

Isolation and identification of *Listeria* sp from ready-to-eat (rte) kilishi in retail outlet in Bauchi, Nigeria.

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ABSTRACT: The ubiquitous nature and continued presence in food processing environments makes *Listeria monocytogenes* a significant threat in ready-to-eat (RTE) food products. The objectives of this study was to determine the presence of *Listeria monocytogenes* in ready-to-eat (RTE) kilishi. RTE food samples were purchased from different locations in Bauchi metropolis to examine the presence of *Listeria monocytogenes*. The presumptive isolates were characterized biochemically. Out of 20 samples, *Listeria monocytogenes* was detected in 12 (60%) samples, while other species like *Listeria ivanovii*, *Listeria innocua*, *Listeria grayi* were detected 2 (10%), 1 (5%) and 5 (25%) respectively. Among the samples studied RTE kilishi, the beefy part has the highest number 4 (33%) of the positive samples (*Listeria Monocytogenes* isolates) followed by fat and old stock 3 (25%) each, while the least was recorded in freshly prepared kilishi 2 (17%). In conclusion, this work revealed consumption of RTE foods as a potential risk of listeriosis in this region.

Keywords: kilishi, *Listeria monocytogenes*, listeriosis, ready to eat food, Isolates, Food

I. INTRODUCTION

Listeriosis is a bacterial infection caused by a gram-positive, rod-shaped, motile bacterium, *Listeria monocytogenes*. *Listeria monocytogenes* has been known as a human pathogen since 1929 but the route of transmission only became clear in 1980s when chains of outbreaks developed which led to the discovery that it is food related, (1). It is a facultative intracellular bacterium that has an affinity for the central nervous system of humans and domesticated mammals.

Listeria monocytogenes is known globally to be one of the most significant food-borne pathogens of worry for the food industries. It is a universal microorganism and it is commonly isolated from foods of animal source, primarily meat and milk products (2,3 and 4), it is also known to be present in fresh produce, for instance salads (5).

Nevertheless, human listeriosis outbreaks are most often connected with ready-to-eat food products that are consumed without pre-cooking process (6). Consumption of *L. Monocytogenes* contaminated foods can cause listeriosis, a serious infectious disease that can lead to meningoenzephalitis, abortion, septicemia, and a high fatality rate (30%). Pregnant women, newborns, elderly people and immunocompromised patients are most predominantly vulnerable to listeriosis (7 and 8). Although, the absence of predisposing conditions may also lead to the manifestation of the disease by non-invasive form of listeriosis. (9).

Listeria monocytogenes can survive in temperatures from -7°C to 45°C. 2,4 and has an optimum growing temperature of around 37°C with the ability to grow, albeit slowly, at temperature as low as 0°C. For this reason, it can multiply in properly refrigerated food after contamination.

Listeria monocytogenes has the ability to grow over a wide range of environmental situations such as high salt concentration and low pH. (10). *Listeria monocytogenes*, unlike some other foodborne pathogens can multiply in contaminated refrigerated food, (11). For instance, soft cheese is accepted as a high risk product for listeriosis due to the fact that bacteria may grow in significant numbers during refrigeration. The fecal-oral route is the main source of transmission when contaminated food is consumed.

Kilishi is prepared using a beef. It is prepared by cutting lean meats into thin sheets and sun dried on raised wooden table covered with rush matting for about four hours, sometimes due to the weather condition could be dried for two days. The sheet of meat is then immersed in a slurry of groundnut cake and seasoning including salt, pepper, 'karago' in Hausa (12). After the seasoning the meat is returned to the rush matting for further sun drying for twelve hours, the product is roasted briefly over the fire, even though some producers do not employ the final roasting stage. Killishi production is not standardized and there are many variations in the method described above (13).

Due to the fact that the report of *Listeria* infection in Africa is scanty and Nigeria in particular despite many pathogenic signs and symptoms suggestive of listeriosis, it makes diagnosis and chemotherapy of the infection difficult and could in most cases result in treatment failure (14). The objective of the work is to isolate and identify the *Listeria* Sp from 'Kilishi sample'.

II. MATERIALS AND METHODS

AREA OF STUDY.

The samples were collected from different locations within Bauchi metropolis. These included Ahmadu Bello way, from Bakin Kura, Yandoka and Yelwa.

SAMPLE COLLECTION

A total of twenty (20) samples of Killishi were aseptically collected in a sterile container and were taken to the laboratory for immediate analysis. Where this was not possible, the sample was stored in the fridge until ready for use.

PREPARATION

AOAC(15) sampling plan format of preparation was followed. Many microorganisms were present in very low numbers and require one or more enrichment steps. Where cell injury was anticipated, a non-selective enrichment medium was used to resuscitate the cells followed by a more selective enrichment.

CUTTING AND WEIGHING

- a) Strict aseptic technique was followed throughout the experiment.
- b) Sterile instrument was used for cutting, removing and manipulating all samples.
- c) The samples were weighed on a balance, placed in an area free from strong air currents.
- d) The area where weighing was carried out disinfected and cleaned. All instruments, containers, gloves and other materials that may have been in contact with the product were thrown away.

SAMPLE PREPARATION

About 10g of each sample of Killishi was weighed using the mettle weighing balance. 1g of the sample was taken into 10ml of the enrichment broth, after homogenization for 5 minutes, the broth was incubated for 24 hours at 37°C. At the end of the incubation period, the culture was streaked onto a selective Listeria agar (Oxford agar) and was incubated at 37 °C for 24, 48 and 72 hours (center for food safety and applied nutrition and Bacteriological Analytical Manual 2003),

MACROSCOPIC/MICROSCOPIC OF CULTURE PLATES

The colonial morphology of Listeria species on the solid media (Oxford Listeria agar) were observed after a period of 24, 48 and 72 hours, at 37°C. Presumptive identification of Listeria species were based on colors, sizes and growth temperature, colonial morphology (macroscopic) pattern and appearance on the solid media compared with the reference existing culture of Listeria species. However, other microorganisms that grew on the plates were not ignored but were also identified and characterized.

The microscopic feature, biochemical and physiological examination of the Listeria involves the following tests; gram stain reaction., catalase reaction motility, sugar fermentation (Glucose, Rhamose, Xylose, Lactose, Sucrose and Manitol).

III. RESULTS

A total of 20 samples of dried 'Kilishi' were collected from different points namely: Ahmadu Bello Way, Ahmadu Bello WayII, Bakin Kura, Yandoka and Yelwa. Four samples each were collected from the 4 locations composing beef, fat, freshly prepared and old stock were all collected and analyzed and the results of the study were compiled in tables shown below. The Morphological, Physiological and Biological characterization of Listeria isolates from Kilishi sample are shown on table 2 and table 1 show the sampling location.

Table1 Sampling location, number of collected samples, physical properties of samples at the time of collection, incubation temperature, color change at the end of incubation..

S/N	Cod e	Location	No of Sampl es	Nature of sample	Physical properties	Color of plate after incubation
1	A1	Ahmadu Bello way I	4	Beef	Dried	Brown Black
	A2			Fat	Dried	Brown Black
	A3			Freshly prepared	Dried	Brown Black
	A4			Old stock	Dried	Brown Black
2	B1	Bakinkura	4	Beef	Dried	Brown Black
	B2			Fat	Dried	Brown Black
	B3			Freshly prepared	Dried	Brown Black
	B4			Old stock	Dried	Brown Black

3	C1	Yandoka	4	Beef Fat Freshly prepared Old stock	Dried Dried Dried Dried	Brown Black Brown Black Brown Black Brown Black
	C2					
	C3					
	C4					
4	D1	Ahmadu Bello Way II	4	Beef Fat Freshly prepared Old stock	Dried Dried Dried Dried	Brown Black Brown Black Brown Black Brown Black
	D2					
	D3					
	D4					
5	E1	Yelwa	4	Beef Fat Freshly prepared Old stock	Dried Dried Dried Dried	Brown Black Brown Black Brown Black Brown Black
	E2					
	E3					
	E4					

Table i: morphological, physiological and biochemical characterization of listeria isolates from experimental samples.

No of samples	Code	Gram reaction	Morphology	Biological											Organism
				Physiological	Motility	Catalase	Glucose	Sucrose	Rhaminose	Xylose	Manitol	Slope	Butt	Gas	
4	A1	+	SR	+	+	+	-	+	-	-	R	Y	+	-	Listeria monocytogen
	A2	+	SR	+	+	+	-	+	-	-	R	Y	-	-	Lister monocytogen
	A3	+	SR	+	+	+	+	+	-	+	R	Y	-	-	Listeria ivanovii
	A4	+	SR	+	+	+	-	+	-	-	R	Y	-	-	Listeria monocytogen
4	B1	+	SR	+	+	+	-	-	-	-	R	Y	-	-	Listeria monocytogen
	B2	+	SR	+	+	+	+	-	-	-	R	Y	-	-	Listeria inocua
	B3	+	SR	+	+	+	-	-	-	-	R	Y	-	-	Listeria monocytogen
	B4	+	SR	+	+	+	-	-	-	-	R	Y	-	-	Listeria grayi
4	C1	+	SR	+	+	+	-	-	-	-	R	Y	-	-	Listeria monocytogen
	C2	+	SR	+	+	+	-	-	-	-	R	Y	-	-	Listeria grayi
	C3	+	SR	+	+	+	-	-	-	-	R	Y	-	-	Listeria grayi
	C4	+	SR	+	+	+	-	-	-	-	R	Y	-	-	Listeria monocytogen
4	D1	+	SR	+	+	+	-	-	-	-	R	Y	+	+	Listeria grayi
	D2	+	SR	+	+	+	+	-	-	-	R	Y	-	-	Listeria monocytogen
	D3	+	SR	+	+	+	-	-	-	-	R	Y	-	-	Listeria grayi
	D4	+	SR	+	+	+	-	-	-	-	R	Y	+	-	Listeria ivanovii
4	E1	+	SR	+	+	+	-	-	-	-	R	Y	-	+	Listeria monocytogen
	E2	+	SR	+	+	+	-	-	-	-	R	Y	-	+	Listeria monocytogen
	E3	+	SR	+	+	+	-	-	-	-	R	Y	-	-	Listeria monocytogen
	E4	+	SR	+	+	+	-	+	-	-	R	Y	-	-	Listeria monocytogen

Legend: SR=Short rod, R=Red, Y=Yellow

IV. DISCUSSION

The sole aim of the Microbiological monitoring of food, animals and their products is to protect the health of the community and individuals by preventing the spread of infectious and non-infectious diseases and their causative agents through environmental sources.

The results of this study show the presence of *Listeria* species in the samples collected and following the procedures for isolating *Listeria* from ready-to-eat meat samples by (16) the results was compiled . *Listeriamonocytogenes* as shown in the table have the highest percentage occurrence in the “Kilishi” samples followed by *Listeria*grayi then *Listeria*ivanovii and *Listeria*inocua as shown in table 2.

The results also confirmed that there is room for “Kilishi” contamination which might occur from the improper handling of the meat before preparation.

Microscopically, all the samples after analysis show characteristics of short rods (i.e. Gram-positive bacteria) on Oxford *Listeria* agar, only two samples (Fat and Beef) from Ahmadu Bello way and Yelwa shows cocci in cluster and chain showing staphylococci and streptococci species respectively which was not ignored. Microscopically, discrete colonies where the nature of growth with the color of Brown-black, the temperature of incubating was 37°C for 24, 48 hours.

The number and types of bacteria isolated shows that the contamination organisms may be a function of environment of sun drying the “Kilishi” handling and even seasoning. It is important that a complete study be conducted to get a picture of the profile of organism contaminating this kind of meat product.

V. RECOMMENDATION

In line with the suggestions made by the World Health Organization , (16), effort should be intensified on the personal hygiene among the handlers and sellers of “Kilishi” to avoid contamination of *Listeria*. Contact between bare hands and “Kilishi” should be minimized. Before the process of preparing Kilishi begin hands of the producer should be properly washed with clean water, and the “Kilishi” should be properly cooked to avoid this dangerous infection of *Listeria* called listeriosis.

VI. CONCLUSION

The result suggests that since *Listeria* is the causative agents of listeriosis as discussed in chapter two and three of this research work, the eating of “Kilishi’ should be minimized especially in the first three months in the pregnancy, elderly ones as suggested (16).

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