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Bacteriology of Dried Meat (*Kilishi*) Hawked in Some Northern Nigerian Cities

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Abstract
Bacterial aetiological agents have been implicated in roughly one-third of the food-borne disease outbreaks worldwide. This study is aimed at detecting the presence of bacterial pathogens in dried meat (*kilishi*) in northern Nigeria. A total of two hundred (200) samples of *kilishi* from 12 locations were examined bacteriologically. Two hundred and twenty five (225) bacterial strains of different species were obtained from the study areas. The bacterial count was in the excess of 10 x 10^{-6} cfu/ml. *Bacillus species* and *Staphylococcus aureus* had prevalence rates of 44.9% and 40% respectively. *Escherichia coli* had an occurrence of 5.8% and was only associated with 2 locations from one specific area. *Proteus mirabilis* and *Proteus vulgaris* had percentage occurrence of 5.3% in 3 locations. *Citrobacter freundii* was isolated from 5 samples showing a percentage of 2.2%. *Salmonella paratyhpi A, Providencia species* and *Alcagenes species* had percentage occurrence of 0.4%, 0.4% and 0.9% respectively. All samples examined had at least one isolate. With the total number of isolates in the study, *kilishi* has been identified as possible source of food borne disease outbreaks in Nigeria.

Keywords: Bacterial pathogens, dried meat, Bacteriology, Kilishi, Food-borne disease
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Introduction

The history of meat preservation in West Africa dates back to the records of the 12th century (Jones *et al*, 2001). Sun-drying was the main method of meat preservation used by the medieval Arabic sources which was transferred to West Africa (Alonge and Hiko, 1981). Although modern methods of meat preservation abound and might be preferred by consumers, refrigeration method is expensive and difficult to maintain in tropical developing countries. In most developing countries, meat preservations are handled with minimal refrigeration. Consequently dried meat product is a laudable alternative. The Hausas refer to dried meat as *kilishi* which is usually made of beef and rarely from lamb or goat. It consists of spices, and mainly hawked in the Northern parts of Nigeria (Alonge and Hiko, 1981).

Kilishi is mostly sold in streets by hawkers on the road side, bus stops, market places and areas or places of business attraction. Unfortunately, this delicacy is mostly processed and handled in an unhygienic manner as hawkers sometimes carry this meat in an open wide tray, exposed to flies and dusts. Most hawkers are illiterate, hence they do not have scientific ideas on food handling and environment (Frazier and Westhoff, 1991). A fast turn over system is used and this ensures that any animal slaughtered is processed, sold and consumed the same day. This system is eminently suitable and feasible for rural village and small towns in many developing countries.

Traditionally, meat preserved by drying is sometimes packaged in nylon bags, baskets or pottery to facilitate storage, transport and to provide some kind of protection from dirt, insect etc. Of the estimated 13.8 million cases of documented food-borne diseases, 30% are due to bacterial infection (Mead *et al*, 1999). However, about 2.1 million children in developing countries die as a result of diarrhea-related illnesses annually. It is suspected that food or water is the vehicle for many of these illnesses (Adams and Moss, 1995; WHO, 2002).

The presence of potentially life-threatening pathogens in our environment and their ability to survive or proliferate under refrigeration and in reduced oxygen atmosphere is of great concern. Previous scientific findings have documented *Salmonella species, Campylobacter species, entero-toxigenic Escherichia coli, Listeria monocytogenes, Clostridium botulinum, Yersinia enterocolitica, Clostridium perfringens, Staphylococcus aureus,* and *Vibrio species* have been documented as pathogenic micro-organisms presenting the greatest risk with meat (Anon, 2004; USDA/ERS, 2003).

This study is aimed at evaluating the presence and distribution of bacterial pathogens in *kilishi* with the objective of alerting the public on the danger of acquiring food borne diseases through the consumption of improperly processed and contaminated *kilishi* and to advocate for a modern hygienic processing of this popular delicacy.

Materials and methods

Sample collection: Two hundred (200) samples of *Kilishi* were collected from hawkers in sterile containers from 12 (twelve) locations in Kano, Sokoto and Abuja (Abuja n =30, Kano n = 70 and Sokoto n = 100).

Total viable count: This was carried out according to the surface count method of Miles and Misra (1938).

Bacterial Isolation

Primary culture: About 2.5g meat sample (Fig.1) was inoculated into 20ml of sterile nutrient broth, alkaline peptone water, selenite faeces (SF) and phosphate buffer saline (pH 7.2) (Fluka, Sigma, Aldrich Chemie, GmBH, Germany) and incubated for 18-24 hours at 37^0C. The phosphate buffer saline was incubated for 21days in the refrigerator at 4^0C (Fredriksson-Ahomaa and Korkeala, 2003).

Figure 1: *Kilishi* sample as seen displayed

Secondary culture: From the nutrient broth blood agar and MacConkey agar plates were aseptically inoculated using sterile wire loop. Cultures were incubated at 37^0C aerobically for 18-24 hours according to the method of Cheesbrough (2000).

The SF broth were subcultured asceptically onto deoxycholate agar plate (DCA plate)(Oxoid ,UK) and incubated at 37^0C aerobically for 18-24 hours. Samples in alkaline peptone water were inoculated into thiosulphate citrate bile salt sucrose (TCBS) medium and incubated aerobically at 37^0C . The phosphate buffer saline (PBS) of the primary cultures were similarly subcultured onto selective media (cefsoludin irgasan novobiocin (CIN) for the isolation of *Yersinia* species and incubated at room temperature (25^0C) for 24-48 hours (CFSAN, 2001).

Bacterial identification: The culture plates were all read macroscopically, microscopically and characterized biochemically using API-20E (Biomereux, France) (Archer *et al*, 1987; Sharma *et al*,1990; Okwori *et al*, 2007).

Antimicrobial susceptibility: The sensitivity spectrum of each of the isolates was determined using eight (8) different antibiotics by standardized single disc diffusion method (Bauer *et al*, 1966., Okwori *et al*, 2007).

Data Management and Analysis: Laboratory results were entered and managed using Microsoft Excel (windows 2003, Duxbury press). Descriptive statistical analysis was done using the Kruskal-Wallis test for the comparison of the results.

Results and Discussion

Two hundred and twenty five (225) bacterial isolates were identified, with at least one isolate from each sample. The distribution of samples collected is contained in Table 1. Table 2 shows the viable count of the micro organisms from samples tested, while Tables 3, 4, and 5 shows the bacterial pathogens isolated according to locations, Kano, Sokoto and Abuja respectively. Table 6 shows the percentage distribution of the bacterial pathogens isolated.

Kilishi a meat product, widely consumed in Nigeria by both the rich and the poor. It is evidently clear from this study that *kilishi* is a potential source of food borne diseases. The source of contamination of food substances like *Kilishi,* a meat product with microorganisms could be endogenous or gotten outside the animal which could be from human handlers, water supplies, soil, vegetation and the likes. Thus from the bacteria count of the *Kilishi* samples collected, there is significant count of microorganisms which is in contrast with the findings of Jones *et al* (2001) who reported no significant count.

Table 1: Average Colony Forming Units (cfu/ml) of bacteria in Locations screened

Sample Location	Number screened	CFU/ml
Kano State		
Brigade	40	$26x10^6$
Katsina Road	20	$22x10^6$
Kofar Ruwa	10	$21x10^6$
Sokoto State		
Ahmadu Bello Way Round About	20	$10x10^6$
Emir Yahaya	12	$17x10^6$
University City Campus	30	$19x10^6$
Diplomat	10	$16x10^6$
Jallen Junction	18	$20x10^6$
Dandima Round about	10	$18x10^6$
Abuja (F.C.T)		
Area 1 Shopping Complex	12	$24x10^6$
Eagles Square	8	$22x10^6$
Utako District	10	$21x10^6$
Total	**200**	

Table 2: Distribution of bacterial pathogens in *Kilishi* screened from Kano zones

Organism	Brigade	Katsina Road	Kofar Ruwa	Total
Staphylococcus aureus	20	15	4	39
Bacillus species	12	7	4	23
Citrobacter species	2	-	-	2
Proteus mirabilis	6	-	-	6
Total	**40**	**22**	**8**	**70**

Table 3: Distribution of bacterial pathogens in *Kilishi* screened from Sokoto zones

Organism	Ahmadu BelloWay Round About	Emir Yahaya	University City Campus	Diplomat	Jallen Junction	Dandima Round about	Total
Staphylococcus aureus	10	7	13	5	9	3	47
Bacillus species	14	7	18	3	9	2	53
Total	**24**	**14**	**31**	**8**	**18**	**5**	**100**

Table 4: Distribution of bacterial pathogens in *Kilishi* screened from Abuja zones

Organism	Area 1 Shopping Complex	Eagles Square	Utako District	Total
Escherichia coli	9	-	4	13
Proteus vulgaris	2	-	4	6
Salmonella paratyphi A	1	-	-	1
Citrobacter species	2	-	1	3
Providencia species	1	-	-	1
Alcagenes species	-	-	2	2
Staphylococcus aureus	2	1	1	4
Bacillus species	7	9	9	25
Total	**24**	**10**	**21**	**55**

Table 5: Percentage (%) distribution of bacterial isolates from *Kilishi* Samples screened

Organism	Frequency	Percentage (%)
Staphylococcus aureus	90	40.0
Escherichia coli	13	5.8
Bacillus species	101	44.9
Citrobacter species	5	2.2
Proteus mirabilis	6	2.7
Proteus vulgaris	6	2.7
Salmonella paratyphi A	1	0.4
Providencia species	1	0.4

Alcagenes species	2	0.9
Total	225	100

225 organisms were isolated from the 200 samples and at least one organism was isolated from each sample. Out of the 225 isolates, *Bacillus species* had the highest occurrence of 44.9%. This could be as a result of environmental contamination during processing, handling and storage or packaging of the product. *Staphylococcus aureus* accounted for 40% of all isolates. Food handlers phenomena could be responsible for the contamination of *Kilishi* by *S. aureus* during preparation. Notably, *S. aureus* and *Bacillus species* were isolated from all sample locations which implies a high environmental contamination. This also indicates a low level of hygiene by the handlers of *Kilishi* meat. Further more *E. coli* occurred in 5.8% of all samples and was only associated with 2 locations, traceable to contamination of faecal origin. Although *E. coli* and *Salmonella species* have been shown to exhibit a high desiccation tolerance, contamination of *kilishi* with these pathogens could be prior or during the drying of the meat (Hiramatsu *et al*, 2005).

Proteus species has a percentage occurrence of 5.3% in 3 locations, one in Kano, two in Abuja locations. Isolation of *Proteus species* could be due to soil or water contamination during the processing of *Kilishi*. *Citrobacter species* isolated could be attributed to contamination of water used in the preparation of *Kilishi*, or contamination of carcasses (Cheesbrough, 2000) or meat which often occurs at the time of evisceration in the abattoirs as documented in similar studies by Kapperud, 1991; Adams and Moss, 1995). Thus *kilishi* could be a source of foodborne disease outbreak in a community at large, and could be a possible means of antibiotic resistance development by transferring resistance organisms in a population.

The multiple number of bacterial pathogens isolated in Abuja area may not be far from the fact that Abuja being a Federal capital territory (FCT) harbours large number of people and hence high frequency of bacterial pollution in the environment and among the meat handlers. The high bacterial contamination of *kilishi* being sold in some northern parts of Nigeria calls for relevant authorities to educate handlers. The presence of potentially life threatening pathogens in our environment indicates the seriousness of the potential hazards with which we are faced. Public health workers therefore, should map out strategies to preventing possible outbreaks of food borne diseases. Food regulation agencies in Nigeria should regularly ensure hygienic conditions in the production, storage and handling of *kilishi*.

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References

Adams, M.R., and Moss, M.O (1995) Food Microbiology. Cambridge: *The Royal Society of Chemistry,* 214-218.

Archer, J. R., Schell, R. F., Pennel, D. R. and Wick, P. D (1987). Identification of *Yersinia* spp. with the API 20E system. *J. Clin. Microbiol.* 25: 2398-2399.

Alonge, D. O and Hiko, A. A (1981).Traditional methods of meat preservation and preparation in Nigeria. West African farming, March/April, 19-20.

Anonymous (2004) Institute of Food Technologists (IFT).Bacteria Associated with Food borne Diseases. Scientific Status Summary.

Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M (1966).Antibiotic susceptibility testing by a single disc method. *American Journal of Clinical Microbiology*, 45: 493.

Cheesbrough Monica (2000): District Laboratory practical in tropical countries test, 2: pp. 171-73. FDA/CFSAN/BAM (2001). *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* Bacteriological Analytical manual online Chp. pp.1-7*. US food and Drug Administration, Center For Food Safety and applied nutrition*.

Frazier, W. C. and Westhoff, D. C (1991) Food Microbiology text 10th edition. pp. 205.

Fredriksson-Ahomaa Maria and H. Korkeala (2003) Low occurrence of pathogenic *Yersinia enterocolitica* in clinical food and environmental samples; A methodological problem. *Clinical Microbol. Reviews* 16: 220-229.

Harrison, J. A. and Harrison M. A (1996) Fate of *Escherichia coli* 0157; *Listeria*

Hiramatsu, R., Matsumuto M., Sakae K. and Miyazaki Y (2005): Ability of Shiga Toxin-production *Escherichia coli* and *Salmonellaspp.* To survive in a Desiccation Model System in Dry Foods. *Applied and Environmental Microbiology.* 71 (11): 6657-6663.

Jones, M.J., Tanya, V. N., Mbofiing, C. M. F., Fonkem D.N. and Silverside.D.E (2001) A Microbial and Nutritional evaluation of the West Africa dried meat product, *Kilishi*. *The Journal of Food Technology in Africa*. 6 : (4) pp. 126-129.

Kapperud, G (1991) *Yersinia enterocolitica* in food hygiene. *Int.J.food Microbiol*. 12:53-65

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., and Tauxe, R.V (1999): Food-related illness and death in the United States. *Emerg. Infect. Dis*. 5: 607-625.

Miles, A.A and Misra, S.S. (1938). The estimation of bactericidal power of the blood. *J. Hyg.* 38:732- 49

Okwori, A. E. J., G.O.A. Agada., A.O. Olabode., S.E. Agina., E. S. Okpe., J. Okopi (2007). The prevalence of pathogenic *Yersinia enterocolitica* among diarrhea patients in Jos, Nigeria. *African Journal of Biotechnology* Vol. 6 (8): 1031-1034

Sharma, N. K., Doyle, P. W., Gerbasi, S. A. and Jessop, J. H (1990). Identification of *Yersinia* species by API 20E. *J. Clin. Microbiol.* 28: 1443-1444.

UDSA/ERS (2003) Economics of food borne disease: Feature, US Department of Agriculture Economic Research Service,Washington, DC. www..ers.usda.gov/briefing/foodborne Disease/features.htm

WHO (2002) Fact Sheet 237: Food Safety and Foodborne Illness. World Health organization, Geneva, Switzerland. *www.who.int/mediacentre/factsheets/fs237/.*