparation was sealed using a petroleum jelly to prevent it from drying. The preparation was observed microscopically (Olympus Microscope, Zeiss 080600 Zeiss, and Germany) for motile organisms using the X10 and X40 objectives.

2.11 Coagulase Test

A small speck of growth from different plates were picked with a sterile loop and dropped on glass-slides. Few drops of plasma were applied on the inoculum and a smear was made and slides were rocked for 2 minutes. Positive result produced clumps while negative results produced no clumps.

2.12 Oxidase Test

Oxidase test strips were used, by inserting it into a 24-hour broth culture of isolates and withdrawing and kept for 5 minutes for color change. For Positive result, color change from yellow to dark purple while negative result produced no color change.

Other biochemical tests carried out were citrate test, indole test, urease test, starch hydrolysis test, methyl- red test etc.

2.13 Data Collection Methods

2.13.1 Questionnaire survey

The study involved a questionnaire survey in order to assess the risk factor contributing to contamination of beef in the abattoir to be. A structured questionnaire was administered to selected abattoir workers. And analysis was done using simple percentage, tables and charts.

3. RESULTS

3.1 Questionnaire Survey Responses

A total of 50 copies of Questionnaire were distributed to abattoir attendants in Jos south Local Government area of Plateau State, Nigeria and 43 of these were retrieved. This was considered sufficient for analysis in this study and conforms to [16] stipulation that a response rate of 50% is adequate for analysis and statistical reporting; a response rate of 60% is good while a response rate of 70% and over is excellent. This commendable response rate was due to extra efforts that were made via follow-up visits to remind the respondents to fill-in and

return the questionnaire and also helping the uneducated ones to fill the form based on their oral responses. Thus, the results presented in this work are based on those 43 respondents.

3.2 Socio-Demographic Characteristics of Respondents

This study collected the general information on the respondents such as gender, age, and educational status, in which all the respondents (100%) were males. A total of 50 males were interviewed during the study of which all were abattoir workers. All of the respondents in

abattoir were males with age ranging between 18-44 years (Fig. 1).

3.3 Bacteriological Analysis

The collected meat samples from the butcheries showed considerably high numbers of colonies as shown in Table 3.

3.4 Detection of Pathogens in Meat Samples from Different Sources

Twenty samples were collected from four locations in the abattoir, five from each location and four species of bacteria were isolated.

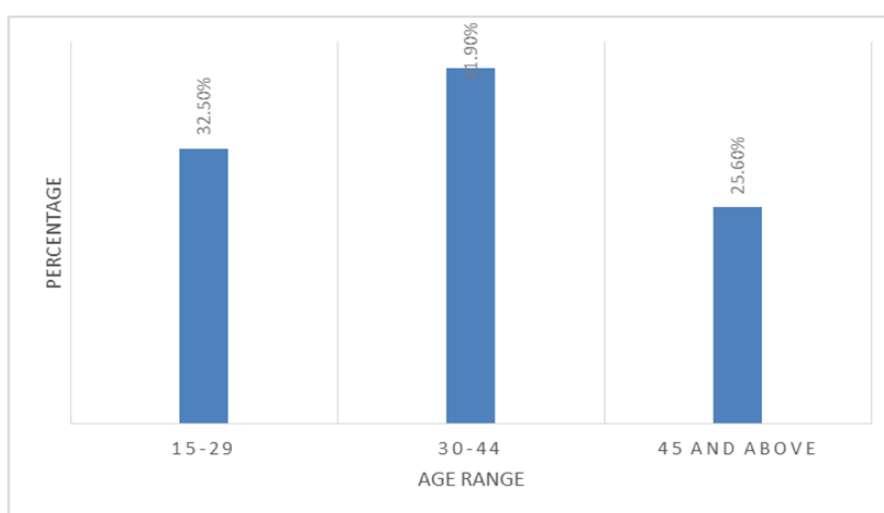


Fig. 1. Distribution of respondents by age

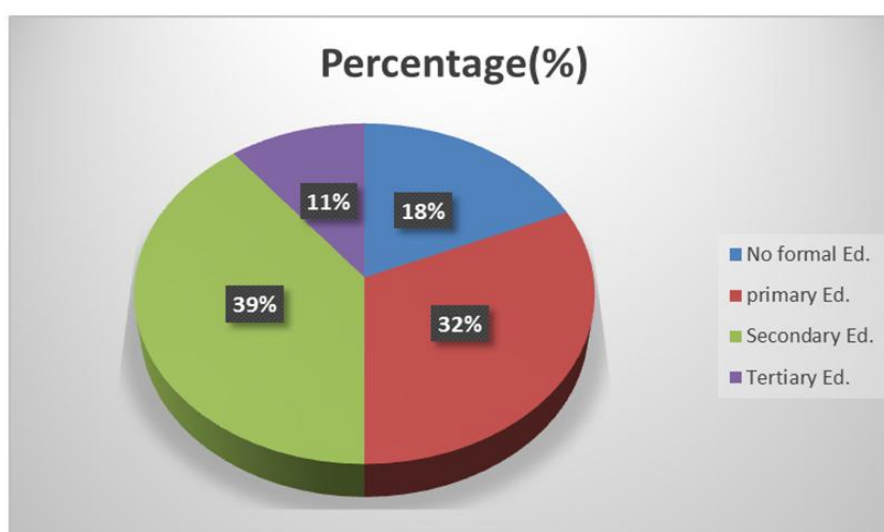


Fig. 2. Distribution of respondents according to level of education

Table 1. Disinfectants used by various respondents to wash floor, hands and containers in the Abattoir

Disinfectant used	Number of respondents (Percentage)
Dettol	15 (34.9%)
Ethanol	3 (7.0%)
Formaldehyde	6 (14.0%)
Others	0 (0%)
Don't use any disinfectant	19 (44.1%)

Table 2. Hygienic condition of abattoir workers

Activity	Yes	No	Undecided
Discard Water Waste Close to Abattoir	32	11	0
Wash hands before slaughtering	39	2	2
Sufficient drainage to carryout waste products	12	24	7
Butchers wear protective cloths	16	27	0
Wash hands after toilet	38	3	2
Toilet close to abattoir	12	22	9

Table 3. Result of plate count for the four different locations

Samples	No. of colony formed	No. of Innoculum/ml	Dilution factor	Total no. of colony formed
1	273	1.0	10^5	2.78×10^7
2	191	1.0	10^5	1.91×10^7
3	206	1.0	10^5	2.06×10^7
4	276	1.0	10^5	2.76×10^7
5	260	1.0	10^5	2.60×10^7
Total	1211			
6	288	1.0	10^5	2.88×10^7
7	284	1.0	10^5	2.84×10^7
8	279	1.0	10^5	2.79×10^7
9	289	1.0	10^5	2.89×10^7
10	277	1.0	10^5	2.77×10^7
Total	1409			
11	300	1.0	10^5	3.00×10^7
12	251	1.0	10^5	2.51×10^7
13	225	1.0	10^5	2.25×10^7
14	300	1.0	10^5	3.00×10^7
15	273	1.0	10^5	2.73×10^7
Total	1349			
16	187	1.0	10^5	1.87×10^7
17	108	1.0	10^5	1.08×10^7
18	103	1.0	10^5	1.03×10^7
19	201	1.0	10^5	2.01×10^7
20	141	1.0	10^5	1.41×10^7
Total	740			

4. DISCUSSION

In the abattoir, 32.5% of workers had an average age of 15-29 years, 30-44 years (41%), and 45 years and above, (25%). Findings from this study are different from what was reported by [5], who

found 45% of the abattoir workers were within the ages of 41-50 years, followed by 31-40 (23%), 51-60 (16%) and 21-30 (13%). Only one abattoir worker (3%) was 60 years old and was the head and coordinator of all activities in the slaughter house. Most of workers in abattoir were

Table 4. Mean value for total viable count (CFU/g/ml) in meat samples collected from four different locations in the Abattoirs

Location	Mean No. of Colonies/g/ml	Dilution factor	Mean total viablecount
L1	242.2	10 ⁵	2.422x10 ⁷
L2	281.8	10 ⁵	2.818 x 10 ⁷
L3	269.8	10 ⁵	2.698 x 10 ⁷
L4	148	10 ⁵	1.48 x 10 ⁷

Table 5. Distribution of four bacterial meat contaminant isolates

Location (L)	Sample size	Isolates			
		<i>Salmonella</i> spp.	<i>Shigella</i> spp.	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
L1	5.00	5	0	4	2
L2	5.00	3	1	2	2
L3	5.00	4	3	4	5
L4	5.00	2	2	5	0
Total	20.00	14	6	15	9

Table 6. Total percentage of organisms found

S/N	Organism	Percentage
1	<i>Salmonella</i> spp.	31.8%
2	<i>Shigella</i> spp.	13.6%
3	<i>Escherichia coli</i>	34.1%
4	<i>Staphioccoccus aureus</i>	20.5%

males with age between 18-40 years which falls within an active age group; this is in consonance with [5] who reported that the butchering activity is more dominated by the youth and middle aged men who are more energetic as the butchering business requires much physical strength. In this research, only 11% attended tertiary institutions, there were 39% of the respondents with secondary education, 32% with primary education and 18% with no formal education. The level of education and training of food handlers about the basic concept and requirements of personal hygiene and its environment plays an important part in safeguarding the safety of products to consumers. During the study it was revealed that, the abattoir workers had low level of education and this could make difficult in acceptability of modern slaughtering practices as well as adherence to strict hygienic and standard slaughtering practices that contribute to microbial contamination. From the survey conducted at Makelle City, Ethiopia by [17], it was found that out of 26 abattoir workers interviewed, 7.7% were illiterate, and 61.5% had no any training regarding meat hygiene. [18] reported that workers working in the abattoir in most cases in developing countries are untrained and thus, they pay no attention to the hygienic standards and as a result contribute immensely to bacterial contamination. This might be the reason for the

meat contamination and with a good number of the respondents in the abattoir (44.1%) don't use disinfectants for washing of floor, 34% make use of Dettol, and 3% make use of ethanol. The floor is a major depositing point of microbes as workers match in and out; packing microbes on the way and depositing them on the floor were the slaughtering takes place. Not using disinfectant to clean the floor allows the pathogens to get in contact with the meat during slaughtering and hence, a good source of contamination. These findings are similar to those reported by [5] that 65% of abattoir workers dressed carcasses on bare floor in the abattoir, 16% dressed carcasses on unclean slaughter slabs and 19% on both the slaughter slabs and bare floor, whereas most of these slaughter floor and slabs were smeared with blood, rumen contents and other wastes from previously dressed animals which increased the risk of contamination of subsequent carcasses. [19] Found that animals were often slaughtered and eviscerated on the floor because of the absence of mechanical or manual hoists, a factor which contributed to a major source of contamination. The sanitation and hygiene status of the butcheries was generally poor as most of them did not meet the sanitary requirements for operation as stipulated in [20]. Meat hygiene was not properly observed as factors that directly affect it such as discarding wastes; sufficient

drainage system and hand washing practices were not adhered to. Additionally, practices such as use of protective wear were low and other requirements such as medical examination of meat handlers and hand washing after toilet was neither consistent nor verifiable. Observation showed that domestic flies were abundant in most parts of the abattoir. It is important to control flies since they feed on meat and other wastes, where they pick up and transport various diseases causing agents with potential to causes such as enteric and eye infections. Based on findings from this study, the sources of meat contamination originated from the slaughter process in the abattoir and poor hygienic environmental conditions, unhygienic handling of meat by the abattoir workers and cross-contamination from the floor and other equipments were used in the abattoir.

From this study the main contaminants were *Escherichia coli* [15] with the highest, followed by *Salmonella spp.* [14], *S. aureus* [9] and *Shigella spp.*[6] was the lowest. These organisms have been found to be associated with food handlers [21]. They were isolated from meat samples in various locations of the abattoir. These finding is slightly different from that previously reported by [22]. These isolated microorganisms were significantly different from those isolated from fresh meat samples from previous studies with similar patterns [9; 23].

5. CONCLUSION AND RECOMMENDATION

The results obtained from this study show that there was high microbial load in the meat processed in the Jos municipal abattoir. This may be due to the poor meat processing methods at the abattoir because the eviscerations were done on the floor, as well as low level of personal hygiene by the butchers, as well as poor abattoir sanitation. Hence, there is need to educate and advocate for good sanitation and meat handling practices in the abattoir via public awareness campaigns.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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