

Research Article

# Identification of Pathogenic Bacteria from Kilishi Meat Sold in Kashere Municipal: Repercussions for Public Health and Food Security

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## Abstract:

Kilishi, a popular dried meat snack predominantly consumed in northern Nigeria, is often accompanied by lacking hygienic practices during its preparation, management, and storage. This study aimed to identify the pathogenic bacteria in Kilishi sold in the Kashere municipal, Gombe State, Nigeria. A total of 30 Kilishi samples were collected from three different locations: Bakin Tasha (Spot 1), Bakin Cross (Spot 2), and Bubabani (Spot 3). The samples were examined through standard microbiological methods, including bacteriological analysis, Gram staining, and biochemical tests. The results disclosed significant bacterial infection across all samples, with Spot 3 unveiling the highest coliform count of  $2.9 \times 10^{10}$  CFU/mL, followed by Spot 2 ( $2.5 \times 10^9$  CFU/mL) and Spot 1 ( $1.7 \times 10^9$  CFU/mL). Six bacterial species were recognized, with *Bacillus* species being the most predominant (28.1%), followed by *Escherichia coli* (23.4%), *Staphylococcus* species (17.2%), *Pseudomonas* species (12.5%), *Proteus* species (4.7%), and *Salmonella* species (14.1%). The presence of these pathogenic bacteria highlights a serious public health risk. The study underscores the need for improved hygienic practices during the manufacture, management, and storage of Kilishi to reduce microbial contamination and ensure user safety.

**Keywords:** Microbial contamination, Kilishi, pathogenic bacteria, Gombe State, food security

## Introduction

Meat is a known source of protein in the human diet. However, it is extremely vulnerable to microbial contamination, leading to food spoilage and foodborne illnesses, causing financial and health burdens (1). Encompassing primarily water, protein, and fat, meat is typically consumed after cooking, seasoning, or processing. While raw meat can be consumed, it is more commonly prepared in various forms to enhance flavour and prolong shelf life. However, natural meat is prone to rapid decay due to bacterial and fungal activity, leading to its putrefaction within hours or days (2). Processed meat refers to products that have undergone cures such as drying, curing, or smoking to improve flavour and extend storage life, while ready-to-eat meats, like dried, roasted, or smoked meat, are often consumed in the form they are sold (2).

One conventional meat product in Northern Nigeria is kilishi, a dried beef marinated with spices and groundnut paste (3). To make kilishi, thin slices of beef are partly dried in the sun, seasoned, and then roasted (3). However, meat's high water content characteristically with a water activity of 0.99 creates an environment favourable to microbial growth, leading to spoilage and the risk of foodborne ailments (4). Research has revealed that the quality of kilishi varies significantly among producers and even between batches from the same producer (5). Factors such as high temperatures, low humidity, inadequate water supplies, and improper handling add to microbial contamination and prompt deterioration of meat (6).

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The World Health Organization (WHO) reports that contaminated food and water trigger millions of children to suffer from diarrheal diseases annually (7). In many developing countries, including Nigeria, poor food handling practices, lack of sanitation, weak regulatory systems, and inadequate resources contribute to widespread contamination (8). In Nigeria, mostly in urban areas, meat is often stored in unsanitary conditions, with butcher shops and equipment, including knives and scales, as potential contamination sources (2;3). During the production of kilishi, meat can become contaminated from sources such as unclean utensils, contaminated water, or improper handling by food workers. Furthermore, flies and other environmental factors can introduce pathogens during the sun-drying process (9).

Meat is a usual vector for pathogens such as Salmonella, Campylobacter, E. coli, and S. aureus (4). These bacteria thrive in the favourable conditions of meat, where factors like pH, temperature, and mineral content support their growth (10). Unsuitable handling and contamination control can lead to foodborne illnesses. The FAO and WHO stress that foodborne diseases are among the most prevalent health concerns, contributing significantly to economic losses (11). Explicitly, E. coli contamination is often associated with faecal contamination in meat products, often due to poor hygiene practices during meat processing. While many E. coli strains are safe, others are pathogenic and may carry genes that bestow antimicrobial resistance, posing further health risks (11).

Known these concerns, the devotion to food safety protocols like Hazard Analysis Critical Control Points (HACCP) is vital for reducing microbial contamination in meat products and to mitigate such dangers. Therefore, the present study aims to evaluate the microbiological quality of raw meat from Kashere Abattoir and local retail meat shops in Kashere, Nigeria. This study will provide perceptiveness into the hygienic conditions of the meat consumed in the community and feature potential risks to public health.

## Materials and Methods

### *Study Design*

This study was conducted in the Kashere municipal in the Akko Local Government Area of Gombe State, Nigeria. Kashere is located in the south-western part of Gombe and consists of six cluster areas: Ungwan Tafida, Ungwan Tumburu, Ungwan Santuraki, Ungwan Wakili, Ya'adda, and Ungwan Ubadoma.

### *Materials*

The following supplies were used: normal saline, Lugol's iodine, crystal violet, safranin, immersion oil, distilled water, and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Laboratory equipment included a weighing balance, micropipettes, autoclave, incubator, microscope, wire loop, refrigerator, bijoux bottles, slides, Petri dishes, conical flasks, hot air oven, and beakers.

### *Media Preparation and Sterilization*

The following media were made according to the manufacturer's instructions: MacConkey agar, Mannitol salt agar, Nutrient agar, Salmonella-Shigella agar, Eosin Methylene Blue (EMB) agar, and peptone water.

### *Sample Collection*

50 g of dried, roasted, sliced meat samples were collected from three well-known meat vendors in the Kashere municipal. The health status of the animals used to prepare the Kilishi (dried meat) was not evaluated. Two samples were collected from each of the three sampling points. The samples were placed aseptically in sterile aluminium foil instantly after collection and transported to the laboratory for examination.

### *Isolation of Bacteria from Freshly Collected Raw Meat*

#### **Serial Dilution**

A 10g portion of each sample was blended and mixed with 100 mL of peptone water. The mixture was homogenized and pre-incubated for 1 hour. Serial dilution was performed using a ten-fold dilution method (12). A 1mL aliquot of the pre-incubated sample was

transferred into 9mL distilled water to achieve a  $10^{-1}$  dilution. Further serial dilutions were performed to achieve dilutions up to  $10^{10}$ .

### **Inoculation and Isolation**

The 6th and 8th dilutions were selected for inoculation from the serially weak samples. One millilitre of each dilution was inoculated onto freshly prepared agar plates, including Nutrient Agar (NA), MacConkey Agar (MCA), Mannitol Salt Agar (MSA), Salmonella-Shigella Agar, and Eosin Methylene Blue (EMB) Agar, using the pour plate technique. The inoculated plates were incubated at  $37^{\circ}\text{C}$  for 18 to 24 hours. A loopful of the first diluted sample was streaked onto MCA and MSA, as described by (12).

The colony-forming units (CFU) per millilitre were calculated using the formula:

$$CFU/mL = \frac{\text{Number of colonies}}{\text{Volume plated}} \times \text{Dilution factor}$$

All obvious colonies from each agar plate were subculture onto their particular media to obtain pure cultures for identification.

### **Identification of Bacterial Isolates**

Pure bacterial cultures were conserved on agar slants for further identification.

### **Microscopic Examination Based on Cultural Characteristics**

The morphological features were observed and recorded, including shape, texture, pigmentation, margin, and elevation of the bacterial colonies on culture plates.

### **Microscopic Examination Based on Gram Staining**

Gram staining was achieved following the method described by (12). A colony of bacteria was emulsified in a drop of sterilized distilled water on a clean slide, air-dried, and heat-fixed. The smear was stained with crystal violet (primary stain) for 30-60 seconds, followed by iodine (mordant) for 30 seconds. The slide was washed with tap water, decolourized with 70% ethanol for 30 seconds, and then counterstained with safranin for 30 seconds to 1 minute. After rinsing with tap water, the slide was air-dried and examined under a microscope using oil immersion at 100x magnification. Gram-positive bacteria seemed purple or blue, while Gram-negative bacteria appeared red or pink.

### **Biochemical Tests**

Biochemical tests were executed further to characterize the bacterial isolates, including the following:

#### **Catalase Test**

The catalase test was executed to detect the production of the catalase enzyme, which decomposes hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) to water and oxygen. A loopful of the bacterial isolate was placed on a sterile slide, and 3% hydrogen peroxide was added. Effervescence indicated a positive result, while no effervescence indicated a negative result (12).

#### **Motility Test**

A drop of normal saline was dropped on a clean glass slide to assess bacterial motility. A colony of the test organism was combined in the saline and covered with a coverslip. The slide was observed under the microscope using 10x and 40x objectives. Movement in numerous directions indicated motility (12).

### Indole Test

The indole test was executed to reveal the ability of the bacteria to generate indole from tryptophan. A pure bacterial culture was inoculated into 5mL of peptone water and incubated at 37°C for 24 hours. A drop of Kovac's reagent was added, and a red ring shown a positive result (12).

### Coagulase Test

A drop of distilled water was dropped on two clean slides to reveal coagulase activity. A bacterial colony was emulsified in the water on one slide, and a loopful of plasma was added to one suspension. Clumping within 10 seconds indicated a positive coagulase test (12).

### Oxidase Test

The oxidase test was executed to detect the production of the cytochrome oxidase enzyme. A piece of filter paper was placed in a Petri dish, and 2-3 drops of fresh oxidase reagent were added. A loopful of the bacterial colony was smeared onto the paper. A positive result was indicated by developing a blue-purple colour within a few seconds (12).

### Citrate Test

The citrate utilization test was executed by inoculating a bacterial isolate into Simon's citrate agar slant and incubating for 24-72 hours. A change to a deep blue colour suggested a positive result (12).

### Urease Test

The urease test was executed by inoculating a bacterial isolate into a urea agar slant and incubating for 24-72 hours. A positive result was suggested by the development of a bright pink or red colour (12).

## Results

The result disclosed that pathogenic and non-pathogenic microbes inhabited the ready-to-eat meat. Microorganisms such as bacteria include *Staphylococcus species*, *Pseudomonas species*, *salmonella species*, *Bacillus species* and *Protens species*. Tables 1-4 show the total coliform count of samples of kilishi meat gathered from three different Kashere market spots.

**Table 1: The result of colony count and CFU/ml of serial dilution of 10 samples of handled meat (Kilishi) collected from Spot 1 cultured on nutrient agar. Kilishi Meat Sold at spot 1 (Bakin Tasha).**

Sample	CFU/ml counted ( $10^{-8}$ )	Colony
1	$7.0 \times 10^8$	7
2	$1.4 \times 10^9$	14
3	$2.0 \times 10^9$	20

4	1.9x10 <sup>10</sup>	19
5	2.9 x10 <sup>9</sup>	29
6	3.7 x10 <sup>9</sup>	37
7	5.2 x10 <sup>9</sup>	52
8	6.0x10 <sup>8</sup>	6
9	2.2 x10 <sup>9</sup>	22
10	3.0 x10 <sup>9</sup>	30
<b>OM= 21.60</b>		<b>SD=</b>
<b>17.00</b>		

**Key: OM= Overall Mean, SD= Standard Deviation**

**Table 2: The result of colony count and CFU/ml of serial dilution of 10 samples of handled meat (Kilishi) collected from Spot 2 cultured on nutrient agar. Kilishi Meat Sold at spot 2 (Bakin cross).**

Sample	Colony	CFU/ml counted (10 <sup>-8</sup> )
1	44	4.4 x10 <sup>9</sup>
2	19	1.9 x10 <sup>9</sup>
3	50	5.0 x10 <sup>9</sup>
4	15	1.5 x10 <sup>9</sup>
5	25	2.5 x10 <sup>9</sup>
6	45	4.5 x10 <sup>9</sup>
7	90	9.0 x10 <sup>9</sup>
8	32	3.2 x10 <sup>9</sup>
9	30	3.0 x10 <sup>9</sup>
10	10	1.0 x10 <sup>9</sup>
<b>OM= 36</b>		<b>SD= 25.33</b>

**Key: OM= Overall Mean, SD= Standard Deviation**

**Table 3: The result of colony count and CFU/ml of serial dilution of 10 samples of handled meat (Kilishi) collected from Spot 3 cultured on nutrient agar. Kilishi Meat Sold at spot 3 (Bubabani).**

Sample	Colony	CFU/ml counted ( $10^{-8}$ )
1	11	$1.1 \times 10^9$
2	58	$5.8 \times 10^9$
3	25	$2.5 \times 10^9$
4	100	$1.0 \times 10^{10}$
5	20	$2.0 \times 10^9$
6	65	$6.5 \times 10^9$
7	16	$1.6 \times 10^9$
8	75	$7.5 \times 10^9$
9	85	$8.5 \times 10^9$
10	35	$3.5 \times 10^9$
	<b>OM= 50</b>	<b>SD= 29.64</b>

**Key: OM= Overall Mean, SD= Standard Deviation**

Table 4 discovered that after morphological, physiological and biochemical tests of the bacterial isolates carried out and recorded, six genera of bacterial which include *Escherichia coli*, *Staphylococcus species*, *Bacillus species*, *Proteus species* and *Pseudomonas species*, were isolated from kilishi meat sold in Kashere.

**Table 4: Morphological and biochemical characteristics of bacterial isolates.**

Characteristics	<i>E.coli</i>	<i>Salmonella spp</i>	<i>Bacillus spp</i>	<i>Proteus spp</i>	<i>Pseudomonas Spp</i>	<i>staphylococcus spp</i>
Gram reaction	-	-	+	-	-	+
Motility	+	+	+	+	+	-
Shape	Rod	Rod	Rod	Rod	Rod	Cocci
Colour	Greenish metallic	Red & black centre	Milky	Colorless	Florescent green	Golden yellow
Margin	Entire	Entire	Entire	Entire	Entire	Entire
Elevation	Raised	Raised	Raised	Raised	Raised	Raised

Catalase	+	+	+	-	+	+
Coagulase	-	-	-	-	-	+
Indole	+	+	+	+	-	-
Oxidase	-	-	-	-	+	-
Citrate	-	+	+	+	+	+
Urease	-	-	-	+	-	+

**KEY:** + = Presence, - = Absence

**Table 5. Frequency and percentage of occurrence of bacteria isolated from Kilishi Meat.**

Sample	Bakin	Bakin	Bubabani	Frequency of Occurrence	Percentage of Occurrence (%)
	Tasha	cross			
<i>Bacillus spp</i>	4	6	8	18	28.13
<i>Escherichia coli</i>	2	5	8	15	23.44
<i>Staphylococcus spp</i>	2	4	5	11	17.19
<i>Salmonella spp</i>	0	2	7	9	14.06
<i>Pseudomonas spp</i>	0	3	5	8	12.50
<i>Proteus spp</i>	0	1	2	3	4.69
<b>Total</b>	<b>10</b>	<b>21</b>	<b>35</b>	<b>64</b>	<b>100</b>

## Discussion

In this study, the results discovered that all kilishi samples analyzed covered at least one bacterial species, suggesting that these samples could be probable sources of foodborne diseases. This surveillance aligns with a study by Jabaka et al. who found risky bacterial species in all samples from the Kashere market (13). Numerous factors could contribute to this high level of contamination, including poor meat quality, improper handling, and lack of formal education among the vendors. In addition, the unhygienic practice of using sick animals for kilishi production due to their lower cost may further exacerbate the contamination risk. The role of improper handling and acquaintance to environmental pollutants during meat processing has also been noted by (3), who emphasized that unsanitary practices during production and retail could surge bacterial contamination.

The bacterial contamination levels were highest in the Bubabani location (Spot 3), which had a coliform count of  $2.9 \times 10^{10}$  cfu/ml, followed by Bakin Cross (Spot 2) with  $2.5 \times 10^{10}$  cfu/ml. In contrast, Bakin Tasha (Spot 1) showed the lowest contamination, with a  $1.7 \times 10^{10}$  cfu/ml count. This pattern may be ascribed to improper hygienic practices at Bubabani, which led to the utmost contamination levels. The meat quality, coupled with the handling methods, likely contributed to the increased bacterial load in this area. This result is consistent with

findings by, (13), who also identified contamination of ready-to-eat beef products, such as kilishi, in different regions.

The bacterial species found in the kilishi samples included *Bacillus* species (28.1%), *Escherichia coli* (23.4%), and *Staphylococcus* species (17.2%). Notably, *Pseudomonas* species (12.5%), *Proteus* species (4.7%), and *Salmonella* species (14.1%) were absent in Spot 1 but present in Spots 2 and 3. These results align with previous research by (14), who isolated *Bacillus* species from meat samples, stressing their ability to survive harsh environmental conditions due to their spore-forming capabilities. However, *Staphylococcus aureus* and *Salmonella* species, as identified by (15), have also been related to kilishi contamination in other studies. *Escherichia coli*, a faecal contaminant, reveals potential contamination during processing or at the retail point (14).

*Staphylococcus* species, which are normal human skin flora, likely entered the kilishi samples through improper handling by vendors. *Salmonella* and *Proteus* species in the samples from Spot 2 and Spot 3 could indicate faecal contamination from the animals or environmental sources during processing. Studies note that *Proteus* species can be introduced through soil, dust, or contaminated water, further supporting the role of unhygienic processing in contamination (16).

The occurrence of *Pseudomonas* species, which are often linked with food spoilage, may suggest post-production contamination, mainly as these bacteria cannot survive the high temperatures typically intricate in the roasting and drying processes of kilishi (5). The potential for contamination in the study area's kilishi samples was further confirmed by (14), who emphasized that microbial growth in meat, mainly from *Pseudomonas* species, indicates spoilage.

Furthermore, the microbial load in the kilishi samples exceeded the microbiological safety standards suggested for meat products. According to set standards, the microbiological standard for meat should not exceed  $1 \times 10^2$  cfu/ml for coliforms, with any value above this indicating a conceivable health risk (17). Similarly, various international food safety guidelines consider counts between  $1 \times 10^6$  and  $1 \times 10^7$  cfu/ml acceptable, while levels exceeding  $10^6$  cfu/ml are considered unsatisfactory (18). The results of this study, with coliform counts ranging from  $1.7 \times 10^{10}$  to  $2.9 \times 10^{10}$  cfu/ml, far outstrip these standards, underscoring the need for enhanced meat handling and hygiene practices in the kilishi production process.

In contrast to other studies, such as those by Daminabo and Agarry (19), Adabara et al. (20), and Odey et al. (21), which found lower levels of bacterial contamination in kilishi samples from different regions, this study suggests that microbial contamination in kilishi may vary reliant on local practices and environmental conditions. Variations in handling, storage, processing methods, and environmental factors such as temperature and humidity could explain these differences (22).

Inclusively, the findings of this study indicate that kilishi samples in the study area are extremely contaminated with pathogenic bacteria, posing a significant food safety risk. The contamination is likely due to a combination of unsanitary handling practices, poor meat quality, and environmental factors, consistent with the findings of previous research on microbial contamination in meat products. More rigorous hygiene practices and better vendor education on safe meat handling are essential to reduce bacterial contamination and protect public health.

## Conclusion

This study showed that Kilishi sold in the Kashere market is significantly contaminated, with all the samples confirmed showing evidence of bacterial contamination. This highlights the urgent need to improve the sanitary conditions surrounding these meat products' production, packaging, and handling. The presence of these harmful contaminants postures health risks not only to indigenous consumers but also to tourists who may consume the meat. It is crucial to address these microbial hazards to safeguard public health by implementing and adhering to proper sanitary practices and food safety standards. The findings stress the need

for intervention measures, including public health awareness campaigns and the strict enforcement of regulatory policies concerning the sale of food products.

## Recommendations

To ensure the well-being of meat products, veterinary and public health authorities should proactively monitor the quality of meat being slaughtered and retain stringent oversight of municipal abattoir environments. Public health movements should also be implemented to raise consciousness about the health risks of consuming contaminated foods, particularly meat, and the harmful pathogens they may harbour. It is essential to firmly adhere to Hazard Analysis Critical Control Point (HACCP) food safety guidelines to prevent foodborne illnesses and reduce microbial contamination in both raw and processed meats. Additionally, meat scrutiny should not be limited to urban areas; regulatory authorities must lengthen their inspection efforts to rural regions to ensure that all meat products meet safety criteria.

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