# Microbiological Analysis Of Kilishi Sold In Port Harcourt, Nigeria

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**Abstract:** The microbial quality of kilishi obtained from two different producers (Local and Princess Rose Logg Company) in Port Harcourt City metropolis, Nigeria was determined to ascertain its safety for consumption. The total bacteria count was determined for both samples. The sample from the Local producer had the higher bacteria count of  $4.32 \times 10^3$  cfu/g, while the sample from the company had a lower bacteria count of  $2.1 \times 10^2$  cfu/g. The bacteria isolated were *Bacillus* species and the fungi isolated were *Botryodiplodia theobromae*. No *Vibrio* species, *Staphylococcus aureus* and *Escherichia coli* was isolated from the Kilishi used in this study. The presence of *Bacillus* species is used as an index of bacteriological quality. The diversity of isolates from the samples tested is an indication of its low microbiological quality and poor hygienic practices. Microbiological analyses are therefore recommended for both pre-and post-production stages. [Okonko IO, Odu NN and IGBOH IE. **Microbiological Analysis of Kilishi Sold In Port Harcourt, Nigeria.** *N Y Sci J* 2013;6(7):37-43]. (ISSN: 1554-0200). http://www.sciencepub.net/newyork. 8

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# **1. INTRODUCTION**

Kilishi is a ready-to-eat meat product obtained from sliced lean muscles of beef, goat or lamb. It is made on a large scale under hot and dry weather conditions prevailing from February to May. It is produced by sun drying the slices of meat. However, recent experience indicates that kilishi can also be produced industrially by tray-drying in a warm air oven. Connective tissues and adhering fat materials are trimmed of the meat, this is cut with a trimming knife into thin slices of about 0.5cm thickness, 15cm length and as much as 6cm width.

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. 2003). 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 meat have high water content corresponding to the water activity approximately 0.99 which is suitable for microbial growth (Rao et al., 2009). Public concern has risen due to numerous food scandals such as those surrounding bovine spongiform encephalopathy and food and mouth disease epidemic (Ellis and Goodacre, 2001; Tauxe, 2006).the wide spread distribution of the meat product makes the consequence of contamination with food poisoning microorganisms more serious. The health status of animals prior to slaughtering and prevailing circumstances in the slaughter contributes to the quality of meat from such animals (Whyte et al, 2004).

Foodborne microbiologic hazards may be responsible for as many cases of illness as possible

each year and are thus an important food safety challenge. To lower the incidence of foodborne disease, many experts and stakeholders urge the development of a science- and risk-based food safety system, in which decision makers prioritize hazards and interventions using the best available data on the distribution and reduction of risks (Batz et al., 2005). Such a system requires an understanding of the many risk factors between the point of production and the point of consumption and the ability to systematically target intervention efforts along this "farm-to-fork" continuum (Batz et al., 2005). The preservation of meat as a perishable food usually is accomplished by a combination of preservation methods which greatly lengthen the keeping quality the meat. So, to increase meat quality assurance in accordance with microbial load assessment is deemed necessary (Yousuf et al., 2008).

The possible sources of contamination during the preparation of kilishi includes using contaminated raw meat, washing the raw meat with contaminated water, using contaminated utensils (knives for slicing meat before sun drying), adding contaminated spices, ingredients and the handler's hands. Sun drying before and after dipping in spices allows for contamination from the environment, (flies perch on the product), and bacterial growth. Roasting of the sun dried product gently decontaminates it by destroying vegetative bacterial cells, but may not destroy some spores. The critical control point during toasting is roasting and temperature (Umoh, 2005). The aim of this study is to determine the bacteriological quality of dried sliced beef (kilishi). This is important because this meat product serves as ready to eat snacks and could be a source of public health threat.

In Nigeria, particularly in rural communities and towns, 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 like kilishi to microbial contamination and rapid deterioration.

## 2. MATERIALS AND METHOD

## 2.1. Sample collection

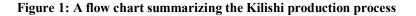
Freshly prepared dried meat samples (100g each) were brought from two different producers, the local producers (at the abattoir) and the kilishi prepared by Princess Rose Logg Company No. 402 Enekia road in Port Harcourt. The health status of the animals used in preparing the meat product was not established and only the already prepared product was analysed. The samples were transported to the laboratory in clean, sterile polyethylene bags after collection.

## 2.2. Kilishi preparation

Traditionally, Kilishi is prepared using quality beef. The lean beef is skilfully cut into thin sheets [1-2mm thick]. These sheets of meat are sun dried on a raised wooden table covered in rush matting. However, the rush matting may lead to hygiene problems especially after repeated use.

Therefore, easily washable corrosion free wire nets or plastics are recommended for horizontal drving. Drying the meat vertically is also recommended in this case. In the first stage of drying, which takes 2 -6 hours, the moisture of the meat is reduced to about 40 - 50%. The slices are then put into an infusion containing defatted wet groundnut cake paste as the main component [about 50%], and which is composed of water [30%], garlic [10%], salt [2%], spices such as ginger, pepper and onions. The dry slices of meat should absorb the infusion until they are almost three times their original weight. After infusion, the wet product is exposed to the sun to dry. This drying stage is faster than the first drying stage. When the moisture content of the slices has been reduced to about 20 - 30%, a process which takes 2 - 30%3 hours depending on the weather condition, the slices are then roasted over fire for 5 minutes. This roasting helps to enhance the desired flavour and to kill contaminating microbes. Roasted kilishi is superior in flavour to the unroasted kilishi. After roasting, the moisture content ranges between 10 -20%. This will reduce during storage at room temperature to as low as 7%. When the meat is sealed in low density plastic bags, the product remains stable at room temperature for a period of about 1 year. Figure 1 shows a flow chart summarizing the Kilishi production process.

Fresh meat excised from hind limb of carcass Cleaning and trimming off of fat, bone and connective tissues Slicing of meat First air drying Dried meat slices weighed Dried meat slices in infusion slurry Second drying of infused slices Dried kilishi Light roasting Finished kilishi Packaging



## 2.2. Determination of moisture content

Ten grams of dried sliced meat sample from each producer was weighed and dried in the oven at 80°c to a constant weight. The moisture content was determined as the difference between the weight of the fresh sample and that of the sample after drying to a constant weight. This was expressed as the percentage of the total weight of the sample (Raji, 2006).

Mc,  $\% = \frac{Lws \times 100}{Wfs}$ 

(Where Mc = Moisture constant %, Lws = Loss in weight of sample, Wfs = Weight of fresh sample)

## **2.3. Microbiological Analyses of Samples 2.3.1. Sample Preparations**

Ten grams (10g) of each kilishi sample was weighed out and homogenized into 90 ml of sterile distilled deionized water using a sterile warring blender. Ten fold dilutions of the homogenates were made using sterile pipettes as described by the methods of Fawole and Oso (2001).

## 2.3.2. Culturing of kilishi samples

All the chemicals and reagents used were of analytical grade, obtained from Sigma chemical co. Ltd, England. Media used in this study included: Nutrient Agar (NA) and Peptone Water (PW) as general and enriched media. Other media with selective and differential characteristics used were Mac Conkey agar (MCA), Eosin Methylene Blue (EMB), Kligler Iron Agar (KIA), Citrate Agar (CA), Christensen's Urea Agar (CUA), Mueller Hinton Agar and Mannitol Salt Agar (MSA). All media were prepared according to the manufacturer's specification and sterilized at 121°C I bar for 15 min. Aliquots (0.1ml) of each dilution were transferred in replicate into corresponding differential and selective media (in duplicates), and were spread uniformly using a hockey stick. The plates were then incubated aerobically at 37°C for 18 to 24hrs (for bacteria) and at 25°C for 72hrs (for fungi). Discrete colonies were sub-cultured into fresh agar plates aseptically to obtain pure cultures of the isolates. Pure isolates of resulting growth were then stored for further identification.

# 2.3.3. Enumeration and Isolation

From the 10-fold dilutions of the homogenates; 0.1ml of  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  dilutions of the homogenate was plated on different media, using pour plate method. The plates were then incubated at  $37^{\circ}\text{C}$  for 24 - 48 h. Mac Conkey agar was used for coliform enumeration while Mannitol salt agar was used for the isolation of *S. aureus*. Total viable aerobic bacteria count was performed on Nutrient Agar. At end of the incubation periods, colonies (30-300) that appeared on each plate for the different dilution were counted using the illuminated colony counter (Gallenkamp, England). The counts for each plate were expressed as colony forming unit of the suspension (cfu/g).

## 2.3.4. Determination of total heterotrophic count

This method was adapted from a manual of laboratory exercise for introductory and general microbiology. The plates which contained between 30 and 300 colonies after 18 to 24 hours of incubation were counted. The number of colonies was multiplied by the reciprocal of the dilution factor and the volume plated. Colony forming unit/gram = number of colony x 1/dilution x 1/volume plated.

# 2.3.5. Identification and characterization of the isolates

Colonies identifiable as discrete on the Mueller Hinton Agar were carefully examined macroscopically for cultural characteristics such as the shape, color, size and consistency. Bacterial isolates were characterized based on microscopic appearance, colonial morphology and Gram staining reactions as well as appropriate biochemical tests for example Kligler's Iron Agar (KIA) test, Indole production test, Methyl Red (MR) test, Voges-Proscauer (VP) test. Citrate utilization test, Motility Indole Urea (MIU) test, Carbohydrate fermentation test and salt tolerance test as described by Cheesbrough (2003) and Oyeleke and Manga (2008) were carried out. The isolates were identified by comparing their characteristics with those of known taxa, as described by Bergey's Manual for Determinative Bacteriology (Buchanan and Gribbons, 1974). Statistical analysis: Data were analyzed using the general linear model procedure and ANOVA.

# 3. RESULTS ANALYSIS

## 3.1. Preparation of kilishi

Table 1 shows the composition of ingredients used for kilishi slurry preparation (kg/100kg) and Figure 2 shows drying of kilishi under the sun during the kilishi production process.

Table 1: Composition of ingredients used for kilishi slurry \_preparation [kg/100kg]

| preparation [kg/100kg] |                       |                  |  |  |  |  |
|------------------------|-----------------------|------------------|--|--|--|--|
| Ingredient             | Other name            | Composition (kg) |  |  |  |  |
| Ginger                 | Zingiber officinale   | 3.80             |  |  |  |  |
| Cloves                 | Eugenia caryophyllata | 2.60             |  |  |  |  |
| Black pepper           | Piper guineense       | 3.40             |  |  |  |  |
| Red pepper             | Capsicum frutescens   | 1.90             |  |  |  |  |
| Sweet pepper           | Capsicum annuum       | 1.90             |  |  |  |  |
| Alligator pepper       | Aframomum melegueta   | 2.10             |  |  |  |  |
| Onion                  | Allium cepa           | 8.40             |  |  |  |  |
| Garlic                 | Allium sativum        | 0.10             |  |  |  |  |
| African nutmeg         | Monodora myristica    | 0.40             |  |  |  |  |
| Curry                  | Murraya koenigii      | 0.70             |  |  |  |  |
| Salt                   | Sodium chloride       | 0.70             |  |  |  |  |
| Maggi seasoning        | Monosodium            | 5.80             |  |  |  |  |
|                        | glutamate             |                  |  |  |  |  |
| Sugar                  | Sucrose               | 3.50             |  |  |  |  |
| Groundnut paste        | Aradus hypogeal       | 28.50            |  |  |  |  |
| Water                  | Hydrogen II oxide     | 36.20            |  |  |  |  |



## Figure 2: Dried Kilishi during production process

#### 3.2. Moisture content of kilishi

The moisture content of the dried sliced meat samples collected varied depending on the producer. Samples from the abattoir had a higher moisture content of 6.0% and the samples from the company had a lower moisture content of 3.0% (Table 2).

## Table 3.2: Moisture content of the kilishi samples

| Samples                  | Moisture (%) |
|--------------------------|--------------|
| Company Produced Kilishi | 6.0          |
| Locally produced Kilishi | 3.0          |

## 3.2. Bacteriological quality of kilishi sample

The microbiological quality of kilishi produced by Princess Rose Logg and the kilishi produced at the Abattoir were analysed. The total bacteria count was determined for both samples. The locally produced kilishi had the higher bacteria count of  $4.32 \times 10^3$  cfu/g while the kilishi produced by Princess Rose Logg Company had the lower bacteria count of  $2.1 \times 10^2$  cfu/g. The organism isolated from the sample was Bacteria.

Table 3.1: Microbial count of the kilishi samples

| Samples                  | Total viable count<br>(cfu/ml) | Staphylococcus<br>count (cfu/ml) | Vibrio count<br>(cfu/ml) | Total coliform<br>count (cfu/ml) |
|--------------------------|--------------------------------|----------------------------------|--------------------------|----------------------------------|
| Company produced Kilishi | $2.1 \times 10^2$              | $0.0 \ge 10^2$                   | $0.0 \ge 10^2$           | $0.0 \ge 10^2$                   |
| Locally produced Kilishi | $4.32 \mathrm{x} \ 10^3$       | $0.0 \ge 10^2$                   | $0.0 \ge 10^2$           | $0.0 \ge 10^2$                   |

# **3.3. Isolation and Identification of isolates**

There was no growth observed on the TCBS, MSA and EMB agar after 24-48 hours of incubation at 37°C. This signifies the absence of *Vibrio, Staphylococcus aureus* and *Escherichia coli* respectively from the Kilishi. The bacteria isolated included *Bacillus species* and the fungi isolates were identified as *Botryodiplodia theobromae*.

## 4. DISCUSSION

It has been reported that gram negative bacteria account for approximately 69% of the cases of bacterial food borne disease (Clarence et al., 2009). Turtura (1991) reported that the most frequently coliform identified on meat were C. freundii, E coli, En. agglomeram and less frequently strains are of the genera Klebsiella, Shigella sonnie and Proteus. E. coli and S. aureus are normal flora in human and animals, their presence in foods are indications of excessive human handling (Clarence et al., 2009). Members of the gram negative bacteria e.g. E. coli are widely distributed in the environment contaminated food and water are the major sources by which the bacteria are spread (Clarence et al., 2009). Selected strains can cause a wide variety of infections in hospitals and community setting (Donnenberg, 2005). Escherichia coli is commonly used as surrogate indicator, its presence in food generally indicate direct and indirect fecal contamination (Clarence et al., 2009). Bacterial gastrointestinal infections continue to cause illness and death and contribute to economic loss in most

parts of the world, including high-income countries that have developed surveillance and control programs (Ternhag et al., 2008).

However in Nigeria, a number of foods (meat inclusive) have been reported to have high incidence of bacteria (Nkanga and Uraih, 1981; Okonko et al., 2008a, b, c, 2009a, b, 2010; Clarence et al., 2009). But there is limited information on the health challenges from food borne diseases from fresh meat retailed within a highly populous community. It was on this basis that it became necessary and essential to give useful information about the bacterial loads in most meat sold in Calabar, Nigeria, which is an indication of this sanitary condition in such area.

The extent to which a product is contaminated by microorganism depends on the level of hygiene and sanitation of the people involved and materials used in the production chain. The degree of humidity of a food material is responsible for the initiation or inhibition of the growth of microorganisms. Traditionally dried or low moisture foods are those which contain no more than 25.0% of moisture or water activity ( $a_w$ ) of 0.0 to 0.6 (g). Bacteria require relatively high levels of moisture for growth. The moisture content and water activity attributes of the two samples of kilishi obtained from the Abattoir and Princess Rose Logg have been studied and are within the range of 6.0% to 3.0%respectively. Their levels indicate that kilishi is a very dry and stable quality product.

In this study, the minimum detection of growth of bacteria is 2.1 x  $10^2$  cfu/g which is an insignificant growth (by a standard of 2.5 x  $10^5$  cfu/g, colony forming units of 30 or less are insignificant). There has been a large debate concerning the limit for the total viable bacteria count in a meat product at the point of consumption. ICMSF (2011) put the limit between 2.5 x  $10^5$  to 1.0 x  $10^8$  cfu/g of consumable meat products. Fresh kilishi samples from the locally processed vendors yielded marked growth of bacteria  $(4.32 \times 10^3 \text{ CFU/ml})$ . The presence of these organisms on kilishi parts could be attributed to the fact that meat contains an abundance of all nutrients required for the growth of bacteria in adequate quantity. The high total viable counts recorded in this study showed the microbial diversity (differences in form or species) in these locations, condition of the production location and the hygienic practice employed by meat sellers and butchers and the kilishi processors and handlers. This determined the variation of bacterial contamination. On comparing the bacterial contamination between the Watt and Marin markets, the result obtained is on the high side. This is an indication of recontamination in food handling and hygiene techniques (Clarence et al., 2009). Similar values were reported by Yousuf et al. (2008) and Okonko et al. (2008c,d, 2009a,b, 2010).

The bacteria isolates were identified as Bacillus spp. and the fungi isolates were identified as Botryodiplodia theobromae by comparing their morphological and biochemical characteristics with standard reference organisms (Buchanan and 1974; Cheesbrough, Gribbons, 2003). Microorganisms isolated from fresh meat samples in this study have been earlier found in foods, environment and other places, and their pattern is similar to previous reports (Nkanga and Uraih, 1981; Agbeyegbe and Uraih, 1982; Enabulele and Uraih, 2009; Sobukola et al., 2009; Clarence et al., 2009; Oyeleke, 2009; Okonko et al., 2008a, b, c, d, 2009a,b, 2010). The presence of Bacillus in some of the samples can be attributed to the fact that samples may have been contaminated before production at the raw meat stage. Bacillus has been reported as the most pathogenic organism that can survive intermediate moisture meat (IMM), thermal processing and osmotic equilibration because it is aerobic together with the fact that no anaerobic have been isolated from IMM.

Though no *Vibrio*, *Staphylococcus aureus* and *Escherichia coli* were isolated the Kilishi used in this study; Agbeyegbe and Uraih (1982) reported high prevalence rate of *E. coli* in raw meat samples. Enabulele and Uraih (2009) reported *E. coli* prevalence rate to be 85.65% in a study with the fresh meat samples from abattoir and traditional open market each, recording 100% *E. coli* prevalence.

Clarence et al. (2009) and Oyeleke (2009) reported the presence of *S. aureus, E. coli, Bacillus* spp, *Enterobacter, Pseudomonas* and *Klebsiella* in meat pie and yoghurts respectively. It also disagrees with Ghaderpoori et al. (2009) who reported *Klebsiella sp., Streptococcus faecalis and P. aeruginosa* and no average indicator organism (*E. coli*) in 88% of rural water in Saqqez, Iran and Okonko et al. (2008a, b, c, d, 2009a,b, 2010), who isolated almost similar organisms from water, seafood products and seafood processors in Nigeria.

Though, Kabir (2009) did not report Streptococci, Bacilli, Micrococci and Salmonella in his study on stored meat samples from probiotics-fed broilers, higher percentage of Staphylococci, *E. coli*, Pseudomonas, and others (unidentified) were reported. Yousuf et al. (2008) reported presence of *S. auerus, Salmonella* sp., *Shigella* sp., *Flavobacterium* sp. and *Vibrio* sp. in the muscle of locally available tiger shrimp (*Penaeus monodon*) and giant water prawn from Bangladesh, Iraq while Okonko et al. (2008b) reported *E. aerogenes* and *S. aureus* in their study. In the United States, the food vehicle for 75 (41%) foodborne outbreaks was ground beef (Schroeder et al., 2005; Okonko et al., 2010).

The possible source of contamination by *Bacillus* spp. *Botryodiplodia theobromae* could be due to the unhygienic manner of handling meat from the slaughters to the markets. This also implies that these meats are viable source of various diseases. Some of these diseases could spread and acquire epidemic status which poses serious health hazards. Since improper handling and improper hygiene might lead to the contamination of fresh meats and this might eventually affects the health of the consumers (Okonko et al., 2008b,c,d, 2009a,b, 2010), it is therefore suggested that fresh meat processors and sellers should be educated on the adverse effect of contamination.

However, the processors/handlers/sellers should observe strict hygienic measures so that they may not serve as source of chance inoculation of microorganisms and fecal contamination of fresh meats and other meat products (Okonko et al., 2010). It demonstrates a potential health risk as the organism is pathogenic and causes complications in children (Taulo et al., 2008; Okonko et al., 2010).

## 5. CONCLUSION

This evaluation of the microbiological quality of ready to eat meat product kilishi shows that Kilishi from Princess Rose Logg Company is safe and acceptable for consumption. However, good precautionary measures must be taken to prevent or reduce its contamination by microorganisms, especially during the handling of the product. To achieve this only meat of good microbiological

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quality should be used for processing. During transportation of carcass and preparation of the product, measures should be taken to avoid contamination. Pieces of meat should be thin enough to ease fast drying in order to minimize the growth of external mesophilic microbial contaminants. The final product must be dried to sufficient low water content and protected by employing suitable packaging techniques.

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