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Determination of Bacterial Qualities of Cow, Chicken and Goat Meat Sold in Owerri Municipal, Imo State, Nigeria

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Abstract

The poor hygienic state of our abattoirs and markets where meats are sold have posed a major threat to public health. The microbial quality of meats sold in Owerri municipal Imo State, Nigeria was investigated with the aim of determining the microbial loads of meat sold in these areas. Samples numbering 110 were collected from 11 meat samples types sourced from cow, goat, and chicken. The eleven (11) meat sample types were fresh cow muscles (FCM), fresh cow intestine (FCI), fresh cow liver (FCL), and fresh cow towel (FCT). Fresh goat muscles (FGM), fresh chicken muscle (FCM), fresh goat intestine (FGI), fresh chicken intestine (FCI), Fresh goat liver (FGL), fresh chicken liver (FCL), fresh goat towel (FGT) etc. The samples were collected with sterile containers and swab sticks. The organisms isolated were *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, *Klebsiella species*, *Coliform species*. Fresh chicken intestine has the highest total mean bacteria count of 1.1×10^7 (cfu)/ml, followed by fresh cow towel 7.0×10^6 (cfu)/ml and lastly fresh chicken liver 8.2×10^5 (cfu)/ml. The presence of all these organisms can pose a major threat to public health. This calls for regular inspection of animals, abattoir environments and regular health check of abattoirs workers and butchers.

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INTRODUCTION:-

Meat contains high quality of protein, fat, carbohydrate, vitamins and minerals which are delicious and easily digestible food. All these nutritional requirements can be met easily if reasonable amount of meat is included in a diet. Meat is an animal flesh that is eaten as food and is an excellent source of protein in human diet. Its chemical composition is ideal for the growth of a wide range of spoilage and pathogenic bacteria. Chemical composition includes 72-75% of water, 21% Nitrogen compounds (19% proteins and 1.6% non-protein Nitrogen), 25-50% lipids, 1% of non-nitrogenous compounds (vitamins) and carbohydrate (very small amount of glycogen). Contaminated raw meat is one of the main sources of food-borne illnesses. According to Paul *et al.* [1], food borne diseases result from ingestion of bacteria, toxins present in the meat. The intensity of symptoms varies with the amount of contaminated meat ingested and the susceptibility of the individual to the toxin. These can result in economic and health losses.

Meat is gotten from animal like goat, cattle, chicken, etc., and they have essential parts that are used as meat e.g. beef (red meat), intestines (small and large intestine), and the skin which is also called node. Muscles of healthy animals do not contain micro-organisms; meat tissue get contaminated during the various stage of slaughter and transportation. The health status of animals prior to slaughtering and prevailing circumstances in the slaughter house contributes to the quality of meat from such animals [2]. In Nigeria particularly, Owerri in Imo state, slaughtering of animals usually takes place under very unhygienic conditions. Other primary sources of microbial contaminations are the equipment and physical facilities (stock, knives, containers, retail tables) used in each operation before the final is eaten [3].

Spoilage is caused by the practically unavoidable infection and subsequent decomposition of meat and by bacteria and fungi, which are borne by the animal itself, and the people handling the meat and their equipment. A great diversity of microbes inhabits fresh meat generally, but different types may become dominant depending on pH composition, textures, storage temperature and transportation method involves [4]. Therefore, this work is targeted to determine the bacterial qualities of cow, goat and chicken meat sold in Owerri municipality, Imo state, Nigeria.

MATERIALS AND METHODS

STUDY AREA

The study was conducted in Owerri Municipal Council area of Imo State, Nigeria.

SAMPLE COLLECTION

Fresh beef, chicken and goat meat will be purchased from different butchers, slaughter houses, open shops, markets within Owerri Municipality which includes Ekeonuwa market, Relief market, New market, etc. Samples will be promptly transported to Medical Laboratory Microbiology Laboratory, Imo State University, with insulted ice container from microbiological analysis.

FRESH MEAT SAMPLE PREPARATION

Ten grams of each of the solid samples will be weighed and aseptically taken into a sterilized jar containing 90mls sterile distilled water to produce a stock solution or homogenized solution through blending at 300rpm for 10 minutes. 1ml aliquot of homogenized solution will be transferred for a test containing 9ml of sterile water to make a tenfold serial dilution and will be shaken vigorously. Sterile dilutions up to 10^{-5} will be prepared for microbiological analysis.

Identification of bacteria isolates

The bacterial isolates from the plates will be identified by gram staining other biochemical tests such as catalase, oxidase, insole, etc. according to [5].

GRAM STAINING TECHNIQUES

Staining procedure

Smear isolates were prepared and heat fixed on a clean grease free glass slide. The slide was gently placed on the staining rack and flooded with the primary stain (Crystal Violet) for 1 minute and was rinsed in tap water. The smear was flooded with Logol's iodine for 60 seconds which serves as a mordant. It was rinsed with tap water. The stained slide is flooded with acetone (decolourizer) for 10 seconds and rinsed immediately with water. The smear was counterstained with neutral red or safranin for 60 seconds and rinsed in tap water. The counterstained smear was allowed to air dry. The smear was examined under the microscope using ×100 oil immersion objective lens after adding a drop of immersion oil. Gram positive organisms appeared purple color while gram negative rods appeared pinkish [5].

BIOCHEMICAL TEST

Oxidase test

The oxidase test is used to assist in the identification of oxidase producing organism e.g. Pseudomonas, Neisseria, Vibrio and other groups.

Methods

A loopful of the reagent was added to filter paper in Petri dish.
The isolated colony was smeared with a plastic loop on the wetted filter paper. The color change was observed within 10 seconds Oxidase positive organism appears purple.

CATALASE TEST

This test is used to differentiate those bacteria that produce the enzyme catalase such as staphylococcus, from non-catalase producing bacteria such as streptococci.

Method

2ml of hydrogen peroxide was placed in a test tube.
Some colonies of the organism were picked with the aid of a sterile wire loop and immersed into the hydrogen peroxide. Immediately gas bubbling indicates a positive test.

CAOGULASE TEST

The coagulase test is used to assist in the differential identification of coagulase producing organism. The test aids in the differentiation of *staphylococcus aureus* from other staphylococcus species.

Method

Two drops of saline were placed on a slide. Two colonies of the organism were emulsified in each of the drop of saline to make a thick suspension. A straight wire loop was dipped into the

undiluted plasma and mixed into one of the bacteria suspensions and no plasma was added to the other suspension as it serves as negative control. Clumping of the mixture was observed immediately within 10 seconds. *Staphylococcus Test* are positive for this test [5].

INDOLE TEST

This test was carried out as an acid to distinguish among members of the gram-negative bacilli i.e. the Enterobacteriaceae e.g. *Escherichia coli*, *Vibro cholera*, *Klesbsiella*, *Salmonella* and *Shigella.*

Method

The organism was grown in peptone water**.** The culture was shaken with equal volume of xylene and ethyl ether. 1ml of Ehrlich's reagent was added A red rose ring between the layer of ether and the peptone water was observed. *Escherichia coli* give a positive reaction.

CITRATE TEST

Method

A light suspension of the organism was made in saline. The Koser's citrate medium was inoculated with a straight wire loop growth indicates a positive test. *Klebsiella* pneumonia are positive for this test while *Escherichia coli* is negative.

MOTILITY TEST

Method

A Vaseline was used to make a ring of 2cm in diameter on a clean, grease free slide. A loopful of the culture was placed on the centre of a cover slip. Cover slip was placed on the Vaseline with it not touching the slide. The slide was quickly inverted so that the cover slip is uppermost. It was then examined under the microscope using ×10 and ×40 objectives. Motile bacteria swarm and give a diffuse growth.

STATISTICAL ANALYSIS

The data obtained from this study were analyzed using frequency distribution.

RESULTS

A total of 110 fresh meats samples from 11 meat sample types sourced from cow, goat and chicken. The Eleven (11) sample type namely fresh cow muscles (FCM), fresh cow intestine (FCI), fresh cow liver (FCL), fresh cow towel (FCT), fresh chicken muscles (FCHM), fresh chicken intestine (FCHI), fresh chicken liver (FCHL), fresh goat muscles (FGM), fresh goat intestine (FGI), fresh goat liver (FGL), and fresh goat towel (FGT) were collected from different meat vendors at abattoir and major markets in Owerri Municipal, Imo State, Nigeria and analyzed bacteriologically.

In Table 1: Shows the variation of microbial loads of fresh meat samples. Fresh chicken intestine has the highest total mean bacteria count of 1.1×10^7 (cfu)/ml followed by fresh cow towel 7.0×10^6 (cfu)/ml and lastly fresh chicken liver 8.2×10^5 (cfu)/ml. This may be as a result; chicken is been raised in a large-scale farm and come in contact with many other chickens which increase the spread of bacteria.

According to Bacterial Analytical Manual (BAM) and American Society for testing materials says that 25 – 250(cfu)/ml plate and 100,000 colonies /ml are significant for infection respectively. Therefore, the result from table below is significant for infection.

TABLE 1: SHOWS THE VARIATION OF MICROBIAL LOADS OF FRESH MEAT SAMPLES

MEAT SAMPLES	TMVBC (cfu/m)	TMCC	TMSSC	TMEC(cfu/m)	TMSC	TMKC
FCM	1.1×10^4	ND	3.6×10^4	2.0×10^4	ND	ND
FCL	2.0×10^4	ND	4.0×10^3	1.5×10^4	5.0×10^3	ND
FCI	6.0×10^4	ND	4.3×10^3	9.3×10^3	1.0×10^6	ND
FCT	7.0×10^6	ND	7.5×10^3	6.0×10^6	8.0×10^5	2.5×10^4
FGM	3.8×10^5	ND	1.1×10^5	5.9×10^5	ND	3.1×10^5
FGL	5.0×10^5	ND	3.5×10^3	3.0×10^5	ND	ND
FGI	4.1×10^5	ND	5.0×10^3	4.1×10^5	ND	5.9×10^4
FGT	9.0×10^4	ND	4.9×10^5	6.5×10^4	ND	1.3×10^5
FCHM	1.6×10^6	3.5×10^5	3.4×10^5	41×10^5	ND	4.4×10^5
FCHL	8.2×10^5	4.8×10^5	9.1×10^3	3.3×10^5	ND	6.0×10^5
FCHI	1.1×10^7	1.8×10^5	4.5×10^5	2.0×10^5	ND	1.0×10^6

KEY:
TMVBC: Total Mean Viable Count
TMCC: Total Mean Coliform Count
TMSSC: Total Mean Salmonella shigella count
TMEC: Total Mean Escherichia coli count
TMSC: Total Mean Staphylococcus count.
FCM: Fresh Cow Muscles, FCL: fresh cow liver,
FCI: Fresh Cow Intestine, FCT: fresh cow towel
FGM: Fresh Goat Muscles, FGL: fresh goat liver
FGI: Fresh Goat Intestine, FCHM: fresh chicken muscles
FCHL: fresh chicken liver, FCHI: fresh chicken intestine
Cfu/ml: Colony Forming Unit per Milliliter

TABLE 2(a): SHOWS THE PREVALENCE OF BACTERIA SPECIES ON GOAT MEAT SAMPLE TYPE
GOAT PARTS

PATHOGENS	**GOAT MUSCLES**	**GOAT LIVER**	**GOAT INTESTINE**	**GOAT TOWEL**	**FREQUENCY**	**PERCENTAGE DISTRIBUTION**
No. of samples	10	10	10	10	40	
Escherichia coli	3	3	3	3	12	43%
Klebsiella spp	1	2	2	2	7	25%
Salmonella spp	3	3	1	2	9	32%
					28	

TABLE 2(b): SHOWS THE PREVALENCE OF BACTERIA SPECIES ON CHICKEN SAMPLE TYPE
CHICKEN PARTS

PATHOGENS	CHICKEN MUSCLES	CHICKEN LIVER	CHICKEN INTESTINE	FREQUENCY	PERCENTAGE DISTRIBUTION
No. of samples	10	10	10	30	
Escherichia coli	2	2	1	5	35%
Klebsiella spp	2	1	1	4	29%
Salmonella spp	2	2	1	5	35
				14	

TABLE 2(c) SHOWS THE PREVALENCE OF BACTERIA SPECIES ON COW SAMPLE TYPE
COW PARTS

PATHOGENS	COW MUSCLES	COW LIVER	COW INTESTINE	COW TOWEL	FREQUENCY	PERCENTAGE DISTRIBUTION
No. of samples	10	10	10	10	40	
Escherichia coli	1	0	0	1	2	8%
Klebsiellaspp	3	1	5	1	10	41%
Salmonella spp	0	1	2	3	6	25%
Staph aureus	0	1	2	3	6	25%
					24	

Table 3 shows the bacteria isolated and their percentage distribution. *Escherichia coli* has the highest frequency and percentage distribution 36 (43%) followed by *Salmonella* species 24(28%), *Klebsiella* 13 (15%), *Staphylococcus aureus* 6 (7%) and lastly *Coliform species* 5 (6%)

TABLE 3: SHOWS THE BACTERIA ISOLATED ON DIFFERENT MEAT SAMPLES AND THEIR PERCENTAGE DISTRIBUTION

BACTERIA ISOLATED	GOAT	COW	CHICKEN	FREQUENCY	DISTRIBUTION $^0/_0$
No. of samples	40	40	30	110	
Eschericia coli	12	19	15	36	$43^0/_0$
Klebsiella spp	7	2	4	13	$15^0/_0$
Salmonella spp	9	5	10	24	$28^0/_0$
Coliform spp	--	---	5	5	$6^0/_0$
Staphylococcus aureus	--	7	---	6	$8^0/_0$
				84	100%

TABLE 4: SHOWS TOTAL NUMBER OF BACTERIA SPECIES ISOLATED ON FRESH **MEAT SAMPLE PARTS**

Names of animal	**Number of samples**	**No. of bacteria species**	**Percentage distribution**
Live goat	40	12	$32^0/_0$
Live chicken	30	12	$32^0/_0$
Live cow	40	13	$35^0/_0$
		37	

TABLE 5: SHOWS THE BIOCHEMICAL TEST OF THE ORGANISMS INVOLVED

Biochemical tests	***Eschericia coli***	***Coliform***	***Kleb spp.***	***Staphylococcus***	***Salmonella spp.***
Catalase	+	+	+	+	+
Coagulase	---	---	---	+	---
Oxidase	---				+
Gram reaction	---	---	---	+	---
Indole	+	---		+	
Citrate	---	---	+	+	---
Motility	+		+	+	+
Methyl red	+				+
Macroscopic examination color	Milky	Creamy	Creamy white	Golden yellow	Cream white
Microscopic	Rod	Circular	Circular	Cocci	Rod

examination shape

DISCUSSION

In this study, a total of five (5) species of bacteria were seen in goat, chicken and cow. These species are identified as *Escherichia coli, Klebsiella spp, coliform, Staphylococcus aureus and Salmonella spp*. The frequency and percentage distribution of bacteria isolates from fresh meat marketed in Owerri municipal showed that cow muscle parts have the highest total bacteria species 13 ($35^0/_0$) while chicken and goat have the same frequency and percentage distribution of 12 ($32^0/_0$). Among these bacterial species, *Escherichia coli* has the highest percentage distribution of 43% and highest total mean viable count in cow towel, chicken intestine, goat towel and cow towel (7.0 x 10^6(cfu)/ml), (8.2 x 10^5(cfu)/ml), (9.0 x 10^4 (cfu)/ml) and 6.4 x 10^4 (cfu)/ml) respectively. *Escherichia coli* is assumed to be an indicator of faecal contamination. This is in agreement with This may be attributed to improper sanitary condition during processing of meat from water supply, unsterilized utensils and contaminants from flies [6-10].

Salmonella species has the second highest percentage distribution of 28% and highest total mean viable count in fresh goat towel 4.9×10^5(cfu)/ml, chicken intestine 4.5×10^5(cfu)/ml and chicken muscle 3.4×10^5(cfu)/ml etc. This result is in contrast with the work of Mgbemena *et al.* [11] that reported that no S*almonella* and *Shigella* were seen in the samples examined. *Salmonella species* such as *Salmonella typhi* is a bacterium that causes typhoid fever, and acute life-threatening ulcer. The disease is a cause of concern and major public health problem in underdeveloped countries (Asia and Africa) especially in Nigeria due to poor sanitary conditions and lack of portable water. *Coliforms* was seen in fresh chicken muscles (1.6×10^6(cfu)/ml), intestine (1.1×10^7(cfu)/ml) and liver (8.2×10^5(cfu)/ml). This may be as a result of reused dirty water in washing meat as well as dirty environment where the chicken is slaughtered.

This work has revealed that microbial qualities of meat sold in Owerri municipal market are grossly contaminated by gram positive and gram negative but mostly gram-negative bacteria. The presence of all these organisms on meat is indicative of public health hazard and gives a signal of possible occurrence of food borne intoxication and infection. This also implies that these meats are viable source of various diseases. The presence of *Staphylococcus aureus* in this study is as a result of cross contamination from many butchers before it gets to the final retailers. *Staphylococcus* being a flora of the body indicates contamination from the handlers. The organism can pass onto meat during slaughtering, butchering and evisceration.

CONCLUSION

The findings in this study have shown high microbial load in meat sold in Owerri Municipal in which *Eschericia coli, Gardia lambia* and *Candida spp* have the highest percentage distribution in bacteriological, parasitological and mycological assessment respectively.

Eschericia coli, Staphylococcus aureus and others have been the aetiological agents of food-borne diseases. According to WHO, these organisms are “priority organisms”. They are involved in multidrug resistant bacteria which increases mortality rate. All these poses great challenge to public health as it may serve as a source of food poisoning and death. Therefore, consumers should apply proper cooking methods to reduce microbial load.

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