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MICROBIAL ASSESSMENT OF ROASTED STREET VENDED BEEF, CHICKEN AND FISH SOLD IN LOKOJA, KOGI STATE

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ABSTRACT

Microbial Assessment of Roasted Street Vended Beef, Chicken and Fish Samples Sold in Lokoja, Kogi State was carried out in April and May 2016. Samples of roasted beef, chicken and fish were collected from two locations and analysed for their microbial load with the aim of ascertaining their quality and suitability for consumption. Pour Plate method on Nutrient Agar (for bacteria), MacConkey Agar (for coliform) and Potato Dextrose Agar (for fungi). The organisms isolated from the samples include: Bacillus sp, Staphylococcus sp, Escherichia sp, Aspergillus flavus, and Rhizopus stolonifer. Analysis of the food samples revealed the mean total bacterial count which range from 3.5x106 cfu/ml(beef) to 1.97 x107 cfu/ml(chicken) in the samples from T-Square joint while it ranged from 1.27 x104 cfu/ml(chicken) to 1.97 x107 cfu/ml(beef) in the samples from Nigerian Union of Journalists joints. Mean coliform count ranged from 3 x106 cfu/ml(chicken) to 4 x106 cfu/ml(Fish) in the samples from the T-square joints while it ranged from 9.3 x106 cfu/ml(beef) to 2.8 x107 cfu/ml(chicken) in the samples from Nigerian Union of Journalists joints. Fungal count ranged from 5 in chicken samples to 15 in fish in the samples obtained from Nigerian Union of Journalists joints while the samples obtained from T-square joints had no fungal growth. The results obtained from the study showed the presence of microorganisms in various degrees in the food samples. All the microorganisms isolated are capable of causing food poisoning. There is no significant difference in the microbial load of roasted street vended beef, chicken and fish ($P > 0.05$). Based on the result of this study, street vended food should be handled properly after preparation to avoid contamination with pathogenic microorganisms and they should not be displayed openly to avoid contamination with spores of fungi in the air.

Keywords: *Microbial, Beef, Chicken, Fish, Fungal-count and Coliform-count*

1.0 INTRODUCTION

Street foods are an extremely heterogeneous food categories, encompassing meals, drinks and snacks. They also show great variety in terms of ingredients, methods of retail, processing and consumption and are sold on the street from "pushcarts, baskets or balance poles or from stalls or shops having fewer than four permanent walls" (FAO, 2007)

Street foods are common in developing countries with high incidences of food poisoning outbreaks. This has an obvious economic consequence both for the individual and the nation. While food borne diseases remain an important public health problem worldwide, one of the most significant food safety hazards is associated with foods from animals (Maripandi and Al-Salamah, 2010)

Beef, Chicken and Fish are common delicacies to many people. Roasted chicken, beef and fish vendors are found in almost every neighbourhood in developing nations with a dense population for various daily formal and informal economic activities. They are street processed, roasted and

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vended for consumption. In Nigeria, the sale of beef (suya) first started before that of Chicken and Fish which emerged just recently and is not as widespread as the former. These foods are bought for consumption without further processing or cooking (Ologhobo et al., 2010)

Street vending of food is a common characteristic of countries with high unemployment, low salaries and poor social security programme (Bryan et al., 1988). However consumers of street vended meat and fish are little aware of the high health risks they face because such foods are exposed to various forms of contamination at every stage of handling. Street foods, particularly in developing countries, have been reported to be contaminated by different pathogenic bacteria (Arambolu et al., 1993). Etok (1998) identified insufficient roasting/heating duration, uneven temperature distribution and exposure to unhygienic environments as crucial factors of infection and contamination of foods. The aim of the vendors is to minimise shrinkage of the meat and fish during roasting to maximise profit but at the same time satisfy the demand and appetite of the buyers. In some cases, the foods are usually prepared in a rush or they are roasted and kept exposed and cold to await the potential consumers (Ologhobo et al., 2010).

Poor environmental sanitation and poor personal hygiene particularly among food handlers accounts specifically for food contamination while improper storage leads to the multiplication of pathogens in food in infective doses. In the resource-poor tropical countries of the world particularly in sub-saharan Africa, foods are often preserved at ambient temperatures long before consumption. Improper handling by food vendors who sold these products in streets in the dirty unhygienic environment also contribute to food contamination (Chukwuemeka et al., 2011). With low power supply in most parts of Nigeria, storage of already processed food is virtually impossible, hence any leftover may encourage microbial growth.

The conditions described above prevail in Lokoja, the capital of Kogi State in north central Nigeria and are compounded by inadequate access to pipe borne water, poor drainage systems and lack of appropriate waste disposal facilities. This lack of basic social amenities and municipal utilities has in no small measure contributed negatively to poor personal and environmental hygiene of food vendors in this locality. There are recent alarms and reports on the social media and other scholarly sources of food poisoning especially street vended snacks which have similar processing methods with fish, chicken and beef. From different reports, it appeared street vended foods are note hygienically safe for consumption. It is against this background that the assessment of the microbial quality of prepared beef, chicken and fish sold by vendors on the streets of Lokoja was carried out.

2.0 MATERIALS AND METHODS

2.1 Study Population and Sites

Thirty (30) vending points from two popular sites comprising Nigeria Union of Journalists (NUJ) office environs (site 1) behind Lokoja Township Stadium and T square hotel (site 2) both in Lokoja. These sites were selected because the NUJ environs is located in the heart of the town and is made up of bars, restaurants and clubs where people go to relax and is always highly populated during the day and at night. The T- square hotel is located at zone 8 junction along a major road and it serves as a relaxation spot for residents from Lokongoma, Baracks, Crusher and environs and is always highly populated during the day and at night. Figure 1 is the map of Lokoja where the study was conducted.

The sample size was determined following the formula described by Thrusfield (1995). By considering the expected prevalence of 50 and 5% absolute precision with 95% confidence level.

The study samples were collected by simple random sampling method in April and May 2016 from the vending points with sterilised polyethylene bags and transported to the Biology laboratory of Federal University Lokoja for analysis within 24 hours.

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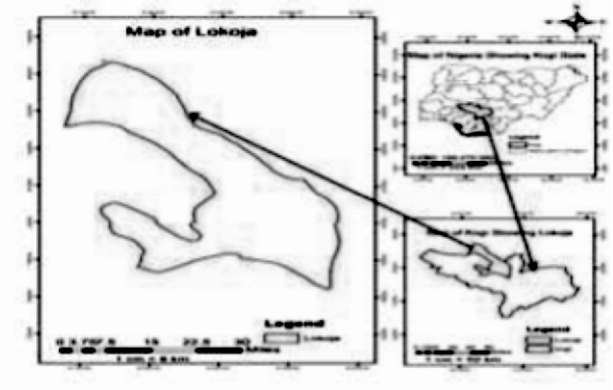

Figure 1: The Map of Lokoja, Kogi state where the study was conducted

2.2 Isolation and Identification of Bacteria and Fungi

The culture media (Nutrient Agar, MacConkey Agar, and Potato Dextrose Agar) were used for the isolation and identification of the microbial load of the samples. Fifty grams (50g) of each sample (beef, chicken and fish) were weighed using digital weighing balance and blend in electrical blender and later homogenised in 90ml of sterile distilled water following the method adopted by Clarence *et al*. (2009).

Ten-fold dilutions of the homogenate was made to 10-5 ml of 10-3 (1:1000) , 10-4 (1:10000) dilution of each sample was transferred into the petri dish using a micropipette and inoculated using the pour plate method on Nutrient agar (For Total Viable bacteria), MacConkey agar (For coliform) and Potato Dextrose agar (For fungi). The plates were prepared in duplicates and incubated in the incubator under aerobic condition at 37°C for 48 hours for bacteria and at 25oC for 5 days fungi following the procedures adopted by Madueke et al. (2014).

Grown colonies of bacteria and fungi on the Nutrient and MacConkey agar media were examined microscopically after 48 hours of incubation. Gram staining was performed for the identification of bacteria. Fungi colonies that developed on Potato dextrose agar were examined to see the type and number of hyphae and the production of spores. Colony morphology, shape, size, elevation, colour and odour were noted. The numbers of colonies formed on the plates were counted using a colony counter. They were expressed as colony forming unit per ml of sample homogenate (cfu/ml) (Clarence et al., 2009).

Typical colonies were picked aseptically with inoculating loop and purified by sub-culturing and bacterial count was done. Bacteria were identified on the basis of colonial morphology, gram stain reaction, positive catalase test, coagulase production and fermentation of mannitol, in accordance with Cowan and Steel (1993).

2.3 Biochemical test

The isolates to be cultured were characterized biochemically using Gram stain reaction, positive catalase test, coagulase test and DNA-ase test were performed on the isolates for identification purposes as described by Cheesbrough, (2006).

2.3.1 Gram Stain test

Gram's stain was performed to determine if the organism is Gram negative or Gram positive. The staining was performed on the isolates according to the known procedures. A smear of the test organism was made on a clean slide, dried, heat fixed and covered with crystal violet for 30-60 seconds. It was washed off with clean water and covered with Lugol's iodine for 30-60 seconds and washed off with clean water. The slide was decolourized with acetone, and washed immediately with clean water and covered again with neutral red stain for 2 minutes, and washed off with clean water (Adeleke et al., 2012). The back of the slide was wiped clean and placed in a draining rack for the smear to air dry. The slide was examined microscopically with the oil immersion lens after the application of the oil on the slide. Gram positive bacteria indicated a dark purple colour while gram negatives gave a red colour.

2.3.2 Catalase test

The catalase test was done to differentiate the bacteria that produce the enzyme catalase, such as staphylococci from the non-catalase producing bacteria such as streptococci (Cheesbrough, 2006).

2.3.3 Coagulase test

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Coagulase test was done to identify Staphylococcus aureus from coagulase negative Staphylococcus. A drop of distilled water was be placed at the end of a slide; a colony of the test organism was emulsified on it to make a suspension. A loopful of plasma was added to it and mixed gently; presence of clumping within 10 seconds indicates the presence of Staphylococcus aureus, while absence indicates presence of Escherichia coli or Staphylococcus epidermidis (Mandel, 2013).

2.3.4 DNA-ase test

It helps to identify Staphylococcus aureus (Cheesbrough, 2006).

2.4 Statistical Analysis

Statistical analysis was carried out using ANOVA with Microsoft Excel 2010 at 5% level of confidence to compare microbial load between food types and between locations.

3.0 RESULTS

3.1 Bacteria and Fungi Taxa Recovered During the Study

Staphylococcus sp, Bacillus sp and Escherichia sp were isolated from the beef, chicken and fish samples obtained from the vendors in the two sites. Two fungi species (Aspergillus flavus, and Rhizopus stolonifer) were isolated from the samples obtained from Nigerian Union of Journalists joints and there was no fugal growth on the samples obtained from T-square joint. Bacteria and fungi species isolated from the samples from Nigerian Union of Journalists joints are shown in Table 1. The three bacteria and two fungi species isolated in this study were all present in the fish, chicken and beef samples. Table 2 shows the microbial taxa isolated at the T-Square joint with only bacteria present.

3.2 Total Viable Count (TVC) of Bacterial Growth of the Samples

The Total Viable Count is the total number of bacteria able to grow in an aerobic environment in moderate temperature. It is an indicator of quality, not safety. In the samples obtained from the T-square environs joint the TVC was highest in chicken (1.97 x107) followed by fish (3.9x106) and beef (3.5x106) (Table 3). In the samples obtained from the Nigerian Union of Journalists joints, the TVC was highest in beef (1.45 x107) followed by fish (1.08x107) and chicken(1.27 x104) had the least TVC as shown in Table 3. From the two sites, chicken (1.97 x107) obtained from the T-Square Joint had the highest TVC while chicken (1.27 x104) obtained from the Nigerian Union of Journalists environ Joints had the least TVC.

Table 1: Bacteria and Fungi Species isolated from Nigerian Union of Journalists Joints ((+) =Present, (-) =Absent)

Bacteria			
Staphylococcus sp	+	+	+
Bacillus sp	+	+	+
Escherichia sp	+	+	+
Fungi			
Aspergillus flavus	+	+	+
Rhizopus stolonifer	+	+	+

Table 2: Bacteria and Fungi Species isolated from T-Square Joints samples ((+) =Present, (-) =Absent)

Bacteria			
Staphylococcus sp	+	+	+
Bacillus sp	+	+	+
Escherichia sp	+	+	+
Fungi			
Aspergillus flavus	-	-	-
Rhizopus stolonifer	-	-	-

Table 3: Total Coliform Count of the beef, chicken and fish samples from Nigerian Union of Journalist and T-square joints

Food Sample	NUJ	T-Square Joint
Fish	1.08×10^7	3.9×10^6
Chicken	1.27×10^4	1.97×10^7
Beef	1.45×10^7	3.5×10^6

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The Total Coliform Count of som the T- Square amples of beef, chicken and fish sold by vendors at Nigerian Union of Journalists joints and T-square environ joints in Lokoja is shown in Table 4. The TCC was highest in chicken (2.8 x107) followed by fish (1.68x107) and beef (9.3 x106) from samples collected from Nigerian Union of Journalists joints. In the samples obtained from the T-Square joints; fish (4 x106) had the highest TCC followed by beef (3.1 x106) and chicken (3 x106) had the least TCC. From both sites, chicken (2.8 x107) samples collected from Nigerian Union of Journalists joints had the highest TCC while chicken (3 x106) obtained frenviron joints had the least TCC.

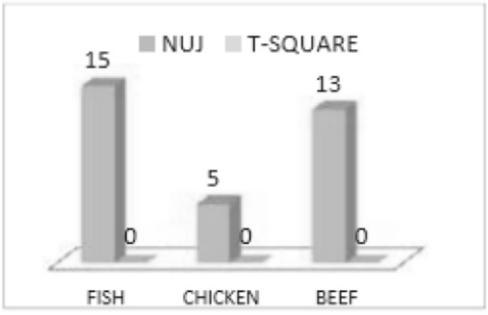

Figure 2: Fungal Count on Food Samples from the Two Locations Using Potato Dextrose Agar as Growth Medium

4.0 DISCUSSION

The microorganisms isolated and identified in this study (Staphylococcus sp, Bacillus sp, Escherichia sp, Aspergillus flavus, and Rhizopus stolonifer) were also reported by Madueke et al. (2014) in a similar work along Lokoja-Abuja express way. However, more organisms were reported in their study. The types and numbers of microorganisms observed in their study could be due to difference types of samples and the sampling site which was a highway with higher vehicular traffic all day making the area always dusty. It could also be due to improper handling by the vendors who mostly live at the vending sites or the resale of leftovers in which the number of microorganisms would have increased due to improper storage.

Figure 2 shows the fungal count on the samples. Fish had the highest fungal count (15) followed by beef (13) while chicken (5) had the least. The samples obtained from the T-square joints had no fungal growth.

Table 4: Total Coliform Count of Fish, Chicken and Beef Sold by Vendors at the Nigerian Union of Journalists Joints and the T-Square Joints

Fish	$1.68x10^{7}$	$4 x10^{6}$
Chicken	$2.8 x10^{7}$	$3 x10^{6}$
Beef	$9.3 x10^{6}$	$3.1x10^{6}$

The bacterial counts obtained may probably be due to post production contamination since the foods are prepared with high amount of heat. Post treatment contamination has been reported by Ogugbue et al. (2011). This can occur during cooling and exposure to the air which has been identified as the main source of microbial contamination of most street foods. Higher microbial growth was recorded from the beef samples from Nigerian Union of Journalists joint than that of T-square joint. At T-square joint, beef were kept in glass containers and the area was less populated when compared to Nigeria Union of Journalist joint which is more densely populated with exposed samples. There was no fungal growth recorded in the samples obtained from T-square and this could be due to reduced exposure of these samples to air.

The presence of Staphylococcus sp in the samples is indicative of human contamination after production. This could be from direct human contact such as fingers or indirectly through additives or utensils. The organism is associated with endotoxin characterized by short incubation period (1-8 Hours), violent nausea, vomiting and diarrhoea (Madueke et al., 2014). Bacillus sp isolate is associated with the production of toxin; diarrheal and emetic in food which causes food poisoning. It is found in dust, soil and raw food and can survive normal cooking as a heat resistant spore (Rajkowski and Bennett, 2003). The presence of Escherichia sp suggested faecal

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contamination and can also be attributed to the use of contaminated water during the different stages of processing because water is a major means by which E. coli is spread although some Escherichia sp are harmless (Madueke et al., 2014). Aspergillus flavus and Rhizopus stolonifer observed in the food samples were as a result of their mode of dispersal through spores which were abundant in the environment and introduced as dust and soil contaminants as reported by Apinis (2003). Their presence in these food samples is of serious public health concern as these fungi have all been implicated with the production of mycotoxin (Makun et al., 2009).

Microbial guideline for cooked food stipulated that "the plate count must be < 10 7cfu/g, for meat < 1.0 x 10 5 cfu/g, for plant products < 10 x 10 5cfu/g, for ready to eat frozen meals < 1.0 x 104 cfu/g and for coliforms, the plate count must be <10 5 cfu/g (Gilbert et al., 2000). The Health Protection Agency (2009) stated that Ready-to-eat foods with Staphylococcus sp that is >104 is high and unsatisfactory while Bacillus sp that is >105 is high and unsatisfactory and food samples with Escherichia detected in it is unsatisfactory. The microbial load of the food samples were higher than the stipulated threshold, hence their presence constituted a health risk; it can be adjudged that the street food retailed in most location at NUJ and T-square as obtained in this study are not fit for consumption.

It is recommended that roasted street vended beef, chicken and fish should be handled properly after preparation to avoid contamination with pathogenic microorganisms and they should not be displayed openly to avoid contamination with spores of fungi which abound in the environment. Vendors should maintain personal hygiene, minimize their contact with food samples after production and ensure proper cleaning of utensils before and after use. For continuation of the present study the following research topic may be advocated to be conducted in future: Identification of the bacteria to species level and Pathogenic characterization of the bacteria. These could not be done due to lack of sophisticated equipment and facilities.

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