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Listeria monocytogenes: Prevalence and contamination profile in different categories of ready-to-eat foods within Ibadan metropolis, Oyo State, Nigeria

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Abstract

The present investigation encompasses the incidence of Listeria monocytogenes in some ready-to-eat food samples, a human pathogen in the outbreak of listeriosis worldwide. A total of 104 food samples, comprising of chicken-pie, apple, shrimps, dried fish, fura, kilishi, salad and yoghurt were analyzed. Samples were tested using standard bacteriological methods to detect the presence of Listeria monocytogenes. Twenty-five suspected Listeria species were isolated from the samples except chicken pie, dried fish, yoghurt, and their percentage occurrence were 8% (kilishi), 28% (shrimps), 24% (fura), 32% (salad) and 8% (apple). Nineteen of these isolates showed α and β haemolysis, however, only 16 were positive to CAMP test, and as such, identified as L. monocytogenes. Shrimp had the highest number (6) of L. monocytogenes, followed by salad, with 5, fura had 4 and 1 from apple. The results indicated that some of the food samples analysed were contaminated with Listeria monocytogenes and posed a threat to consumers.

Keywords: Ready-to-eat food; Listeria monocytogenes; Listeriosis; Food contamination

1. Introduction

Listeria, ubiquitous in the environment (Leong *et al*., 2016), currently has six recognized species: *Listeria monocytogenes, L. innocua, L. ivanovii, L. seeligeri, L. welshimeri* and *L. grayi*, only two of which are generally considered to be pathogenic, *L. monocytogenes* in humans and *L. ivanovii* in other mammals (Orsi and Wiedmann, 2016). *Listeria monocytogenes* is a human pathogen, causing illness in pregnant women, newborns, elderly and immunocompromised persons (Takarada *et al*., 2004). The organism causes listeriosis, a fatal human disease that is considered a public health issue because of its high mortality rate that reaches 20-40%, most of which are foodborne (Wan Norhana *et al*., 2010), thus, has been isolated from numerous food products (Jami *et al*., 2014). Its incidence however, seemed small, when compared with other food-borne diseases (Noordhout *et al*., 2014). Prevalence of *L. monocytogenes* in ready-to-eat food is commonly reported in high rates globally, constituting a threat to food safety, as it can grow at refrigerated temperature (4˚C - 0˚C), wide pH ranges as well as high salt concentrations (Pérez-Trallero *et al.,* 2014). They have also been reported to contaminate processing environments in which ready-to-eat foods are produced and served (Jarvis *et al*., 2016). Consumption of certain RTE foods, that have been exposed for prolonged period of time and those that were contaminated during processing and via cross-contamination, could increase the risk of listeriosis (Elbashir *et al.,* 2018).

Several selective enrichment and culture based methods have been employed in the detection of these organisms, both in food and the environment generally. Most times, the organisms remain undetectable in samples, thus, reason for an enrichment, to allow them grow and be detectable, prior to inoculation on selective media. Confirmation of identities could however be by PCR, API kits, Microbact and sequencing (Leong, 2017; Law *et al*., 2015). High prevalence of *L. monocytogenes* in RTE food is commonly reported in different parts of world. Ibadan, a city in Nigeria, is the largest city in West Africa and the second largest in Africa, after Cairo. A city of such population is of high relevance in public health studies, moreso, the state is struggling with its waste management, which could be a factor in cross-contamination between raw materials, equipment, utensils, humans, rodents, insects, animals and birds, thus, contributing to the spread of *L. monocytogenes*. Studies like this provides regional information, which constitutes the basis for baseline survey of certain regions, which can be used to analyse the incidence and conduct risk assessment analysis, because it has been reported that, despite Food Safety Criteria (FSC) for *L. monocytogenes* in RTE foods, there is still high incidence in listeriosis cases. Such studies are needed in evaluating most recent information on *L. monocytogenes* in RTE foods and further establishing the effectiveness of guidelines and general principles of food hygiene.

The objectives of this study were to detect, isolate and identify *Listeria monocytogenes* from selected ready-to-eat foods in Ibadan metropolis.

2. Materials and Methods

2.1 Sample Collection

One hundred and four randomly sampled foods were used for this study. The samples were categorised into meat and meat products, fish and fish products, milk and milk products, fruits and vegetables. Chicken-pie, Apple, Shrimps, Dried fish and locally fermented milk drink 'fura' were purchased from retailers at a central food market (Bodija) in Ibadan, Oyo State, Nigeria, while Kilishi, Salad and bottled yoghurt were purchased from Sabo Community and University of Ibadan, Nigeria. All food samples were transported to the laboratory in sterile glass jars.

2.2 Media Preparation

Materials such as test tubes, McCarthney bottles, conical flasks, beakers, were autoclaved at 121˚C and 15 psi for 15 min to completely eliminate all microorganisms, including spores that may serve as contaminants. Listeria Selective Media (LSM) (LabM, UK) was prepared according to formulations on the jar. It was homogenised for 5 min and sterilized at 121˚C and 15 psi for 15 min. The sterile LSM was allowed to cool to about 45˚C.

2.3 Isolation Procedures

Twenty grams of each solid food samples were thoroughly mashed with sterile laboratory mortar and pestle. Ten gram each, were aseptically introduced into 90 ml of sterile peptone water (LabM, UK) as an enrichment medium. Similarly, 10ml of each liquid food samples were aseptically dispensed into 90 ml of sterile peptone water. The enrichment cultures were then incubated at 37˚C for 24 h. After incubation, a loopful was taken from each bottle and streaked on sterile LSM plates, then labelled appropriately. Inoculated plates were incubated at 37˚C for 48 h. Distinct representative colonies were picked and sub-cultured repeatedly to obtain pure cultures which were transferred onto Listeria Selective Medium as well as tryptic soy agar (containing 0.6% yeast extract) slants, aseptically and refrigerated at 4˚C.

2.4 Characterization and Identification of Isolatees

Cultural features of each isolate on LSM plates such as size, elevation, edge, shape, colour, degree of growth and opacity of colonies were studied and observations noted for comparison with appropriate compendium. Pure cultures were also Gram stained as described by Norris and Ribbond (1971). Biochemical tests, such as Catalase, Voges-Proskaeur and Methyl red tests were done according to the method of Harrigan and McCance, (1976). Oxidase (Seeley and Van Demark, 1972) and motility (Olutiola *et al*., 2000) tests were also carried out. A rapid API *Listeria* (bio-Merieux, Marcy-Etoile, France), with identification strips that contains eleven sugar utilization test and a rapid heamolysis test was also utilized.

2.5 Confirmatory Test for *L. monocytogenes*

Haemolysis test

Modified method of Alsheikh *et al.* (2013) was used. 7g of Nutrient agar (LabM, UK) was dissolved in 250 mL of distilled water and sterilized at 121°C and 15psi for 15 minutes. The medium was allowed to cool, then, 10mL sheep blood was aseptically introduced with the aid of a syringe. The resultant blood agar was homogenized for 5 min before it was aseptically poured into plates. Test cultures were streaked on blood agar plates and were incubated at 25˚C for 24 h. The presence of clear zones of haemolysis around bacterial cultures indicated a β-haemolytic isolate, while partial zones of haemolysis (with the agar underneath the colony appearing dark and

greenish) was considered to be α-hemolytic and absence of visible clear zone was recorded as non-haemolytic or γ-haemolytic cultures.

Christie, Atkins, Munch–Petersen (CAMP) test

3.5 g of Nutrient agar (LabM, UK) was dissolved in 125 mL of distilled water, homogenized and sterilized at 121˚C and 15 psi for 15 min. The medium was allowed to cool, then, 5 mL of sheep blood was aseptically introduced with the aid of a syringe. The resultant blood agar was homogenized for 5 min before it was aseptically poured into plates. A single streak of *Staphylococcus aureus* was made across the center of the plate. A single colony of selected β-haemolytic *Listeria* was picked with a sterile loop and used to make a single streak perpendicular but not touching the *S. aureus* streak. A 2-3 mm space was maintained between the streaks. Plates were incubated at 35˚C for 24 h. A positive result showed an enhanced and very visible zone of haemolysis (which is usually triangular in shape) in the region between the two cultures (Groves and Welshimer, 1977)

Mean values were obtained and differences between percentages of contaminated samples were estimated.

3. Results and Discussion

3.1 Results

According to growth on LSM, colonies presented as creamy and brown in colour, shapes ranging from spherical to irregular morphologically, the degree of growth also ranged from profuse to moderate and rare for some food samples, as shown in Table I.

A total of 25 suspected *Listeria spp*. was isolated from the 104 samples analysed, with seven (7) *Listeria* spp. obtained from shrimps, six (6) from *fura*, eight (8) from salad, two (2) each, from *kilishi* and apple. No *Listeria* growth was seen on plates inoculated with chicken pie, dried fish and yoghurt samples. Their frequency and percentage occurrences were shown in Table II.

Results of microscopic and biochemical characterisation showed that all twenty-five isolates were positive to catalase reaction, majority were also rod shaped under the microscope with a positive Gram's reaction. Fifteen (15) out of the twenty-five isolates were β-haemolytic and few, α-haemolytic, with the remaining showing no zones of haemolysis (γ-haemolytic) as shown in table III. Isolates that showed non haemolytic zones were discarded while isolates which showed both partial (α) and complete (β) zones of haemolysis (Fig 1) were selected for further confirmatory *Listeria* characterization.

Utilisation of various mono- and poly- saccharides indicated that all selected isolates hydrolysed glucose, mannose, sucrose, maltose, fructose and rhamnose. However, majority did not utilise xylose and sorbitol, as carbon source. Of the 19 isolates selected for further identification, 16 were positive to CAMP test, as indicated in Table IV, and as such, identified as *Listeria monocytogenes,* thereby, bringing the overall prevalence of *L. monocytogenes* to 64% of the total suspected *Listeria* spp.

The prevalence of identified *L. monocytogenes* indicated the highest number (6, 38%) from shrimps, followed by (5, 31%) from salad, (4, 25%) from fura and (1, 6%) from apple (Fig 2).

Table 1 Representative colonial morphology on Listeria Selective Medium

Sample		Number sampled	*Listeria* spp. growth	
			Freq. of occurrence (n)	Percentage occurrence (%)
Meat and meat product	Chicken pie	14	-	-
	Kilishi	10	2	8
Fish and fish product	Shrimp	25	7	28
	Dried fish	15	-	-
Milk and milk product	*Fura*	10	6	24
	Yoghurt	10	-	-
Fruit and Vegetable	Salad	9	8	32
	Apple	11	2	8
Total		**104**	**25**	**100**

Table2 Frequency of occurrence of isolated *Listeria* spp. from different food samples

Isolate code	Shape	Colour	Opacity	Elevation	Surface	Edge	Degree of growth
SaA	Spherical (small)	Cream	Opaque	Concave	Shiny	Entire	Moderate
SaB	Spherical (small)	Cream	Opaque	Concave	Shiny	Entire	Moderate
SaC	Spherical (small)	Cream	Opaque	Concave	Shiny	Entire	Moderate
SaD	Spherical (small)	Cream	Opaque	Concave	Shiny	Entire	Moderate
SaE	Spherical (small)	Cream	Opaque	Concave	Shiny	Entire	Moderate
SaF	Irregular	Cream (with brown/black halo)	Opaque	Flat	Dry	Undulating	Moderate
SaG	Spherical (large)	Cream (with brown/black halo)	Opaque	Flat	Dry	Crenated	Moderate
SaH	Spherical (small)	Cream	Opaque	Concave	Shiny	Entire	Moderate
ApA	Spherical (small)	Cream (with brown/black halo)	Opaque	Concave	Shiny	Entire	Profuse

Table 2 Frequency of occurrence of isolated *Listeria* spp. from different food samples (Continue)

Isolate code	Shape	Colour	Opacity	Elevation	Surface	Edge	Degree of growth
ApB	Spherical (small)	Brownish (with brown/black halo)	Opaque	Concave	Shiny	Entire	Profuse
KlA	Irregular	Cream	Opaque	Flat	Dry	Undulating	Moderate
KlB	Spherical (small)	Brownish	Opaque	Concave	Shiny	Entire	Profuse
ShpA	Swarmy	Cream	Opaque	Flat	Dry	Rhizhoid	Profuse
ShpB	Spherical (large)	Cream (with brown/black halo)	Opaque	Flat	Dry	Entire	Rare
ShpC	Spherical (small)	Brownish (with brown/black halo)	Opaque	Concave	Shiny	Entire	Moderate
ShpD	Irregular	Brownish (with brown/black halo)	Opaque	Flat	Dry	Undulating	Moderate
ShpE	Irregular	Cream	Opaque	Flat	Dry	Crenated	Profuse
ShpF	Spherical (small)	Brownish	Opaque	Concave	Shiny	Entire	Rare
ShpG	Irregular	Irregular (with brown/black halo)	Opaque	Raised	Shiny	Entire	Rare
FuA	Spherical (medium)	Cream	Opaque	Concave	Shiny	Entire	Rare
FuB	Irregular	Cream	Opaque	Flat	Dry	Undulating	Rare
FuC	Spherical (small)	Cream	Opaque	Concave	Shiny	Entire	Rare
FuD	Irregular	Cream (with brown/black halo)	Opaque	Flat	Dry	Undulating	Profuse
FuE	Irregular	Brownish (with brown/black halo)	Opaque	Flat	Dry	Undulating	Profuse
FuF	Spherical (small)	Cream	Opaque	Concave	Shiny	Entire	Moderate

Table 3 Biochemical characterisation of selected isolates

Isolate code	Gram Staining	Catalase	Motility	Methyl red	Voges Proskeur	Oxidase	Heamolysis test
SaA	+	+	+	+	+	-	β
SaB	+	+	+	+	+	-	α
SaC	+	+	+	+	+	-	β
SaD	+	+	+	+	+	-	β
SaE	+ cocci	+	-	+	+	-	α
SaF	+	+	+	+	-	+	γ
SaG	+	+	+	+	-	+	γ
SaH	+	+	+	-	-	+	γ
ApA	+ cocci	+	-	+	-	+	γ
ApB	+	+	+	+	+	-	β
KlA	+ rod	+	-	-	+	-	β
KlB	+	+	+	+	+	-	β
ShpA	+	+	+	+	+	-	β
ShpB	+	+	+	+	+	-	β
ShpC	+	+	+	+	+	-	β
ShpD	+ cocci	+	+	+	+	-	β
ShpE	+	+	+	+	+	-	β
ShpF	+	+	+	-	+	+	α
ShpG	+	+	+	-	+	-	γ
FuA	+	+	+	+	+	-	β
FuB	+	+	+	+	+	-	β
FuC	+	+	+	-	+	-	α
FuD	+	+	+	-	+	-	β
FuE	+	+	+	+	+	-	β
FuF	+	+	+	-	+	-	γ

Key: + positive; - negative; α partial haemolysis; β complete haemolysis; γ no haemolysis

Table 4 Sugar fermentation pattern and CAMP heamolytic test of selected isolates

Isolate code	Glucose	Mannose	Sucrose	Maltose	Xylose	Lactose	Sorbitol	Galactose	Fructose	Rhamnose	CAMP Test
SaA	+	+	+	+	-	+	-	+	+	+	+
SaB	+	+	+	+	-	+	-	+	+	+	+
SaC	+	+	+	+	-	+	-	+	+	+	+
SaD	+	+	+	+	-	+	-	+	+	+	+
SaE	+	+	+	+	-	+	+	+	+	+	+
ApB	+	+	+	+	-	+	-	+	+	+	+
KlA	+	+	+	-	+	-	-	-	+	+	-
KlB	+	+	+	-	+	+	+	-	+	+	-
ShpA	+	+	+	+	-	+	-	-	+	+	+
ShpB	+	+	+	+	-	+	-	-	+	+	+
ShpC	+	+	+	+	-	+	-	-	+	+	+
ShpD	+	+	+	+	-	+	-	+	+	+	+
ShpE	+	+	+	+	-	+	-	+	+	+	+
ShpF	+	+	+	+	-	+	-	-	+	+	+
FuA	+	+	+	+	-	+	+	+	+	+	+
FuB	+	+	+	+	-	+	+	+	+	+	+
FuC	+	+	+	+	-	+	-	+	+	+	+
FuD	+	+	+	-	+	-	-	-	+	+	-
FuE	+	+	+	+	-	+	-	-	+	+	+

Key: + positive; - negative

Fig 1 Representative isolate showing β (complete) heamolysis on blood agar.

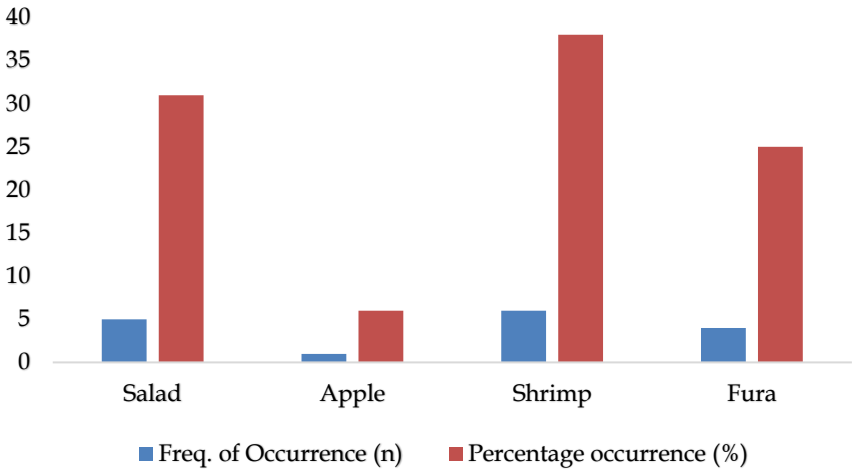

Fig 2 Prevalence of identified *Listeria monocytogenes*

3.2 Discussion

Several outbreaks of listeriosis have been associated with contaminated commercial foodstuffs, such as vegetables, milk, and meat products, on which the causal organism can multiply even at low temperatures (Schuchat, *et al.*, 1991). A foodborne pathogen, that contaminates food-processing environments, *Listeria monocytogenes* persists as biofilms on equipment, utensils, floors and drains, ultimately reaching final products by cross-contamination. Even though, with incidence, lower than most enteric illnesses, *L. monocytogenes* infections have high mortality rate (Camargo, 2017). The use of selective agar in *Listeria spp*. isolation gives an initial result that is presumptively positive, but confirmatory test is very essential (Leong, 2017).

Isolation of *Listeria spp*. from most of the sampled ready- to-eat foods indicated that they were contaminated. The percentage occurrence of *Listeria spp*. among the samples, showed that salad had the highest contamination with *Listeria spp*. and this could be the result of not being subjected to any heat treatment. Furthermore, contamination during harvesting of salad vegetables e.g cabbage, lettuce etc., is a major factor, which can't be overlooked (Ponniah *et al*., 2010). This was closely followed by shrimps (28%). Cross-contamination is a possibility for this, since they were not really processed and were mostly without packaging medium at the point of purchase, thus, been exposed for a prolonged period of time. *Fura,* on the other hand, is a locally produced milk drink, usually processed in poor, unhygienic environment.

Furthermore, cross-contamination after foods were cooked (Kornacki and Gurtler, 2007; Tompkin, 2002), as in the case of *kilishi,* in this study, could not be ruled out because, in some cases, the cooking process is not sufficient to inactivate these organisms. In a study done by Wong *et a*l. (2011), *L. monocytogenes* was not detected after 6 min cooking of chicken burger patties but it was isolated when the cooking was done in 4 min, however, the absence of *Listeria spp* in sampled bottled yoghurt may solely be due to the presence of lactic acid bacteria (LAB) present in this milk product, and as a result of microbial competition, have been able to synthesize certain substances (e.g organic acids, hydrogen peroxide, diacetyl and bacteriocin) which does not favour *Listeria* growth (Daeschel, 1989; Analie and Bennie, 2001; Liu, 2003) or these LABs may have a faster mechanism of utilizing available nutrients than *Listeria* species.

Biochemical tests measure the phenotypic characteristics of Listeria bacteria, their performance can be influenced by external factors that affect bacterial growth and metabolic mechanisms (Liu, 2006). However, this study revealed that most identified isolates were Gram positive, catalase positive, oxidase negative and non-motile bacteria. These biochemical characteristics of *Listeria* reported in this study were similar to the findings of Bayoub *et al*. (2010) and Islam *et al*. (2016).

The prevalence of L monocytogenes in the current study (64%) when compared to other spp., was higher than earlier reported 40% and 37.65% from fresh fish ((Ikeh *et al*., 2010; Lennox *et al*., 2017). However, lower cases of occurrence (7.7% and 7.6%) were also detected by Momtaz and Yadollahi (2013) and Jamali *et al*. (2015) respectively, while working with raw fishes.

4. Conclusion

In conclusion, this study reveals that consumption of RTE foods is a source of potential risk of listeriosis. The high contamination rate of *L. monocytogenes* in some food samples used for this study is of great public health concern. The scenario warrants further surveillance and action by local authorities to control the incidence of *L. monocytogenes* contamination in RTE foods through regulations and sanctions.

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