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ISOLATION AND IDENTIFICATION OF MICROORGANISMS FROM RAW BEEF SOLD IN DUTSIN-MA METROPOLIS, KATSINA STATE, NIGERIA

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ABSTRACT
The spoilage of fresh meat comes from the activities of different microorganisms usually via Salmonella spp. through feacal contamination, poor sanitary condition of abattoir, insufficient washing and sterilization of the sellers table, improper handling during slaughter and dressing of the meat. Samples of beef were collected aseptically and transported to Microbiology laboratory in a new polythene bag for analysis. Total bacterial count, isolation and identification of bacterial and fungal strains were identified from the beef samples using standard procedures. The mean total bacterial count of meat sample from different locations showed that Wednesday market (4.16 x 10 [4] cfu/ml) had the highest bacterial count while Yara Dole (1.51 x10 [4]cfu/ml) had the least. The bacterial species isolated had Escherichia coli (53.66%), Pseudomonas aeruginosa (7.32%), Salmonella spp., (2.43%), Bacillus subtilis (21.96), and Staphylococcus aureus (14.63%). Fungal strains identified were Mucor spp. (36.3%), Aspergillus fumigatus (36.3%), while Aspergillus niger (18.2%) an d Aspergillus flavus (27.3%). The presence of these pathogenic microorganisms in beef shows poor hygienic conditions and sanitary practices in both slaughter houses and selling points.
Key words: Dutsin-Ma, Escherichia coli, Beef, Aspergillus spp., Salmonella spp.

INTRODUCTION

Meat is the skeletal muscle associated with fat and other tissues and an animal flesh generally eaten as food. It is significant in human nutritional needs because it is very rich in protein and also has complete and balanced essential amino acids. Meat is easily damaged because it contains approximately 75% water, 19% protein, 1.2% carbohydrate, other substances such as vitamins, minerals and cholesterol, and pH of 5.7 which is the acceptable range of contamination (Heinz and Hautzinger, 2007). Meat is a foodstuff that can be spoiled extremely quickly. Certain species of bacteria multiply easily thanks to its chemical composition, favorable water activity, value and pH value. Their numbers soon reach levels that cause sensory deviations and lead finally to spoilage of the meat (Adam and Moss 1999). Microbial contamination of meat leads to its spoilage resulting to economic losses. Microorganisms, also called microbes, are extremely tiny organisms that can only be seen under a microscope. They are one of the most diverse organisms and they include bacteria, fungi, archaea, protozoa, algae and viruses. They are ubiquitous in distribution therefore are mostly associated with food spoilage such as meat. The damage rate of meat depends on the number of initial microbes, the higher the number of initial microbes, the easier the spoilage of the meat. The initial microbial population on meat depends on physiological stage of the animal when it is slaughtered and on the level of environmental contamination in the slaughterhouse and areas in which the subsequent handling of the meat is performed, including the level of hygiene of employees and the tools and equipment used (Gill, 1998). However, less attention is given to utensils used and are intermediate for microbial transfer if it is cause of meat spoilage which could lead to a lot of people being infected with different diseases. Sources of fresh meat contaminated by microbes includes poor hygienic practice, use of unsterilized equipment such as knives, business table (meat seller) and handling of meat with contaminated hands etc. this means that get contact with fresh meat, the best way of reducing microbial contamination is by proper sanitation because there is no substitute for good sanitation**.** The main factors having the greatest influence on the growth of microorganisms in meat and meat products are the storage temperatures, moisture and oxygen availability.

Raw meat quality is reported to be severely affected by the stress conditions during slaughtering process and the slaughtering methods. This research is aimed at isolation and identification of microorganisms both bacteria and fungi in raw beef sold in Dutsin-Ma Metropolis**.**

SAMPLE COLLECTION AND SAMPLE PROCESSING

Sample Collection

A total of 60 samples of raw beef within Dutsin-ma metropolis were collected for the purpose of isolating and identifying of bacterial and fungal species. Fifteen samples of raw beef were collected from 4 different locations (Hospital road, Hayin Gada, Yara Dole and Wednesday market) aseptically and transported to Microbiology laboratory in a sterilized polythene.

Sample Processing

The samples were pounded using sterile mortar and pestle. Twenty five gram (25g) of each sample was weighed using analytical weighing balance and the analytical portions were placed in separate sterile test tubes containing 225ml of normal saline (for each). The test tubes were shaken vigorously and 1ml of the sample was collected using sterile micropipette and transferred to the next tube containing 9ml of sterile normal saline and shaken, the same procedure was repeated for each of the sample in 10 folds (Cheesbrough, 2003).

Total Bacterial Count

One milli liter (1ml) of diluted sample was collected from specific dilutions using sterile micropipette and dispensed into sterile petri dish followed by the addition of Nutrient agar for the isolation of bacteria species and 1ml of sample into sterile petri dish The media was swirled and allowed to solidify and the Nutrient agar plates were incubated at 37 ^{0}C for 24 hours (Cheesbrough, 2003).

Isolation of Bacterial species using Streak Plate Method

The day old colonies were further sub-cultured on to Eosine Methylene Blue Agar to isolate *Escherichia coli*, *Salmonella - Shigella* Agar for the isolation of *Salmonella* and *Shigella* spp., Mannitol Salt Agar to isolate *Staphylococcus* spp*.,* Casein digest Mannitol agrose media to isolate *Bacillus* spp. and Cetrimide agar to isolate *Pseudomonas aeruginosa,* streaking method (Cheesbrough, 2003).

Identification of Bacterial Isolates by Microscopy, Morphology and Biochemical Methods

Identification of the isolates was achieved by initial morphological examination of the colonies on the plate (macroscopically) for colonial appearance, size, elevation, form, edge, consistency, colour, opacity and the result was recorded. The Gram stained colonies on the glass slides were viewed under an oil immersion magnification. Biochemical identification of the bacteria was done by performing specific tests such as indole, methyl red, citrate utilization tests, coagulase, motility test and urease test was (Cheesbrough, 2003).

Isolation and Identification of Fungi

Total 1g of each sample was placed in separate test tubes containing 9ml peptone water. The test tubes were then shaken and 1 ml of the preparation was transferred into petri dishes containing potato dextrose agar (PDA) medium containing streptomycin (100ppm) for each sample (Agrios, 2004). The petri dishes were then incubated at room temperature for 5-7 days for possible fungal growth. After incubating for about 7 days, the growth rate and other colonial characteristics of each sample were examined macroscopically. After 7 days, individual colonies were sub-cultured on PDA plates by picking and streaking the colonies using sterile wire loop on already prepared PDA plates.

Microscopic Examination of Fungi

A drop of Lacto-phenol cotton blue was placed on a clean glass slide and a portion of the fungal colony was collected aseptically using a sterile needle and covered with a cover-slip. The prepared slide was observed under microscope by using X10 and X40 objective. Morphological characterization was done based on morphological characters and compared to standard reference keys (ATLAS) for possible identification.

RESULTS

The mean total bacterial count of the beef sample from different locations; Hayin Gada having the mean total count of (3.65 x 10 ^{4}cfu/g), Yara dole (1.51 x10 ^{4}cfu/g), Wednesday market (4.16 x 10 ^{4}cfu/g) and Hospital road (3.92 x 10 ^{4} cfu/g). Wednesday market had the highest bacterial count while Hayin Gada has the lowest (4.15 x 10^{4} cfu/g) as shown in table 1.

Table 1. Total bacterial count of raw meat

Location	No of sample	Mean value of bacteria(cfu/g)
Hayin Gada	15	3.65x 10^{4}
Yara dole	15	1.51x 10^{4}
Wednesday market	15	4.16 x 10^{4}
Hospital road	15	3.92 x 10^{4}

Key; cfu=colony forming unit, g=gram.

Morphological characteristics on selective media of respective bacteria and Gram reaction were shown in table 2.

Table 2 .Morphological characteristics and gram reaction of bacterial isolates from meat

Cultural characteristics	Gram reaction	Probable organism identified
Green metallic sheen with dark center on EMB	-rod	*Escherichia coli*
Pale coloured colonies with dark center on SSA	-rod	*Salmonella* spp.
Golden colour MSA usually small colonies	+cocci	*Staphylococcus spp*
Greenish pigment with flat colonies on NA	-rod	*Pseudomonas spp*
Creamish, large and rough	+ rod	*Bacillus spp*

KEY; EMB= Eosin Methylene Blue agar, SSA= *Salmonella Shigella agar*, NA= Nutrient agar, MSA=Mannitol Salt Agar, + = Gram positive, - = Gram negative.

Biochemical reactions for the confirmation of isolated organisms is shown in table 3.

Table 3: Biochemical reaction of isolated organism.

BIOCHEMICAL TESTS										**ORGANISM SUSPECTED**
I	M.R	V.P	C	Mot	Cat	Coag	Gas	Butt	H2S	
+	+	-	-	+	N.A	N.A	N.A	N.A	N.A	*Escherichia coli*
-	-	-	+	+	+	-	+	N.A	-	*Pseudomonas aeruginosa*
-	-	+	+	+	+	N.A	-	N.A	N.A	*Bacillus subtilis*
-	+	+	N.A	-	+	+	-	N.A	N.A	*Staphylococcus aureus*
-	+		N.A	+	+	-	-	Y	+	*Salmonella* spp.

Key: I=Indole test, M.R=Methyl red test, V.P=Vorge's proskauer test, C=Citrate utilization test, Mot=Motility test, Cat=Catalase test, Coag. =Coagulase test, Gas=Gas production, H2S=Hydrogen sulphide production, R=acidic pH, N.A=Not applicable

A total of 41 isolates were obtained from the 60 beef samples of 5 bacterial organisms that include *Escherichia coli, Pseudomonas aeruginosa, Salmonella* spp., *Bacillus* spp. and *Staphylococcus aureus.* These include *Escherichia coli* (53.66%), *Pseudomonas aeruginosa* (7.32%) with the highest prevalence, *Salmonella* spp., (2.43%), *Bacillus* spp., (21.96), and *Staphylococcus aureus* (14.63%) (Table 4).

Table 4. Distribution of bacterial isolates

Microorganisms	Number	Percentage (%)
Escherichia coli	22	53.66
Salmonella spp.	1	2.43
Bacillus subtilis	9	21.96
Staphylococcus aureus	6	14.63
Pseudomonas aeruginosa	3	7.32
TOTAL	**41**	**100**

Macroscopic and microscopic identification of fungal isolates in beef samples. Morphological characterization was done based on morphology of the conidia head. Viewed moulds were compared to standard reference keys on ATLAS for identification (Table 4).

Table 5. Macroscopic and Microscopic identification of fungal isolates in meat samples.

Microscopic Identification	Morphological Characteristics	Microorganisms
Short conidiophores and a septate hyphae	Smokey grey-green and a powdery growth with a reverse white, pale-yellow colony	*Aspergillus fumigatus*
Septate and aerial hyphae bearing long conidiophore	Light green or olive green with whitish margin	*Aspergillus flavus*
Long conidiophores arising from septate hyphae	Brown to black colonies terminate in vessels	*Aspergillus niger*
Sporangiospores can be simple or branched and form apical or globular sporangia that are supported and elevated by a column-shaped columella.	White to beige or grey and fast-growing. Colonies on culture medium may grow to several centimetres in height.	*Mucor spp*

Table 6 showed distribution of fungal species isolated and identified from the beef samples. They include *Mucor* spp. (36.3%), *Aspergillus fumigatus* (36.3%), *Aspergillus niger* (18.2%) and *Aspergillus flavus* (27.3%).

Table 6. Prevalence of total fungal isolates

Microorganisms	Number	Percentage (%)
Aspergillus fumigatus	2	18.2
Aspergillus flavus	3	27.3
Aspergillus niger	2	18.2
Mucor spp	4	36.3
TOTAL	**11**	**100%**

DISCUSSION

Bacterial load obtained from this study is not within the acceptable limit of $\geq 10^5$ cfu/gm. Samples analyzed were contaminated with high bacterial load. The highest bacterial count was (4.16×10^4 cfu/g) which is higher than the finding of Raji (2006) who reported a bacterial count of (3.50×10^4 cfu/g) from dried sliced beef but lower than the finding of Ahmad *et al.,* (2013) (5.35×10^4 cfu/g) in Tanzania.

However, the occurance rate of *Escherichia coli* in this study (53.66%) is higher than the finding of Okonko (2020) who obtained an occurrence rate of (10%) for *Escherichia coli* from Marin Market in Calabar, prevalence rate for *Pseudomonas* spp. obtained in this study (7.32%) is lower than the finding of Moro (2020) (13%) who determined the bacteriological quality of meat sold in Ojo, Lagos state. Prevalence for *Staphylococcus aureus* in this study (14.63%) is lower than the finding of Abdullahi *et al.,* (2020) (35.6%) from beef muscles in Danbatta.

Forty one (41) isolates comprising of four (4) different gram negative and one (1) Gram positive bacterial organisms were isolated from the beef samples. The bacterial isolates were identified as *Staphylococcus aureus, Salmonella* spp., *Escherichia coli, Pseudomonas* spp. and *Bacillus* spp. using standard procedures (Cheesbrough, 2003).

Microorganisms from fresh beef samples have been found in foods, environments and other places (Nkanga and Uraih, 1981; Okonko *et al.,* 2008, a, b, c, d, Enabule and Uraih, 2009; Sobukola *et al.,* 2009; Clarence *et al.,* 2009; Oyeleke, 2009; 2009). The presence of these microorganisms in raw beef shows poor hygienic conditions and poor sanitary practices in slaughter houses.

Most of the organisms in meat sample get into meat as a result of poor sanitary condition of abattoir because most abattoirs environment are untidy especially where meat sold in Wednesday market and Hayin Gada slaughtered as stated by Grandin (2000) that untidy environment at abattoir can cause disease to man when such meat on the table were consumed. However, the use of glass boxes can reduce such contamination.

Also, some organisms can come in contact with meat during transportation since most meat are conveyed to the market in open and therefore microorganisms from air come in contact with the meat thereby making it contaminated. Flies deposit a lot of microorganisms on meat especially flies from the toilet which harbors a lot of microorganisms and transfers it to meat when such meat is explored to the air, water used in the washing of meat in most of the Abattoirs are from untreated source, this can also contaminate the meat as water harbors microorganisms.

Meat sample collected from Yara Dole and Hospital Road might be contaminated from intestines of the animal during skinning process as Gills *et al.* 1998 stated that contamination can come from the inside of animal during skinning process some microorganisms such as *Escherichia coli* a species which occurs in the lower portion of the intestine of humans and warm blooded animals, where it is part of the normal flora.

Fungal species isolated from the beef samples from this study are; *Mucor* spp., *Aspergillus fumigatus, Aspergillus niger* and *Aspergillus flavus* with prevalence rates of (36.3%), (36.3%), (18.2%) and (27.3) respectively. Occurrence rate for *Mucor* spp. in this study (36.3%) is lower than the finding of Ike and Akortha (2017) (65%) from raw beef. However, occurance for *Aspergillus flavus* for this study (27.3%) is higher than the finding of Ogundijo *et al.,* (2018) (14.79%) in a study of bacteriological quality of meat markets and abattoirs in Ibadan, Oyo state.

CONCLUSION

The highest mean bacterial count was found from Wednesday Market (4.16×10^4), while the least mean count was found in Yara Dole (1.5×10^4). Other sampling sites that include Hayin Gada and Hospital Road has 3.65×10^4 cfu/gram and 3.92×10^4 cfu/gram respectively.

The bacterial spp. with the highest percentage occurence was *Escherichia coli* (36.6%) and *Salmonella* species with the least occurrence (1.6%). Other species that include *Bacillus* spp. *Staphylococcus* spp. and *Pseudomonas* spp. recorded 15%, 10% and 5% prevalence rates respectively.

Amongst the fungal species isolated from the meat sample, the highest occurrence rate recorded was 36.6% for *Mucor* species while the least was recorded at 18.2% each for both *Aspergillus niger* and *Aspergillus fumigatus*. Whereas *Aspergillus flavus* has 27.3% prevalence rate.

RECOMMENDATION

Activities like keeping of the abattoir clean, the use of clean water for washing the meat, inspection of animals by veterinary workers to know their state of health before slaughtering, carrying out more researches on meat processing, if properly done will reduce the level of contaminants.

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