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Microbiological Quality, Antibiotic Susceptibility Patterns of Bacteria and Safety of Raw Beef Sold in Open Markets

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ABSTRACT

Raw beef offers the right conditions for pathogenic microbes and spoiling agents to thrive. One of the main causes of food-borne diseases is contaminated raw meat. The goal of this study was to determine the microbial load and sensitivity of the bacterial isolates of raw beef sold in open markets to various antibiotics. Fifteen (15) raw beef samples were aseptically collected from three separate markets in some towns (Otuoke, Elebele, and Imiringi) in Ogbia. The beef samples were examined for microbial contamination using conventional microbiological methods. Kirby-Bauer multi-disc diffusion method was used for testing antibiotics susceptibility. The result of total viable heterotrophic bacteria (TVHB) count varied from 2.0 x 10^7 CFU/g to 7.0 x 10^7 CFU/g. The results showed no significant differences ($P<0.05$) between the TVHB of the fresh meat samples collected from the different locations. Isolated bacteria identified and frequency of isolation (%) were; *Escherichia coli* (19.0%), *Enterococcus sp*. (8.0%), *Staphylococcus sp* (23.0%), *Klebsiella sp*. (8.0%), *Salmonella sp*. (23.0%), and *Micrococcus sp*. (19.0%). While isolated fungi and frequency of isolation (%) were; *Aspergillus* sp (29.0%), *Candida* sp (14.0%), *Fusarium* sp (12.0%), *Penicillium sp* (14.0%), and *Fusarium sp*. (43.0%). While *Klebsiella sp*. shown resistance to every antibiotic utilized in this investigation, *Staphylococcus sp*. remained fully susceptible to them all. The existence of *Salmonella*, *Escherichia coli*, and *Staphylococcus* species suggests that the slaughter house facilities' general hygienic conditions and hygiene standards are subpar.

Keywords: Raw beef, open market, microbiological quality, antimicrobial sensitivity, antimicrobial resistance, safety.

Introduction

Microbiological diseases or food poisoning pose a serious threat to public health. Most people living in cities eat a lot of beef, which is a high-protein diet that is fragile and prone to microbial invasion and subsequent degradation. Food-borne illnesses, which are linked to beef and arise from consuming bacteria, toxins, and cells produced by microorganisms found in food, continue to be the biggest global food safety risks (Clarence *et al.*, 2009).

One of the main causes of food-borne diseases is contaminated raw meat (Bhandare *et al.*, 2007). Meat offers the right conditions for pathogenic microbes and spoiling agents to thrive.

Fresh meat normally contains a wide variety of microorganisms, but depending on the pH, texture, composition, storage temperature, and method of raw meat transit, different species may become dominant (Adu-Gyamfi *et al*., 2012). Every step of the meat processing process following slaughter introduces new microorganisms, which eventually contaminate the meat (Ebel *et al.*, 2004; Sumner *et al.*, 2003). Sometimes germs that can be dangerous to humans contaminate fresh meats (Burgess *et al.*, 2005). It is concerning for meat's sanitary quality that harmful microorganisms are present. Therefore, one can assess the microbiological quality of meat and meat products based on their hygienic quality. Food poisoning caused by bacteria spreads quickly and is usually the result of dirty surroundings and improperly stored food.

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The microorganisms that are relevant to meat are viruses, bacteria, and molds. In all of these groupings, bacteria are extremely important. According to Nouichi and Hamdi (2009), the most commonly found bacterial infections linked to the intake of beef products include *Staphylococcus, Salmonella*, *E. coli*, *Listeria*, and *Clostridium.* Because meat and meat products have a significant impact on public health, its microbiological quality is particularly important (Bhandare *et al.*, 2007). Given that raw retail meats have been linked to the spread of food-borne illnesses, there is a need to strengthen the use of hazard analysis critical control point (HACCP) and consumer food safety education programs (Zhao *et al.*, 2001).

The majority of Bayelsa's slaughter houses are ill-equipped to process and butcher beef, which makes it easy for meats to get contaminated and for incidences of food poisoning to occur. In the meanwhile, fresh meat for several homes, food vendors and shops is primarily sourced from these markets. In order to evaluate the microbiological safety of fresh beef marketed in various towns in Ogbia, which may be a reflection of the hygienic state of meat consumed and potential health risks, this study was designed.

Materials and Methods

Sample Collection

Fresh raw beef samples were aseptically collected from five (5) different locations from each of three distinct markets (Otuoke market, Elebele market, and Imiringi market) located in different parts of Ogbia Kingdom in Bayelsa State, Nigeria. A total of fifteen (15) fresh raw beef samples were collected for the study. To avoid cross-contamination, samples were aseptically collected into distinct sterile plastic bags and quickly placed into ice-packed cool box and transported immediately to the Microbiology Laboratory of the Federal University Otuoke, Bayelsa State, for analyses.

Sample Preparation

One gram (1g) of previously weighed beef sample was aseptically transferred to a sterile jar containing 9ml of sterile normal saline diluent was homogenizing for 15 seconds.

Aliquot (1.0 ml) of the homogenate was transferred to 9 ml of sterile distilled water in test tube. This created a 10^{-1} dilution, and the mixture was thoroughly mixed. For the microbiological study, serial dilutions up to 10^{-6} were created (Oso and Fawole, 2001).

Identification of Bacterial Isolates

To obtain the total viable plate count of bacteria (TVC), 0.1 ml of 10^{-6} dilution were obtained and inoculated into a sterile Petri dish along with 25 ml of sterile solidified Nutrient Agar, Salmonella-Shigella Agar to cultivate these organisms, Mannitol Salt Agar for the cultivation of pathogenic staphyloccoci, and Eosin methylene blue agar for detection of Coliforms.

Thereafter, the inoculum was evenly spread out using a flame-tipped bent rod and incubated for 24 to 28 hours at 37°C. The number of viable bacteria cells in a sample of meat was estimated and recorded as colony forming unit (CFU/mL). The different isolates underwent the conventional Gram's staining protocol and biochemical tests as outlined by Cheesbrough (2002).

Identification of Fungal Isolates

Using the pour plate method, aliquots of dilution (10^{-6}) were used to inoculate on sterile Sabouraud Dextrose Agar (SDA) plates. The cultured plates were incubated at room temperature (28 ºC) for 72 to 120 hours. To ensure accurate identification, all fungal isolates were sub-cultured onto freshly prepared sterile SDA plates (Pál *et al.*, 2015).

Antibiotic Sensitivity Testing

The modified Kirby-Bauer multi disk diffusion method was used to investigate the isolates' antimicrobial susceptibility (Andrup *et al.*, 2008). Muller Hinton agar was treated with commercial antibiotic discs (ROSCO) containing the antibiotics.

The following antibiotics were found on the discs (Celtech Diagnostic) and were tested for effectiveness against the isolates: PEF: 10µg of perfloxacin, Z: 20µg of zincacef, CPX: 30µg of ciprofloxacin, E: 10µg of erythromycin, GN: 10µg of Gentamycin, AM: 30µg of amoxicillin, SXT: 30µg of streptomycin, APX: 30µg of ampiclox, R: 25µg of rocephin, and S: 30µg of septrin.

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Data Analysis

Simple descriptive statistics was used to determine the frequency of occurrence of bacterial isolates. Microbial loads of raw beef samples were expressed as means ± standard deviations. Duncan's Multiple Range test was performed to compare microbial loads of samples using SPSS 22 software (SPSS Inc., IBM Corporation, Chicago, USA). Significant difference was set at $p < 0.05$.

Results

The result of the bacteria count from raw beef samples from the markets are presented in Tables 1 and 2. The samples were heavily contaminated with 7.0 x 10^7CFU/g in Otuoke as the highest count recorded and 2.0 x10^7CFU/g in Elebele and Imiringi as the lowest. In terms of both microbial quantity and composition, the fresh beef samples under investigation had generally high bacteria counts.

Table 1: Total Viable Heterotrophic Bacterial Counts from Raw Beef Samples from Different Markets

Sampling point	Total viable heterotrophic bacteria count (CFU/g)		
	Otuoke Market	Elebele Market	Imiringi Market
1	4.0 x10^7	2.0 x10^7	3.0 x10^7
2	4.0 x10^7	4.0 x10^7	2.0 x10^7
3	5.0 x10^7	3.0 x10^7	2.0 x10^7
4	7.0 x10^7	4.0 x10^7	2.0 x10^7
5	4.0 x10^7	3.0 x10^7	4.0 x10^7
Mean ± SE	4.9 ± 1.2	3.35 ± 0.7	2.8 ± 0.7

Table 2: Microbial Counts of Raw Beef on the Selective Media for Bacterial Groups (CFU/g)

Bacteria Group	Otuoke Market,	Elebele Market	Imiringi Market
Total Salmonella-Shigella	4.25±0.96*	2.25±0.5^{a}	3±0.8^
Total Staphylococcal	3.25±0.50*	4.25±0.5$^{\#b}$	2.25±0.5^^{bc}
Total Coliform	6±1.41	4.25±1.0$^{\#}$	6.25±1.3
Total viable heterotrophic bacteria (TVHB)	7.25±0.96	4.25±0.5$^{\#d}$	2.25±1.0^^{de}

Note: There is no significant difference in means with the same superscript

The results for the occurrences (%) of bacteria and fungi isolated from the fresh beef samples from the Markets are presented in Figure 1 and Figure 2 respectively. Their occurrences were; *E. coli* (19.0%), *Enterococcus* sp. (8.0%), *S. aureus* (23.0%), *Klebsiella* sp. (8.0%), *Salmonella* sp. (23.0%), *Micrococcus* spp. (19.0%), *Aspergillus* sp (29.0%), *Candida* sp (14.0%), *Fusarium* sp (43.0%), and *Penicillium* sp (14.0%).

The result of antibiotic sensitivity and resistance patterns the isolated bacteria as observed is presented in Table 3.

It was evident that various antibiotics affected bacteria in different ways. For instance, while *Klebsiella* sp. shown resistance to every antibiotic administered, *Staphylococcus* sp. remained fully sensitive to all of them. Pefloxacin was resistant to only *Klebsiella* sp. and susceptible to other isolates tested. Additionally, all organisms except for S*taphylococcus* sp, which was sensitive, were resistant to ampicillin and amoxicillin.

The most susceptible isolate was *Staphylococcus* sp., followed by *Enterococcus* sp. The most resistant bacteria were *Klebsiella* species, then *Esherichia coli* and species of *Micrococcus*.

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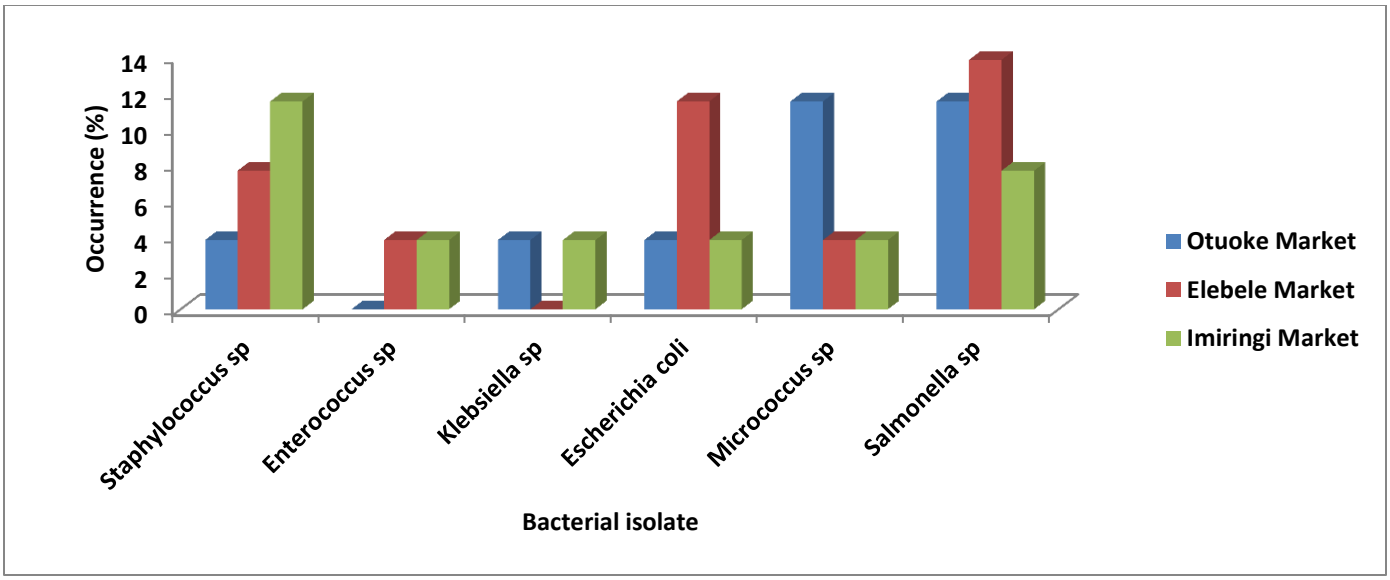

Figure 1: Occurrence of Bacteria Isolated from the Fresh Beef Samples from the Markets

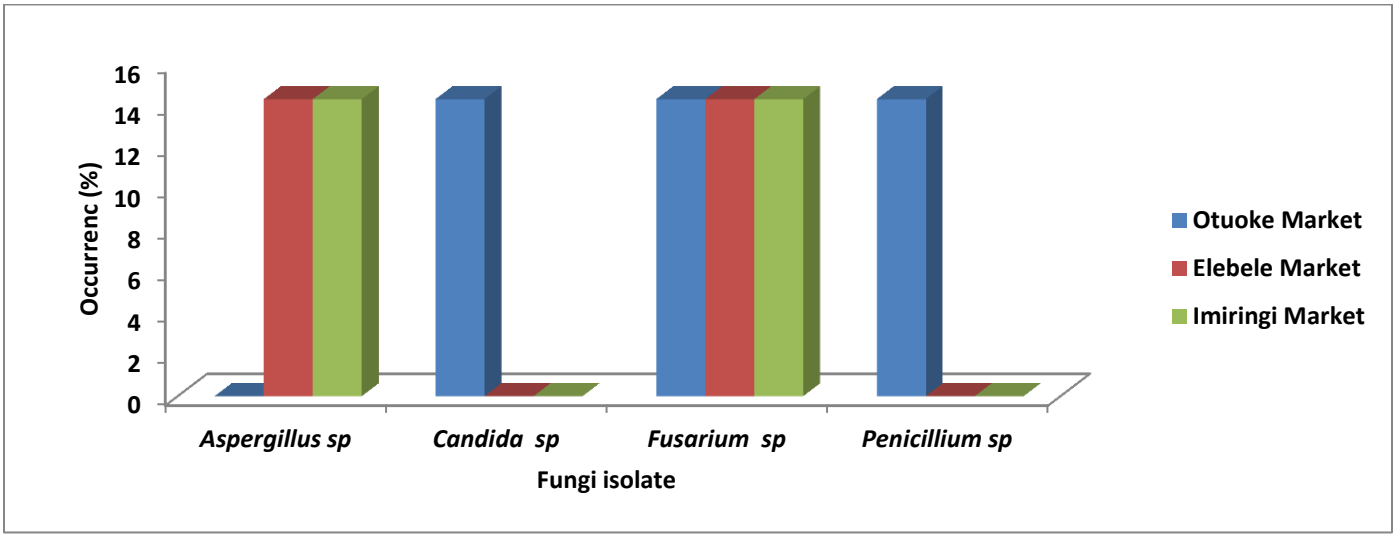

Figure 2: Occurrence of Fungi Isolated from the Fresh Beef Samples from the Markets

Table 3: Antibiotics Sensitivity Pattern of Bacteria Isolated from Raw Beef

Isolates	CN (10µg)	R (30µg)	AM (25µg)	CPX (5µg)	APX (30µg)	PEF (5µg)	S (10µg)	E (15µg)	Z (20µg)
Enterococcus spp	S(17)	R(0)	R(0)	S(45)	R(0)	S(36)	I(10)	R(10)	S(22)
Escherichia coli	R(11)	R(12)	R(0)	S(36)	R(0)	S(32)	S(20)	R(10)	R(0)
Klebseilla spp.	R(12)	R(0)	R(0)	R(5)	R(0)	R(0)	R(0)	R(0)	R(0)
Staphylococcus spp.	S(29)	S(23)	S(20)	S(30)	S(20)	S(30)	S(36)	S(28)	S(28)
Micrococcus spp	R(12)	R(14)	R(0)	S(38)	R(0)	S(36)	R(0)	R(0)	I(16)

Key: Pefloxacin(pef), gentamycin(cn), Ampicillin(apx), Cefuroxime(z), amoxicillin(am), Ceftriaxone(r), ciprofloxacin(cpx), streptomycin(s), erythromycin(e). Resistant (R), Sensitive (S), Intermediate (I).

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Discussion

The study showed the raw beef samples from the various markets in different towns in Ogbia recorded high microbial counts and that Otuoke market meat had the highest level of contamination. These high counts are attributed to the fact that the market places were right next to busy roads and were open, unsanitary markets. Meat contamination can result from insects, roadside dust, cross-infection from chopping tables, and the method used to obtain the meat from the butcher shop to the store.

A high bacterial load in food is a sign of both the overall quality of the meal and the potential level of spoiling. But eating raw meat or improperly prepared meat products poses the biggest threat to human health (Yagoub, 2009). However, according to James *et al.* (2000), every species of bacteria that was isolated—including *Salmonella* species, *Escherichia coli, Staphylococcus* species, *Enterococcus* species, and *Micrococcus sp* species—is known to cause disease. These microorganisms were discovered in the imiringi market. The investigation showed that the beef that was sold was tainted with bacteria from different genera, with *Salmonella* and *Staphylococcus* species being the most prevalent. This is most likely because of the butchers' poor handling, improper storage, and unfavorable climatic conditions. Public health is concerned about the isolation of harmful organisms such as *Salmonella* spp., which are significant food-borne pathogens. Despite the fact that proper cooking will eradicate these infections, consumers are nevertheless at risk while buying beef from the numerous markets surrounding Ogbia Metropolis. Cutting knives, intestinal contents, chopping boards, hides, meat handlers, containers, vehicles used to transport meats, and the atmosphere surrounding the sale of meat are all potential sources of contamination.

In this study, the most prominent bacteria isolated from the raw beef samples were *Staphylococcus* species, followed by *Escherichia coli* and *Salmonella* species.

This is in congruent with earlier reports that isolated *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella* spp, *Klebsiella pneumoniae* and *Staphylococcus epidermidis* from raw and processed meat product (Suya) in varying part of Nigeria (Iroha *et al*., 2011; Falegan *et al*., 2017).

These in turn might be major causes of gastro-intestinal disorders, food poisoning and food borne diseases. From the results obtained, there were high levels of *E. coli*, *Staphylococcus* species, and *Salmonella* species in the fresh meat sample. This is consistent with reports from Fasanmi *et al*., (2010) and Clarence *et al*., (2009), which indicated that these organisms are the primary contributors of contamination. Additionally, the results of this study are consistent with those of Nouichi and Hamdi (2009), who found that *Staphylococcus, Salmonella, E. coli*, *Listeria*, and *Clostridium* are the most commonly found bacterial pathogens linked to the intake of beef products. According to the data, *E. coli* was present in 19% of the samples. This aligns with a study that also found a significant prevalence of *E. coli* (Zhao, 2001).

It had also been observed that the retail meat sector had a high frequency of *E. coli* (Kumar, *et al.,* 2001). Many scientists have investigated the occurrence of *E. coli* strains in meat and meat products (Dutta, *et al.*, 2000). The results of the present investigation also indicated the presence of *Salmonella* sp., which is corroborated by the findings of the study conducted by Soultos *et al.,* (2003), which demonstrated contamination levels of this strain in retail foods. This result is supported by another investigation that found a prevalence of *Salmonella* sp. in several raw meat samples from the local market (Maharjan, *et al*., 2006).

The study's findings regarding the incidence and establishment of multidrug-resistant *Klebsiella pneumoniae* in chickens are consistent with the 8% prevalence of *Klebsiella sp*. in the samples (Kim, *et al*., 2005). Raw meat is still a significant and most likely the predominant source of food-borne bacterial infections in humans. Despite decades of work, it has been challenging to get pathogen-free animals food. In other developing nations, reports of cattle and carcass handling in conjunction with microbial contamination of beef samples have also been made. According to Kumar *et al.* (2010), beef produced and sold in certain regions of the Ethiopian region has a high total aerobic plate (APC) count of 759.16%. *Salmonell*a sp. and *Staphylococcus sp* were most common (23%), followed by *Escherichia coli* (19%) and *Klebsiella* sp. (8%). This is comparable to a report by Moshood *et al.* (2012), who noted a similar pattern from roasted pork products called balangu that were sold in Bauchi.

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Considering that people are carriers of *Staphylococcus aureus* (on the skin, nose, and throat), this may result from the unsanitary circumstances of butchers. These results showed a lower fungus count than those published by Omorodion and Odu (2014), who found a high total fungal count from meats marketed in Nigerian, Port Harcourt Metropolis. The mycoflora level of the beef samples, according to Olufunmilayo and Akeeb (2010), varied from 5.81 to 6.34 log cfu/g, which is likewise less than what was discovered in this investigation. Given that fungi like dry, crevices and cracks, the fissures on the slaughter house floors and tables could be potential origins of the organisms. Table 3 displayed the results of the conventional antibiotic susceptibility test.

This study's findings regarding antibiotic sensitivity are comparable to those of tests conducted on Suya, or sliced roasted beef, which is sold in Ado-Ekiti Metropolis, Ekiti State (Falegan *et al.*, 2017). Additionally, the findings of this investigation are consistent with the antimicrobial resistance profile of raw meat sold in Abakaliki, Ebonyi State, Nigeria, as reported by Iroha *et al.* (2011). The use of inferior antibiotics in developing animals to treat bacterial infections may have led to resistance to these drugs. This may result in long-term sickness and even death in susceptible individuals, such as those with weakened immune systems, raising concerns about public health.

In conclusion, the findings of this study revealed high levels of bacterial contamination from the various meat sources. This could be attributed to contaminated environments and inadequate personal hygiene. The causes of contamination in this case could include the handling and slaughter procedure, or the intestinal contents and meat's handling during transportation to the various markets. It is also possible for other beefs to become contaminated by leftovers from tables, knives, and weighing scales. This study showed how this meat serves as a source of bacteria resistant to antibiotics that can infect humans, posing a long-term threat to public health. In order to prevent the spread of antibiotic resistance, it is hereby recommended that the implementation of strict hygienic measures both before and during the processing of meat as well as the responsible use of antibiotics in animal husbandry be monitored regularly.

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