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ASSESSMENT OF BACTERIOLOGICAL QUALITY OF *KILISHI* (READY-TO-EAT MEAT PRODUCT) SOLD IN GOMBE, NIGERIA

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ABSTRACT

Kilishi is a highly relished ready-to-eat meat product processed and sold in most parts of Nigeria. Its consumption cut across diverse cultures, classes and socio-economic status, though it originated and popular in the northern part of the country. However, the common unhygienic method of processing *Kilishi* depicted by majority of producers has widely queried its microbiological quality and safety for public consumption which prompted this study. This study was aimed at assessing the bacteriological quality of randomly sampled Fifteen (15) *Kilishi* and its spice mix sold in five (5) different locations within Gombe metropolis. *Kilishi* samples were subjected to standard microbiological analyses that include serial dilution, viable plate count, culturing on selective and differential media then morphological identification and biochemical-based characterization of bacterial isolates. Findings revealed higher bacterial count (aerobic mesophilic bacteria) exceeding the minimum allowable limit for a ready-to-eat products thus indicating an unacceptable state of the meat products. Also, coupled with the identification and confirmation of ten (10) diverse bacteria including potentially pathogenic strains (*Salmonella typhi, Shigella* spp, *Staphylococcus aureus, and Pseudomonas aeruginosa, Bacillus cereus*), indicators of faecal contamination (*Escherichia coli, Klebsiella pneumoniae, Citrobacter freundii*) and others. Put together, these showed the low bacteriological quality of *Kilishi* sold in the outlets studied. Therefore, it is highly recommended that best hygiene measures, good sanitation practices and standard food handling rules be strictly adhered to during processing and post-production stages including packaging. This is paramount to guaranty food safety, prevent any outbreak of food-borne diseases and to importantly safeguard public health.

Keywords: *Kilishi*, Bacteria, Plate count, Bacteriological quality, Colony forming unit.

INTRODUCTION

Globally, meat and meat products are essential part of human diet relied upon for the provision of vital nutritional components of balanced diet, specifically as a chief source of protein, fat, minerals, and fat-soluble vitamins (Mgbemere *et al.,* 2011; Igene and Mohammed, 1993). This is aside from its diverse ways it is relished by vast cultures and people in different menu - these and other details have highly portrayed the enormous value of meat. Adams and Moss (1999) defined meat as an edible animal flesh that comprises mostly of the muscular tissue, also includes internal organs such as heart, liver, kidney, intestine and bladder; while Okala and Reedi (2001) added that

bulk of meat is derived from goat, cattle, pig, sheep, and poultry. The high demand for meat has basically led to the production of meat products which was anciently discovered due to lack of meat storage facilities, leading to various preservation methods that could prolong the shelf life of meat. Jones *et al,* (2001) buttressed that the history of meat preservation in West Africa dates back to the records of the 12th century. Explicitly, meat products derived from meat are subjected to combination of several basic processing steps before reaching their final forms. Moshood *et al.,* (2012) stated that meat products are obtained when raw meat is altered in form by grinding, pressing, drying and other processes then augmented in flavour by smoking, spicing or blending with other food components. Different types of meat products exist, ranging from the industrially processed ones such as corned beef, ham, bacon sausage to the traditionally processed ready-to-eat meat products indigenous to Nigeria such as *Kilishi, Dan-bu-nama, Suya, Balangu, Tsire, Jirga, Banda, Ndako,* and others (Yunusa, 2000). Other meat products are continental thus peculiar to certain culture, nation and continent as seen with Beef kebab (Europe), Kyiskiyma (Central Africa), and the Sogo djemine which is peculiar to Mali (Moshood *et al.,* 2012).

Kilishi, a well-known Nigerian and Sub-Saharan African ready-to-eat meat product from lean beef infused with spices and defatted groundnut paste traditionally processed mostly by sun-drying and on few occasions by heat-smoking for an entire expulsion of moisture (Abubakar *et al*., 2011). Equally, prepared by partially drying thin sheets of beef in the sun followed by the addition of ingredients before a second trend of sun-drying and partial roasting (Igene *et al*., 1990). This *Kilishi* meat product has a very long shelf life thus can be preserved in a dry place at room temperature without deteriorating due to its highly dry physical nature attributed to its unique processing techniques. It was reported to contains about 46 % meat and 54 % non-meat ingredients, additionally, the nutritional constituents could be around 50 % protein, 7.5 % moisture, 18 % lipid and 9.8 % fiber or ash content (Igene and Mohammed, 1993). Alonge and Hiko (1981) reported that sun-drying was the primary method applied for the preservation of meat by the medieval Arabic sources and later transferred to West Africa. As a result, this metamorphosed to *Kilishi* production in Nigeria many years ago as a way of preserving meat in the absence of refrigeration by the early Fulani and Hausa herdsmen in northern Nigerian states (Borno, Kano, Sokoto, Kaduna, Gombe and Bauchi). Today, *Kilishi* has gained vast popularity nationwide mostly sold in major cities and rural settings predominantly in the northern Nigeria where it is vended regularly in the streets, motor parks, hotels, recreational spots, party joints, supermarkets and other public places. Consequently, it has become one of the traditional Nigerian meat products to attain such status (Igene *et al.,* 1990).

Advanced knowledge in food safety have caused food experts to raise questions and concern regarding the microbiological quality status of *Kilishi* vended for public consumption. This concern is particularly drawn from insinuations bothered on the poor and unhygienic processing of *Kilishi* which predispose the meat product to insects-carrying germs and dust containing microbial spores (Raji, 2006), post-processing poor handling of the meat product (Moshood *et al.,* 2012), health condition of slaughtered animal (Whyte *et al*., 2004), widespread distribution of *Kilishi*

which makes the consequences of contamination with food poisoning microbes more serious (Macrae *et al.,* 1993). Accordingly, the implication of consuming microbial contaminated *Kilishi* is the potential outbreak of food-borne diseases that could adversely affects general public health. Therefore, this study aimed at assessing the bacteriological quality of *Kilishi* sold within Gombe metropolis - this is in a bid to anticipate the microbial hazards associated to the consumption of the ready-to-eat meat product.

MATERIALS AND METHODS

Study Area

The study areas where samples were collected for microbiological analyses include Bolari, Gombe Main Market, Jekadafari, Pantami and area nicknamed British Cotton Growers Association (BCGA). These were the selected five (5) different *Kilishi* vending outlets where Kilishi were purchased in Gombe Local Government Area of Gombe State, Nigeria.

Collection of *Kilishi* Samples

Triplicates of *Kilishi* samples were randomly purchased from five (5) different selling outlets within Gombe metropolis. These *Kilishi* samples wrapped in newsprint or cement paper were aseptically collected into a new and sterile (unused) pre-labelled sample bags then immediately transported to the laboratory in a sterile and dry container for microbiological analysis. Also, triplicates of the spice ix for the processing of *Kilishi* were collected from the entire five (5) outlets sampled. This implies that a total number of Fifteen (15) *Kilishi* samples and Fifteen (15) spices were sampled for this study.

Bacteriological Analysis of *Kilishi*

Sample Processing, Stock Preparation and Serial Dilution

The *Kilishi* samples were initially processed separately by grinding using a mini laboratory blender to obtain a homogenized granulated *Kilishi* samples. As applied by Moshood *et al.,* (2011), one gram was taken from the grinded meat product and transferred into a sterile test tube containing 10 mL of distilled water then thoroughly vortexed to obtain a stock homogenate for serial dilution protocol. Ten-fold serial dilution was carried out up to 10^{-6} dilutions by taking 1 mL from the prepared stock homogenate then dispensed into 9 mL distilled water for the first dilution (10^{-1}). Afterwards, dilution was continued until 10^{-6} dilutions were reached according to the standard serial dilution protocol in order to obtain discrete colonies. Similarly, stock solution was prepared for the spice condiment mix then preceded with ten-fold serial dilution up to 10^{-6} dilution.

Plating on Solid Culture Media

Culturing of microbes on different purpose solid culture media such as Nutrient agar (NA), Plate count agar (PCA) Mannitol salt agar (MSA), Eosine methylene blue (EMB) and *Salmonella Shigella* agar (SSA) was achieved using pour plating method. One mL inoculum was taken from 10^{-1} to 10^{-6} dilutions and dispensed into an empty Petri dishes prior to subsequent addition of ~20 mL of prepared culture media cooled to 45 °C, then gently swirled to mix uniformly. The inoculated plates were allowed to set firmly for at least 10 minutes then inverted for incubation at 37 ºC for 24 to 48 hours. Plating on NA and PCA was intended for the enumeration of viable cells (bacterial plate count) while plating on other selective and differential media was for easy and

tentative identification of bacterial isolates based on their differential cultural features.

Determination of Viable Plate Count

Discrete colonies obtained in plates for viable plate counts (NA and PCA) were counted using colony counter where only counts within the range of 30-300 were considered valid from several plates incubated. Result of the viable bacterial plate counts regarded as the total aerobic mesophilic bacterial counts were established via calculation according to surface count method by Miles and Misra, (1938), and results expressed in colony forming unit per gram (CFU/g).

Identification and Confirmation of Bacterial Isolates

Macroscopy for colonial morphology and cultural characteristics that includes colour, shapes, elevation, margin and pigment formation on the colonies was achieved especially on selective and differential culture plates for the initial identification of bacteria isolated from the *Kilishi* samples based on experimental microbiology manual by Aneja, (2003). In furtherance to the identification of bacterial isolates, gram staining reaction and microscopy was carried out according to standard microbiological protocols described by Cheesbrough, (2006) to reveal cell's gram reaction and for proper microscopic observation of bacteria cell morphology.

Biochemical-based Characterization of Bacterial Isolates

After the macroscopic and microscopic examination of bacterial isolates, further biochemical characterization of bacteria isolated was done via biochemical a test that includes Catalase, Coagulase, Indole, Citrate Utilization, Motility and Urease tests for genuine confirmation of the various bacteria isolated from the Kilishi samples. A protocol described by Cheesbourgh (2005) was used for all the biochemical tests mentioned in this study. Biochemical test outcomes were checked in Cowan and Steel's manual for the identification of medical bacteria (Barrow and Feltham, 1993) for the final validation of bacterial isolates' identity.

RESULTS

The bacteriological assessment of *Kilishi* samples and its spice mix purchased from five (5) different retail locations within Gombe metropolis produced several results that include the mean of viable bacterial plate count (mostly the aerobic mesophilic bacteria) on PCA and NA for Fifteen (15) *Kilishi* samples analyzed, the mean viable bacterial plate count (on PCA) for the Fifteen (15) spice mix, the biochemical characteristics of bacteria isolated from all the *Kilishi* samples, and the occurrences of bacterial isolates in *Kilishi* across all the five (5) vending outlets.

Table 1 shows mean of total aerobic mesophilic bacterial plate count (TAMBPC) on PCA and NA for all the *Kilishi* samples collected from the five (5) outlets, where samples from Gombe main market produced the highest bacterial plate count of 4.08 x 10^4 CFU/g and 3.36 x 10^4 CFU/g on PCA and NA respectively. This was followed by Jekadefari with viable count of 3.69 x 10^4 CFU/g on PCA and 3.35 x 10^4 CFU/g on NA. While the *Kilishi* samples from Pantami produced the lowest bacterial count of 1.75 x 10^4 CFU/g and 1.03 x 10^4 CFU/g on PCA and NA respectively.

Table 1: Mean of Total Aerobic Mesophilic Bacteria Plate Count (TAMBPC) (CFU/g) of Kilishi sampled from five (5) different locations within Gombe metropolis.

Sample location	Viable plate counts on PCA	Viable plate counts on NA
Pantami	1.75×10^4	1.03×10^4
BCGA	2.42×10^4	1.72×10^4
Bolari	2.78×10^4	1.43×10^4
Jekadefari	3.69×10^4	3.35×10^4
Gombe Main Market	4.08×10^4	3.36×10^4

PCA= Plate count agar, NA= Nutrient agar, CFU/g = Colony Forming Unit per gram.

Table 2 shows the mean of TAMBPC on PCA for (15) spice mix samples from the whole five (5) outlets. The values obtained are 1.23×10^4, 1.52×10^4, 1.65×10^4, 1.70×10^4, 1.85×10^4 in CFU/g for Bolari, BCGA, Pantami, Jekadafari, and Gombe Main Market retail outlets respectively.

Table 2: Mean of Total Aerobic Mesophilic Bacteria Plate Count (TAMBPC) (CFU/g) of spices sampled along with *Kilishi* from five (5) different locations within Gombe metropolis.

Sample location	Viable plate counts on PCA
Bolari	1.23×10^4
BCGA	1.52×10^4
Pantami	1.65×10^4
Jekadafari	1.70×10^4
Gombe Main Market	1.85×10^4

PCA= Plate count agar, CFU/g = Colony Forming Unit per gram.

Table 3 highlight on the various morphological features and biochemical characteristics of Ten (10) bacteria genera/species isolated, identified and confirmed from the all the *Kilishi* samples analysed in this study. These bacteria isolates include *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Citrobacter freundii, Klebsiella pneumonia, Salmonella typhi, Pseudomonas aeruginosa, Enterococcus faecalis, Proteus mirabilis,* and *Shigella* spp. Table 4 shows the occurrences of each bacterium in *Kilishi* samples with respect to the entire five (5) different locations where these samples were collected.

Table 3: Microscopic morphology and biochemical characteristics of bacteria isolated from *Kilishi* (ready-to-eat meat product) sold within Gombe metropolis.

Bacteria	Biochemical tests										
	GR	Morp.	Ur	Cat	Co	Cit	Mot	Ind	MR	Ox	H_2S
E. coli	-	srs	-	+	-	-	d+	+	+	-	-
S. aureus	+	cc	-	+	+	-	-	-	+	-	-
Enterococcus faecalis	+	*cpsc*	-	-	nd	-	-	-	-	-	-
Proteus mirabilis	-	*rps*	+	+	nd	+	+	-	+	-	+
Salmonella typhi	-	srs	-	+	nd	-	+	-	+	-	d+
Shigella spp.	-	srs	-	+	nd	-	-	d+	+	-	-
Pseudomonas aeruginosa	-	srs	d+	+	-	+	+	-	-	+	-
Klebsiella pneumoniae	-	*lrs*	+	+	-	+	-	-	+	-	-
Bacillus cereus	+	*srs*	d+	+	nd	d+	+	-	-	d+	nd
Citrobacter freundii	-	*rps*	d+	+	nd	+	+	d+	+	-	+

Table 3 shows the microscopic morphology and biochemical tests results that confirm bacteria isolated from *Kilishi* samples sold in five (5) different locations within Gombe metropolis. Key: Positive sign + signifies positive reaction, d+ signifies 16-84% strains are positive (mostly positive), Negative sign - signifies negative reaction, nd = Not determined, Ur = urease test, Cat = catalase, Co = coagulase test, Cit = Simmons' citrate test, Mot = motility test, Ind = indole test, MR = Methyl red test, Ox = Oxidase test, Morp: microscopic morphology represented as cc = cocci in cluster, cpsc = cocci in pairs and short chain, srs = short rods in singles, rps = rods in pairs and singles, lrs = long rods in singles. Biochemical test results were checked with Cowan and Steel's manual for the identification of medical bacteria (Barrow and Feltham,1993) for convincing confirmation of bacterial isolates.

Table 4: Occurrences of bacteria isolates in *Kilishi* (ready-to-eat meat product) across five (5) locations within Gombe metropolis.

Bacteria	Frequency and percentage of occurrence across locations						
	BCGA	Bolari	Jekadefari	Main market	Pantami	Total	Perc (%)
E. coli	3	3	3	3	3	15	13.6
S. aureus	3	3	3	3	3	15	13.6
B. cereus	2	2	3	3	3	13	11.8
E. faecalis	2	3	2	3	2	12	10.9
S. typhi	3	0	2	3	3	11	10.0
K. pneumoniae	3	3	1	3	1	11	10.0
C. freundii	0	2	3	3	3	11	10.0
P. aeruginosa	0	0	3	3	2	8	7.30
P. mirabilis	0	3	0	2	2	7	6.40
Shigella spp.	3	0	1	2	1	7	6.40
Total						**110**	**100**

Perc = percentage. Occurrence bacterial isolates checked in plate based on triplicate analysis.

DISCUSSION

In table 1, the bacterial counts of *Kilishi* samples from five (5) different locations in Gombe is considerably high depicting high load of aerobic mesophilic bacteria in the *Kilishi* samples analysed. In considering one of the standard thresholds, the International Commission on Microbiological Specifications for Foods (ICMSF) (Cary, 1996) reported limits in the order of $\leq 10^3$ for total aerobic bacterial and fungal counts for any ready-to-eat foods to be acceptable – this implies that bacterial count beyond this stipulated limit could be termed unacceptable. As expected, finding from this study (Table 1) revealed bacterial counts for *Kilishi* sampled from the selected outlets exceeded allowable limit set by ICMSF standard for a ready-to-eat food thus regarded unacceptable and unsafe for consumption. However, it must be acknowledged that standard for ready-to-eat food could subjectively vary hence inference in some cases depend on the adopted acceptable food regulatory policies and bodies.

Even though *Kilishi* is a ready-to-eat meat product that should be devoid of high bacterial load due to the heat treatment (sun-drying and smoke-heating) applied during processing which is required to kill vegetative cells and reduce the bacteria load. Contrarily, relatively high bacteria counts were obtained in spite of the heat treatment processing and this solely suggest conceivable post-production contamination. Consequent to the objectionable bacterial load, there could be health complications specifically the food-borne diseases arising from the consumption of these highly contaminated *Kilishi* samples. It should be clarified that several microbial spores could escape the processing of *Kilishi*, besides; other post-processing contamination such as unhygienic handling of the meat product could inflate the bacterial counts of the meat product beyond unobjectionable limit. Generally, the overall bacterial load could arise from combined factors that include contamination of meat from slaughter house, during processing or production, and post-processing contamination such as handling and packaging of the meat product (Moshood *et al.,* 2012).

The high bacterial count for *Kilishi* recorded in this study is in accordance with several studies (e.g., Raji, 2006; Dahiru and Maigari, 2019) that also reported high bacterial count and declared the *Kilishi* sample having low bacteriological quality. Nevertheless, other studies have reported better quality of *Kilishi* sold in Abuja (Daminabo *et al*. (2013), Port Harcourt (Okonko *et al*., 2013) and Calabar (Odey *et al*., 2013) than *Kilishi* sold in Kano (Dahiru and Maigari, 2019). Therefore, it could be reasoned that regional factor and socio-economic class of people where the *Kilishi* is produced plays a key role in the quality of *Kilishi* produced. It may imply that good hygiene practices during production and packaging is much likely adhered to in advanced cities than other northern states with trailing socio-economic status. To support this, Abdullahi *et al*. (2016) elucidated differences in processing methods, ingredients used,meat handling practices, and variation in environmental factors may impacton the microbial count of meat products.

In a bid to trace possible contamination via spice mix applied during the processing of *Kilishi*, the bacterial plate count of the spice mix used in the production of *Kilishi* collected from the selected five (5) locations were examined. Interestingly, the spice mix produced bacterial counts in the order of 10^4 (Table 2) though much less than *Kilishi* counts (Table 1) which suggest it could add

up to the contamination of *Kilishi* during processing. This finding corroborates with Frazier and Westthroff, (2006), who stated that spices may serve as source of contamination of processed products thus, may add high number and undesirable kind of microorganisms to food. Also, spice ingredients are thought to have some antimicrobial activities, yet spiced meat products have significant microbial load and contain bacteria such as *E. coli*, *Salmonella* species, *Staphylococci* and *Clostridium perfringens* (Shamsuddeen, 2009).On other hand, contrary studies (e.g., Inusa and Sa'id, 2017; Fonkem *et al*., 2010) reported that spice might help to inhibit the growth and multiplication of some microbes.

Highest bacterial count in Gombe Main market and high counts in other public vending outlets (Table 1) could be attributed to the environmental factor peculiar to these locations. For instance, the Gombe market is an open market place with dusty air and unclean environmental condition which could meaningfully influence contamination by airborne microbial spores and infestation of insects carrying germs such as flies as attested by Monica and Chessbrough (2000).

This study revealed Ten (10) bacteria genera/species isolated from all the *Kilishi* samples bacteriologically examined. These include *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Citrobacter freundii, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Enterococcus faecalis, Proteus mirabilis,* and *Shigella spp*. However, it must be acknowledged that their percentage of occurrences in *Kilishi* across the five locations differs (Table 4). It is worthy to state that presence of these bacteria (Table 3) in *Kilishi* signifies different implication in terms of the sources of bacterial contamination. The diverse bacteria identified corroborate with other work, as similar bacteria have been reportedly isolated from *Kilishi* in other part of the country. Among several studies is the work of Odey *et al*., (2013) who also isolated *S. aureus*, *E. coli*, *Streptococcus spp,Salmonella spp*, *Bacillus spp*, *Pseudomonas spp*and *Proteus spp* from *Kilishi* samples sold in Calabar, Cross RiversState, Nigeria.To elaborate on route of contamination peculiar to these bacteria isolated, the presence of Coliform (*E. coli*, *Klebsiella pneumoniae* and *Citrobacter freundii*) and *Enterococcus faecalis* in the *Kilishi* often signifies contamination from fecal and non-faecal sources like soil origin. The implication of coliform in food indicates the presence of pathogenic *Enterobacteriaceae* in such food by direct and indirect fecal contamination (Clarence *et al.,* 2009; Ibrahim *et al.,* 2020). This is justified by the presence of pathogenic bacterial such as *S. typhi,S. aureus,P. aeruginosa,* and *Shigella spp* and others in the *Kilishi* samples analyzed though the contamination route may vary.

S. typhi and *Shigella spp* causes food borne illness such as typhoid fever and Shigellosis respectively and are both from enteric sources thus its presence is expected at post-processing stage due to poor and unhygienic handling and packaging of meat product since they can be destroyed by heat during processing. Moshood *et al.,* (2012) added that post-processing contamination could arise from unhygienic packaging such as the used old newsprint and cement papers for packaging. The presence of *S. aureus* is also attributed to poor hygiene and improper handling of *Kilishi* after production as Postgate, (2000) reported that *S. aureus* is a normal flora of the skin of man and can be transmitted from person to product through unhygienic practices. Its presence presence could be from nose, hands, skin and clothing

of filthy food handlers, cooking utensils or processing equipment. (Clarence *et al.,* 2009; Moshood *et al.,* 2012).As an evidence of microbial hazard in food, Rasooly *et al*., (1997) reported that *S. aureus* enterotoxin A (SEA) is a leading cause of food intoxication.

Bacillus cereus is an important bacterial contaminant that could escape heat destruction during processing because they are spore formers, this spore could germinate in *Kilishi* at favorable condition. In addition to escaping the processing stage, they could still contaminate *Kilishi* at the post-production stage as aerial spores during sales in some environment such as motor parks and market. Okwori *et al.,* (2007) attributed the highest occurrence of *Bacillus* species to environmental contamination during processing, handling, storage and packaging of the product.*P. aeruginosa* in *Kilishi* could signifies an important food spoiler aside from its attribute of pathogenicity. Borch *et al*., (1996) reported *P. aeruginosa*as dominant meat spoilage organisms.

CONCLUSSION

This study revealed that *Kilishi*, a ready-to-eat meat product sold in the selected studied area within Gombe metropolis produced higher bacterial count as compared to the acceptable limit and this indicate its poor bacteriological quality status rendering it objectionable for consumption. The presence of pathogenic bacterial strains in the *Kilishi* samples portrays a significant public health hazard as these microbes are potential causes of acute and chronic food-borne diseases and food poisoning. To ensure food safety relating to *Kilishi* production, it is recommended that producers of *Kilishi* should modify the processing method by sticking to only excessive heat- or roast-drying and avoid sun-drying which exposes the meat product to multiple microbial contamination sources. Importantly, good hygiene measures and environmental sanitation must be strictly adhered to at all stages of *Kilishi* production - from slaughtering of animal to the actual processing stage, post-processing handling and packaging of the meat product.

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