MICROBIAL DIVERSITY ASSOCIATED WITH DIFFERENT FRESH MEATS SOLD IN ABA METROPOLIS, ABIA STATE, NIGERIA.

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ABSTRACT

The microbial diversity associated with different fresh meat samples: intestine, hide (Kpomo) and beef were analyzed using standard microbiological methods. The microbial loads were high in mean aerobic, coliform and fungi counts (CFU/g) in samples of intestine (3.0×10^5) ±0.12, (2.3×10^4) ±0.09, (0.9×10^4) ±0.14, while the least counts were among samples of beef: (1.2×10^3) ±0.16, (0.4×10^2) ±0.02, (0.6×10^2) ±0.33 respectively. Nine bacterial and nine fungal isolates were identified with their percentage prevalence to include *Aeromonas* (71%), *Pseudomonas* (65%), *Salmonella* (44%), *Clostridium* (43%), *Enterococcus* (43%), *Alcalegenes* (41%), *Staphylococcus* (38%), *Escherichia coli* (35%), *Bacillus* (34%), and fungi: *Mucor* (65%), *Rhizopus* (63%),, *Candida* (59%), *Torulopsis* (55%), *Rhodotorula* (40%), *Aspergillus* (39%), *Cryptococcus* (37%), *Fuarium* (33%), and *Penicillium* species (31%). The presence of these organisms in high thresholds is indicative of serious non-conformity and looming food borne outbreaks, if unchecked. Therefore, there is need for urgent awareness training among stakeholders (butchers/ sellers and teeming consumers) on the inherent risks associated with the crude practices in order to avert the looming food borne outbreaks.

Key words: Meat**,** Microorganisms, Contamination, Microbial thresholds.
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Introduction

Meat has exerted a crucial role in human evolution and is an important component of a healthy and balanced diet due to its nutritional richness. The increase in population with consequent pressing demand for enhanced requirements of food has led to a continued search for novel sources of food and protein. Ruminants as herbivores convert the materials into balanced source of protein and energy for human consumption and are called meat. Meat is an animal flesh that is eaten as food and excellent source of protein in human die. It is highly susceptible to microbial

contaminations, which can cause its spoilage and food borne infections in human, resulting in economic and health losses (Komba *et al.*, 2012). It is normally eaten after it has been cooked and seasoned or processed in a variety of ways.

Meat is one of the most perishable foods and its composition is ideal for the growth of a wide range of spoilage bacteria (Mayr *et al*., 2010). Meat is considered as the most nutritive source of protein consumed by humans. Age and sex of the animal has a major influence on the quality of meat that is produced from animals (Rao *et al*., 2009). Most meats have high water content with corresponding water activity approximately of 0.99 which is suitable for microbial growth (Rao *et al*., 2009). Public concern has risen due to widespread microbial contamination, leading to food poisoning and food borne illnesses.

Meat is gotten from animals like sheep, and cattle etc. The widely used animal for meat is the cattle and it has many essential parts that are also used as meat, such parts are the beef (red meat), intestine, and the skin which is also called hide. Meat is the most common food that provides nutrition to our diets. Although muscles of healthy animals do not contain microorganisms, meat tissues get contamination during the various stages of slaughter and transportation (Ercolini *et al.*, 2006; Jay *et al*., 2005). The health status of animals prior to slaughtering and prevailing circumstances in the slaughter house contributes to the quality of meat from such animals (Whyte *et al.*, 2004).

It may be noted that most of the meats have a final ultimate pH of about 5.6 and above. This makes these products susceptible to bacteria as well as to mold and yeast spoilage. With respect to the keeping quality of meats, it is well established that meat from fatigued animals spoils faster than that from rested animals and this is a direct consequence of final pH attained upon completion of rigor mortis. The death of a well-rested meat animal, triggers conversion 1% glycogen to lactic acid, which directly causes a depression in pH values from about 7.4 to about 5.6, depending on the type of animal (Jay *et al*., 2005). Callow, (1949) found the lowest pH values for beef to be 5.1 and the highest 6.2 after rigor mortis. The usual pH value attained upon completion of rigor mortis of beef is around 5.6 (Jay *et al*., 2005; Bate-Smith, 1948).

The possible sources of contamination are likely to come from the skin (animal hide), gastrointestinal tract, and lymph nodes of the animal from which the meat was obtained. Other primary sources of microbial contaminations are the equipment and the physical facilities (the stick knife, containers, retail tables) used in each operation before the final product is eaten. The clothing, hands of handlers, handling and storage environment are all implicated (Jay *et al*., 2005). Spoilage is caused by the practically unavoidable infection and subsequent decomposition of meat by bacteria and fungi, which are borne by the animal itself, and the people handling the meat and their implements. A great diversity of microbes inhabit fresh meat generally, but different types may become dominant depending on pH, composition, textures, storage temperature, and transportation methods involved (Adu-Gyamfi *et al*., 2012; Ercolini *et al*., 2006; Li *et al*., 2006).

Raw meat may harbour many important pathogenic microbes such as *Salmonella* species, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Escherichia coli*, S*taphylococcus aureus* and to some extent, *Listeria monocytogenes*, making the meat a risk for human health, of which without proper handling and control of these pathogens, food borne illnesses may occur (Norrung *et al*., 2009). The slaughtering of animals usually takes place under very unhygienic conditions. This coupled with high ambient temperature, high humidity, shortage of portable water and poor handling practices exposes meat products to microbial contamination and rapid deterioration. Therefore, the aim of this study is to assess the diversity of microorganisms associated with fresh meat sold in Aba metropolis, Abia State.

Materials and Methods

Study area

The study area is Aba Metropolis, Abia State, in the South-Eastern Nigeria. The Aba town which has been known as a major commercial centre in Eastern Nigeria is of the Igbo tribe and inhabited by Ngwa people. The geographical coordinates are 5.1215^0N, and 7.3732^0E. (Oriji, 2011).

Sources of sample

The different meat samples (intestine, hide and beef) for the study were purchased at random from Ariaria market in Aba metropolis, Abia State. Ariaria market is the largest market in the South-Eastern Nigeria, located in Aba, Abia State, serving other neighbouring States both in the South-

East and South-South Nigeria such as Akwa Ibom, Imo, Rivers, Bayelsa, Delta, Anambra States, and parts of Enugu and Ebonyi States.

Sample collection

A total of ten (10) samples of different meat samples (intestine, hide and beef) were purchased at random from Ariaria market in Aba metropolis. These samples were aseptically packaged in sterile ziploc bags in icebox for microbiological analysis. Samples were analyzed in the laboratory within thirty (30) minutes of collection.

Microbiological analysis of samples

Ten fold serial dilutions of samples were done. Spread plate and streaking techniques (Cappucino and Sherman, 2010) were used to enumerate and isolate bacteria and fungi in the samples. One (1) gramme of each meat sample was mashed in a sterile mortar, transferred to a sterile test tube. 10^{-1} dilution was made by adding 9ml distilled water into the test tube and sample was shaken vigorously to ensure adequate disengagement of microorganisms. Serial dilutions of the homogenates were made to 10^{-2} and 10^{-3} and each dilution was plated in replicates using plate count agar for mean aerobic bacteria enumeration and isolation, tergitol agar for coliform enumeration and isolation, fortified sabouraud dextrose agar (SDA) for fungal enumeration and isolation. Pure bacterial isolates were identified using cultural, morphological and biochemical characterization. Identification of the bacteria to genera level was based on the schemes of Boone *et al*., (2005). The purified fungal isolates were identified on the basis of macroscopic and microscopic characteristics by slide culture technique, and lactophenol staining. The schemes of Barnet and Hunter, (2000) and Watanabe, (2010) were used for the identification. The plates were incubated at 35 ± 2°C for 72 hours and 24 hours for total bacterial and coliform counts respectively and 25 ± 2°C for 120 hours for fungal counts.

Data analysis

Data obtained from this research work were analysed using ANOVA. Descriptive statistics in form of means and standard deviation and Duncan post hoc were also used to assess the data. The analyses were done using SPSS 16.

Results

The mean total bacterial (aerobic plate), coliform and fungal counts of different meat samples sold in Ariaria market in Aba metropolis are shown in Table 1, 2 and 3 respectively**.** It showed that intestine samples had significant high mean bacterial, coliform and fungal counts when compared to other meat samples. This is followed by hide while beef samples had the least mean counts. Results showed that intestine samples took lead in mean bacterial counts – CFU/g (3.6×10^5) ±0.17), followed by hide samples (3.2×10^4) ±0.19), and beef samples (2.0×10^4) ±0.20) in that order.

The mean coliform counts maintained similar trend with bacterial counts, with intestine samples leading, followed by hide and beef samples. Intestine samples took the lead in mean coliform counts – CFU/g (2.3×10^4) ±0.09), followed by hide (1.4×10^3) ±0.05), and beef (1.2×10^3) ±0.07). Mean fungal counts had same pattern with both bacterial and coliform counts. Intestine samples had the highest value in CFU/g (0.9×10^4) ±0.14), followed by hide (1.2×10^3) ±0.12), and beef (1.1×10^3) ±0.02). There is statistical significance among different values obtained in the results ($p<0.05$). Figures 1 and 2 showed the prevalence of bacterial and fungal species isolated from the different meat samples drawn from Ariaria market in Aba metropolis. *Aeromonas* and *Pseudomonas* species had the highest bacteria prevalence (71% & 65% in intestine, 60% & 53% in hide and 54% & 45% in beef samples), while *Mucor* and *Rhizopus* species; *Candida* and *Torulopsis* species had the highest fungal prevalence (65% & 63% in intestine, 53% & 51% in hide, 45% & 41% in beef) and (59% & 55% in intestine, 51% & 49% in hide, 47% & 31% in beef) for mold and yeast respectively. The least in prevalence were species of *Bacillus* species (bacteria) and *Penicillium* species (fungi).

Discussion

Meats are contaminated with pathogens from the intestinal tract or from faecal material deposits (Jay *et al*., 2005). Cross contamination is another problem in the control of pathogens (Singer *et al*., 2007). With high nutritive value, both essential macro and micronutrients, meat is an important part of a balanced diet for human and microorganisms (Mayr *et al*., 2010). Retailed meat and meat products are normally sold in markets in unhygienic conditions (most often in open tables). These are various sources of contamination that attested to the results of high microbial loads recorded

in this study. Most of the bioloads results obtained are in high thresholds and are serious indications of non-conformity in food safety management with looming food borne outbreaks, if unchecked.

In this study, there were high aerobic bacterial, coliform and fungal counts which are indicative of heavy contamination. Meanwhile, muscles and tissues of healthy animals do not contain microorganisms, rather contamination is encountered during the various stages of slaughtering from inherent intestine, prevailing environment, during transportation and handling (Ercolini *et al.*, 2006; Whyte *et al.*, 2004). The results obtained in this work showed high microbial counts in Tables 1, 2 and 3. The mean aerobic bacterial, coliform and fungal counts are highest in samples of intestine, followed by hide and beef. Also, presence of these microbes in higher thresholds could fast track spoilage of the meat. The high microbial loads could be linked to heavy contamination and cross contamination during slaughtering. The presence of these organisms in higher thresholds could be traced to poor hygienic conditions and cross contaminations from the slaughter house to points of sell. The use of bare hands and the open display of meat on contaminated tables by sellers is another issue of concern in troubleshooting sources of huge contamination However, most of the meat sellers and butchers are unaware of the implications of these crude practices, in relation to health hazards of consumers. The presence of coliforms could be traced to cross contamination from the intestine during slaughtering (Jay *et al*., 2005), and poor hygiene practices among butchers/ sellers. Coliform presence is a strong indication of faecal contamination. A number of microorganisms were isolated and they include *Aeromonas*, *Pseudomonas*, *Clostridium*, *Bacillus*, *Alcalegenes*, *Staphylococcus*, *Escherichia coli*, *Enterococcus*, *Salmonella*, *Penicillium*, *Mucor*, *Rhizopus*, *Aspergillus*, *Fuarium*, *Cryptococcus*, *Rhodotorula*, *Candida* and *Torulopsis* species. The results obtained in this study were in agreement with the works of Adu-Gyamfi *et al*. 2012; Ercolini *et al*. 2006; and Li *et al*. 2006.

In Figure 1 and 2, *Aeromonas* and *Pseudomonas* species had the highest bacterial prevalence in meat samples, while *Bacillus* species had the least bacterial prevalence. Similarly, *Mucor* and *Rhizopus* species had the highest fungal prevalence in meat samples, while *Penicillium* species had the least fungal prevalence . The prevalence trend for both bacteria and fungi maintained a pattern among the identified microorganisms with *Aeromonas* species leading, followed by *Pseudomonas, Salmonella, Clostridium, Enterococci, Alcaligenes, Staphylococcus, Escherichia*

coli, and *Bacillus* species in that order for bacteria, while *Mucor* species took the lead, followed by *Rhizopus*, *Candida*, *Torulopsis*, *Rhodotorula*, *Aspergillus*, *Cryptococcus*, *Fusarium* and *Penicillium* species in that order for fungi. *Bacillus* species are known as environmental contaminants and spore formers, inhabiting the air, water and soil and can withstand harsh weather conditions, while *Staphylococcus aureus* is known to inhabit the human skin as normal flora and opportunistic during a break or under depressed immunity (Ike *et al*., 2015). The presence of *Aspergillus*, *Penicillium*, *Rhizopus*, and *Mucor* species could be attributed to the surrounding environment (Chukwu *et al*., 2013). *Enterococci* species and *Escherichia coli* are known as intestinal inhabitants of animals (Jay *et al*., 2005). *Salmonella* species such as *Salmonella typhi* is a bacterium that causes typhoid fever (enteric fever), an acute, life-threatening febrile illness (CDC, 2008). The disease is a major public health problem in developing countries, especially in Nigeria due to poor sanitary conditions and lack of or inadequate potable water (Ibekwe *et al*., 2008). It is mainly transmitted through food or drink or water contaminated with urine or faeces of infected people or a chronic carrier (CDC, 2008; Ibekwe *et al*., 2008).

Conclusion

This study had revealed high microbial loads among the different meat samples, which if left unattended could pose serious health hazards that might lead to serious food borne outbreaks. Therefore, it is advisable that an adequate campaign should be launched for the major stakeholders (butchers/ sellers and teeming consumers) in order to educate them on the inherent risks associated with the crude and non-conforming practices in order to avert food borne outbreaks.

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Table 1: Mean Aerobic Bacteria Count

Sample Numbers	Meat samples (CFU/g)		
	Intestine	**Hide**	**Beef**
S_1	(3.0 $x10^5$) ±0.12[a]	(3.1 $x10^3$) ±0.23[b]	(1.8 $x10^3$) ±0.19[c]
S_2	(3.1 $x10^4$) ±0.55[a]	(3.2 $x10^4$) ±0.19[a]	3.5 $x10^3$) ±0.04[b]
S_3	(4.4 $x10^4$) ±0.01[a]	(2.5 $x10^4$) ±0.08[b]	(2.0 $x10^4$) ±0.20[c]
S_4	(1.8 $x10^5$) ±0.03[a]	(1.3 $x10^4$) ±0.07[b]	(1.6 $x10^3$) ±0.50[c]
S_5	(3.0 $x10^5$) ±0.09[a]	(2.0 $x10^4$) ±0.24[b]	(2.0 $x10^3$) ±0.44[c]
S_6	(3.6 $x10^5$) ±0.17[a]	(2.3 $x10^3$) ±0.21[b]	(1.6 $x10^3$) ±0.05[c]
S_7	(4.0 $x10^4$) ±0.22[a]	(1.8 $x10^3$) ±0.26[b]	(1.4 $x10^3$) ±0.11[c]
S_8	(1.4 $x10^4$) ±0.18[a]	(1.3 $x10^4$) ±0.33[a]	(0.8 $x10^4$) ±0.07[b]
S_9	(2.2 $x10^4$) ±0.06[a]	(1.8 $x10^3$) ±0.03[c]	(1.0 $x10^4$) ±0.06[b]
S_{10}	(2.0 $x10^4$) ±0.02[a]	(1.8 $x10^4$) ±0.02[a]	(1.2 $x10^3$) ±0.16[b]

Within rows, values with the same letters are not significantly different.
Standards: Aerobic bacteria count (ABC) = ≤ 10^5/g, Coliform count (CC) = < 100/g, Fungal count (FC) = ≤ 10^4/g (PHLS, 2000); (FSANZ, 2001).

Table 2: Mean Coliform Count

Sample Numbers	Meat samples (CFU/g)		
	Intestine	**Hide**	**Beef**
S_1	$(2.0 \times 10^3) \pm 0.27^a$	$(0.9 \times 10^3) \pm 0.03^b$	$(1.2 \times 10^2) \pm 0.04^c$
S_2	$(2.2 \times 10^3) \pm 0.05^a$	$(1.3 \times 10^3) \pm 0.09^b$	$1.2 \times 10^3) \pm 0.07^b$
S_3	$(2.3 \times 10^3) \pm 0.11^a$	$1.2 \times 10^2) \pm 0.18^b$	$(1.0 \times 10^2) \pm 0.23^b$
S_4	$(0.6 \times 10^4) \pm 0.13^a$	$(1.1 \times 10^3) \pm 0.17^b$	$(1.3 \times 10^2) \pm 0.05^c$
S_5	$(2.1 \times 10^3) \pm 0.19^a$	$(2.4 \times 10^2) \pm 0.04^b$	$(1.8 \times 10^2) \pm 0.24^c$
S_6	$(2.3 \times 10^4) \pm 0.09^a$	$(2.0 \times 10^2) \pm 0.01^b$	$(1.2 \times 10^2) \pm 0.09^c$
S_7	$(1.4 \times 10^4) \pm 0.20^a$	$(1.5 \times 10^2) \pm 0.06^c$	$(0.3 \times 10^3) \pm 0.31^b$
S_8	$(0.5 \times 10^3) \pm 0.13^a$	$(0.6 \times 10^3) \pm 0.03^a$	$(0.4 \times 10^2) \pm 0.02^b$
S_9	$(1.3 \times 10^3) \pm 0.06^a$	$(0.8 \times 10^3) \pm 0.23^b$	$(1.3 \times 10^2) \pm 0.04^c$
S_{10}	$(1.3 \times 10^4) \pm 0.12^a$	$(1.4 \times 10^3) \pm 0.05^b$	$(1.0 \times 10^3) \pm 0.17^c$

Within rows, values with the same letters are not significantly different.
Standards: Aerobic bacteria count (ABC) = $\leq 10^5$/g, Coliform count (CC) = < 100/g, Fungal count (FC) = $\leq 10^4$/g (PHLS, 2000); (FSANZ, 2001).

Table 3: Mean Fungi Count

Sample Numbers	Meat samples (CFU/g)		
	Intestine	**Hide**	**Beef**
S_1	$(1.4 \times 10^3) \pm 0.07^a$	$(0.7 \times 10^3) \pm 0.05^c$	$(1.0 \times 10^3) \pm 0.11^b$
S_2	$(1.4 \times 10^3) \pm 0.03^a$	$(1.1 \times 10^3) \pm 0.11^b$	$1.1 \times 10^3) \pm 0.02^b$
S_3	$(1.3 \times 10^3) \pm 0.01^a$	$1.1 \times 10^2) \pm 0.13^b$	$(1.0 \times 10^2) \pm 0.23^c$
S_4	$(1.1 \times 10^3) \pm 0.04^a$	$(1.0 \times 10^3) \pm 0.15^a$	$(0.9 \times 10^2) \pm 0.03^b$
S_5	$(2.1 \times 10^2) \pm 0.20^a$	$(1.2 \times 10^2) \pm 0.09^b$	$(1.1 \times 10^2) \pm 0.04^b$
S_6	$(1.4 \times 10^3) \pm 0.14^a$	$(1.0 \times 10^2) \pm 0.21^b$	$(1.0 \times 10^2) \pm 0.03^b$
S_7	$(1.5 \times 10^3) \pm 0.25^a$	$(1.1 \times 10^3) \pm 0.03^b$	$(0.7 \times 10^3) \pm 0.12^c$
S_8	$(0.9 \times 10^3) \pm 0.05^b$	$(1.2 \times 10^3) \pm 0.12^a$	$(0.6 \times 10^2) \pm 0.33^c$
S_9	$(1.0 \times 10^3) \pm 0.03^a$	$(0.8 \times 10^3) \pm 0.20^b$	$(0.7 \times 10^2) \pm 0.05^c$
S_{10}	$(0.9 \times 10^4) \pm 0.14^a$	$(1.0 \times 10^3) \pm 0.02^b$	$(1.0 \times 10^2) \pm 0.06^c$

Within rows, values with the same letters are not significantly different.
Standards: Aerobic bacteria count (ABC) = $\leq 10^5$/g, Coliform count (CC) = < 100/g, Fungal count (FC) = $\leq 10^4$/g (PHLS, 2000); (FSANZ, 2001).

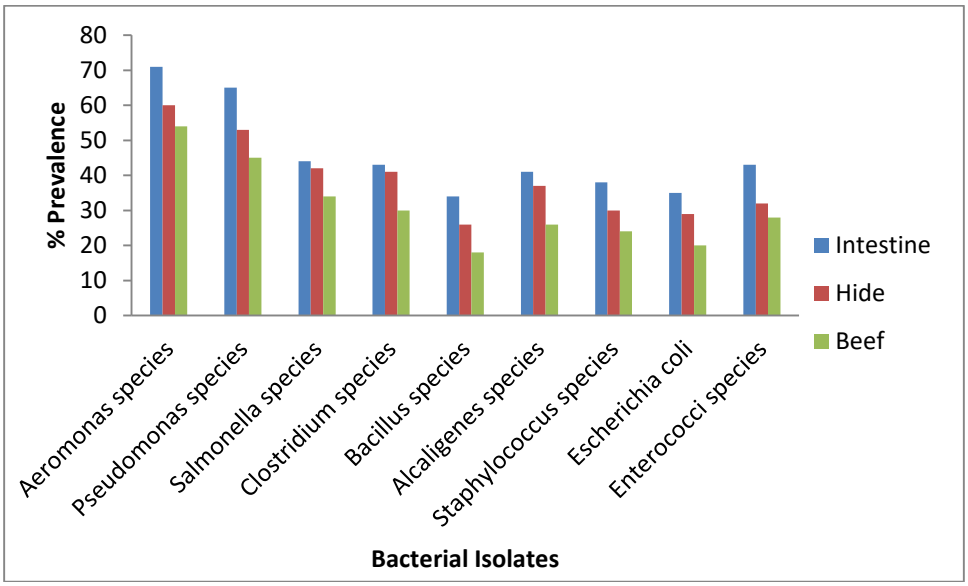

Fig. 1: Percentage prevalence of bacterial isolates from different meat samples

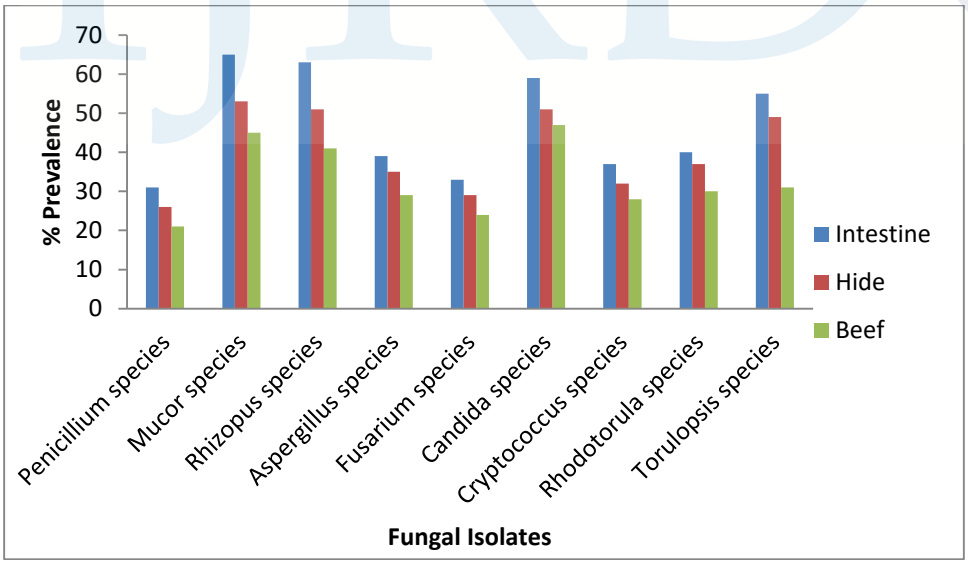

Fig. 2: Percentage prevalence of fungal isolates from different meat samples