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Molecular characterization of *Listeria monocytogenes* isolated from a ready-to-eat fermented milk and cereal product, *Fura-de-Nunu*

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This study was conducted to determine the occurrence of *Listeria (L.) monocytogenes* in *Fura-de-Nunu,* a ready-to-eat (RTE) fermented milk (*Nunu*) and cereal (*Fura*) blend, the serogroups as well as the virulence of the isolates. A total of 75 *Fura* and 75 *Nunu* samples were examined. *Listeria* species were isolated on PALCAM medium and *Listeria* chromogenic agar, and identified phenotypically according to International Standardization Organization (ISO) procedures. Identification of *L. monocytogenes,* serogrouping and detection of virulence genes were carried out by polymerase chain reaction (PCR). *Listeria* spp. were recovered from 23 (30.67%) and 41 (54.67%) samples of *Fura* and *Nunu*, respectively. The bioloads of *Listeria* ranged from 10^3 to 10^5 CFU/ml. Six presumptive species of *Listeria* were identified from the samples, with *L. monocytogenes* accounting for 21.00 and 20.64% of isolates from *Fura* and *Nunu,* respectively. Out of the three major serogroups (1/2a, 1/2b and 4b) associated with human disease, only 1/2a and 4b were identified among the isolates. Some of the isolates tested positive for the presence of virulence genes, *hlyA* and *iap*. Results from this study show that *Fura-de-Nunu*, may represent a risk for transmission of listeriosis to consumers.

Key words: *Listeria monocytogenes*, Fura-de-Nunu, fermented milk, ready-to-eat, Listeriosis.

INTRODUCTION

The genus *Listeria* currently comprises seventeen species of Gram-positive, non-spore forming, and catalase-positive bacteria: *Listeria (L.) aquatica, L. booriae, L. cornellensis, L. fleischmannii, L. floridensis, L. grandensis, L. grayi, L. innocua, L. ivanovii, L. marthii, L. monocytogenes, L. newyorkensis, L. riparia, L. rocourtiae, L. seeligeri, L. weihenstephanensis,* and *L. welshimeri* (Weller et al*.,* 2015). The haemolytic species (*L. monocytogenes, L. ivanovii* and *L. seeligeri*) are associated with human disease, although *L. ivanovii* and *L. seeligeri* are more commonly involved in diseases of animals and are rare causes of human disease (Guillet et al*.,* 2010; Jamali et al., 2013; Abdellrazeq et al., 2014).

L. monocytogenes has emerged as a very important food-borne pathogen, causing the human disease, listeriosis (Marnissi et al., 2013; Mansouri-Najand et al.,

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2015). It has been detected in various foods, including raw milk and other dairy products, such as cheese and ice cream (Jamali et al., 2013). Infectious doses are reported to range from 10^1 to 10^5 CFU/g of food (Maijala et al., 2001; Ooi and Lorber, 2005; Yang et al., 2007; Marnissi et al., 2013; Jamali et al., 2013; Mansouri-Najand et al., 2015; Oyinloye, 2016). However, according to the European food safety regulations, generally, food samples containing more than 100 CFU/g of *L. monocytogenes* are considered unsatisfactory. In foods intended for infants or foods where the shelf life is determined without consideration of the organism, there should be complete absence of *L. monocytogenes* /25 g of the food before the food leaves the immediate control of the producer (Food Standards Agency, 2006; Public Health England, 2014). Listeriosis is a disease of serious public health concern because of the high mortality rate associated with the disease; especially in pregnant women and infants, the elderly and immunocompromised individuals, who are at the greatest risk for the disease (Gillespie et al., 2010; Barton et al., 2011; Abdellrazeq et al., 2014; Oyinloye, 2016). A survey in 2010, by the Europe PMC Funders Group, estimated that listeriosis resulted in 23,150 illnesses, 5,463 deaths and 172, 823 disability adjusted life years (DALYs) globally. The proportion of perinatal cases was 20·7%. This led to a call by the World Health Organization (WHO) for more studies, particularly in developing countries (de Noordhout et al., 2014).

The presence of *L. monocytogenes* in ready-to-eat (RTE) foods and the low infectious dose of the organism are a cause for concern as such foods could constitute health hazards to consumers. *L. monocytogenes* has been reported from humans, environment and various foods in Nigeria, although most of these reports have come from studies based on phenotypic and biochemical characterization of the organism (Nwaiwu, 2015). *Fura-de-Nunu*, a fermented milk-cereal mix, is a street-vended, RTE food, consumed in various parts of Nigeria, especially in Northern Nigeria. In Nigeria, about 90% of the dairy cattle belong to the Fulani agro-pasturalists and their women strictly control the processing and marketing of their milk products (Chukwuma, 2009; Okeke et al., 2014). Most of these women are illiterate and have no knowledge of critical control points of food production. *Fura* is a semi-solid dumpling millet-based meal. The millet is blended with spices and water, molded into dough balls and then cooked. To make the complete meal, *Fura-de-Nunu,* the cooked dough balls are broken up and mixed into the fermented milk (*Nunu)*, to form porridge (Kordylasi, 1990; Jideani et al., 2001).

Fura-de-Nunu, like many other indigenous fermented foods, depends on spontaneous fermentation initiated by natural microorganisms on raw materials and equipment, the hands of producers and the local environment. In addition, some aspects of the production of this widely consumed product, such as use of leftover products as starter for fresh fermentation of the *Nunu* and molding of the *Fura* by hand, all increase the chances of contamination of the product. This study therefore aimed to determine the prevalence of *L. monocytogenes* in RTE *Fura-de-Nunu* samples, the PCR serogroups as well as virulence genes of the isolates.

MATERIALS AND METHODS

Samples collection

A total of 150 samples, comprising 75 *Fura* and 75 *Nunu,* were purchased from vendors in different communities within Nsukka metropolis. *Nunu* samples were collected in a sterile large screw capped bottles while *Fura* balls were placed in sterile polyethylene bags and transported in ice-packed containers to the Microbiology Laboratory, University of Nigeria, Nsukka for analysis.

Isolation and enumeration of organisms

The procedure recommended by the International Organization for Standardization (ISO) 11290 (2004) was used as follows: A 25 g representative portion from each *Fura* sample, mashed into a semi-powdery paste or 25 mL representative portion of each *Nunu* sample was introduced aseptically into a sterile conical flask containing 225 mL of Half Fraser Broth to obtain a 1:10 sample dilution. The samples were homogenized for 1 min and incubated for 24 h at 37°C. After incubation, a 0.1 mL aliquot from each Half Fraser Broth culture was added to 9.9 mL of Fraser Broth and incubated for 24 to 48 h at 37°C. Following incubation, the culture was serially diluted ten-fold and 0.1 mL from 10^{-3}, $10^{-4}$and 10^{-5} dilutions was spread on *Listeria* selective agar (PALCAM; Oxoid, UK). Samples were also inoculated onto plates of Brilliance *Listeria* chromogenic agar (Oxoid, UK), selective for *Listeria* spp. The plates were incubated for 24 to 48 h at 37°C and examined for grayish colonies, typical of *Listeria.* Total viable counts were taken from plates containing countable colonies.

Biochemical confirmation of the isolates

Colonies showing typical morphology of *Listeria* were streaked onto Tryptone Soya Agar supplemented with 0.6% yeast extract powder (TSYEA; Oxoid, UK) and incubated at 37°C for 24 h to further obtain pure cultures for identification and characterization (Alsheikh et al., 2013). The isolates were identified and characterized using the following tests: Gram staining, oxidase, catalase, motility test, sugar fermentation (Glucose, Rhamnose, Mannitol, methyl-D-mannose, xylose), and haemolysis test. Results from suspected *L. monocytogenes* isolates were compared to those of a reference *L. monocytogenes* strain ATCC13932

Haemolysis test

The isolates were inoculated onto 7% Sheep Blood Agar (SBA) by piercing into the medium and incubated at 37°C for 24 h. The haemolytic zones around the colonies were examined after incubation (Alsheikh et al., 2013; Momtaz and Yadollahi, 2013).

Christie, Atkins, Munch-Petersen (CAMP) test

This was performed according to the procedure described by Yadav et al. (2010). A β-haemolytic strain of *Staphylococcus aureus* was

grown overnight on 7% SBA at 37°C and a colony was streaked again on freshly prepared 7% SBA in a manner that the streak allows for streaking of *Listeria* colonies. *Listeria* isolates was streaked at 90° angle and 3 mm apart before incubating them at 37°C for 24 h. A positive reaction was indicated by an enhanced zone of haemolysis at the intersection of the test and indicator strain.

Determination of pH of samples

For the *Fura* samples, 10 g of each sample was homogenized with 20 mL of distilled water in a beaker, for 1 min and the pH of the homogenate determined using digital pH meter calibrated with standard buffer solutions. For the *Nunu* samples, the samples were mixed properly and 25 mL of each sample was poured into a beaker and the pH determined using a digital pH meter.

Determination of titratable acidity of the samples (using phenolphthalein indicator)

Ten millilitres (*Nunu*) or 10 g (*Fura*) of each sample was added to 50 mL of distilled water in a 250 mL beaker and homogenized properly. Then, 3 drops of phenolphthalein were added to the mixture. A 0.1 M solution of NaOH was slowly titrated into the mixture, while carefully swirling the beaker to ensure thorough mixing. The point of neutrality was reached when the indicator changed colour from colourless to pink, which must remain stable for at least 30 s. The amount of NaOH used on the burette was recorded and result expressed as percentage acid, using the following formula:

$$\text{Percent acid} = \frac{\text{Titre} \times \text{acid factor} \times 100}{\text{10 mL of sample}}$$

Acid factor for Lactic acid = 0.0090

Determination of NaCl (salt content) with standard silver nitrate solution

To each sample neutralized as described above, about 1 mL of 5% aqueous potassium chromate solution was added and titrated with 0.1 N $AgNO_3$ solution to produce red-brown end point.

$$\text{NaCl \%} = \frac{\text{Titre value} \times \text{Normality of } AgNO_3 \times 58.4 \times 100}{\text{Weight of the sample} \times 1000}$$

Determination of moisture content

About 5 mL and 5 g of well mixed samples (*Nunu* and *Fura*) were weighed in a well dried oven sterilized glass tray. The tray was placed in a hot air oven maintained at 105 ± 2°C and dried for at least 2 h. It was then cooled in a dessicator and weighed. The process of heating, cooling and weighing was repeated until the difference between two successive weighing was less than 1 mg and the lowest weight recorded.

$$\text{\% Moisture content} = \frac{100\ (M_1 - M_2)}{M_1 - M}$$

Where; M_1 = Weight in gram of dish with material before drying; M_2 = Weight in gram of dish with the dried material, and M = Weight in gram of empty dish

Determination of water activity (a_w)

This was done using water activity meter (Buchi, Switzerland). About 10 g of each *Fura* and 10 mL of each *Nunu* sample was placed on the sample scale pan and readings recorded.

DNA extraction

Genomic DNA was extracted from the isolates using an extraction kit (Zymo Research, U.S.A.) following the procedure of the manufacturer. A 72- h tryptoy soya broth culture of the isolates was transferred in an Eppendorf tube and centrifuged for 5 minutes to pellet the cells. A 200 µL volume of nuclease free water was added to the cell pellet and vortexed for 1 min in a vortex mixer to wash the cells, it was then centrifuged for 1 min and the supernatant was decanted out. A 50 to 100 mg weight of the bacterial cells was resuspended in 200 µL of nuclease free water and transferred to a ZR Bashing Bead Lysis tube. Then, 750 µL of lysis solution was added to the tube. The tube was then vortexed in a vortex mixer vigorously for 5 to 10 min to lyse the cells. The ZR Bashing Bead Lysis tube was then centrifuged for 1 min at 10,000 × *g*. About 400 µL of supernatant was transferred to a Zymo-Spin IV Spin Filter in a collection tube and centrifuged at 7,000 × *g* for 1 min. To the filtrate in the collection tube, 1,200 µL of bacterial DNA Binding Buffer was added. About 800 µL of the mixture was transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000 × *g* for 1 min. The flow through the collection tube was discarded and the process repeated. Then, 200 µL of DNA Pre-wash buffer was added to the Zymo-Spin IIC column in a new collection tube and centrifuged at 10,000 × g for 1 min. Next, 500 µL of bacterial DNA Wash Buffer was added to the Zymo-Spin IIC column and centrifuged at 10,000 × *g* for 1 min. The Zymo-Spin IIC column was finally transferred to a clean Eppendorf tube and 50 µL of DNA Elution Buffer was added directly into the column matrix and centrifuged at 10,000 × *g* for 30 s to elute the DNA.

PCR identification of *L. monocytogenes*

A 25 µL PCR supermix comprising 12.5 µL One Taq Quick-Load 2× Master Mix with Standard Buffer (New England Biolabs, U.S.A.), 0.5 µL of each primer (10 µM), 4.5 µL of nuclease free water and 5 µL of bacterial genomic DNA solution was subjected to thermocycling conditions, in a TECHNE thermocycler (Bibby Scientific, UK), according to the following schedule: Initial denaturation, 95°C for 5 min; 35 thermal cycles of 95°C, 30 s; 58°C, 30 s; 72°C, 45 s); final extension, 72°C for 5 min; and hold, 4°C. The PCR products were analysed on a 1.5% agarose gel containing ethidium bromide (10 mg/mL) and specific DNA bands were visualized using a bench top UV transilluminator (Upland, U.S.A.). Primers used for PCR are shown in Table 1. Identification of *L. monocytogenes* was by detection of the 370 and 456 bp amplicon of the *prs* and *hlyA* genes, respectively.

Serogrouping of *L. monocytogenes* and dectection of virulence genes

Serogroups of the isolates were determined by PCR using specific primers (Table 1) for the three major serogroups (1/2a, 1/2b and 4b) associated with human listeriosis. The PCR conditions were: Initial denaturation, 94°C for 5 min; 35 thermal cycles of (94°C, 40 s; 53°C, 75 s; 72°C, 75 s); final extension, 72°C for 7 min; and hold,

Table 1. Nucleotide sequences of primers used in this study.

Primer name	Primer sequence (5'-3')	Product size (bp)	Primer target	References
prs	F: GCTGAAGAGATTGCGAAAGAAG R: CAAAGAAACCTTGGATTTGCGG	370	Genus *Listeria*	Doumith et al., 2004
lmo0737	F: AGGGCTTCAAGGACTTACCC R: ACGATTTCTGCTTGCCATTC	691	**Lm 1/2a*	Doumith et al., 2004
ORF 2819	F: AGCAAAATGCCAAAACTCGT R: CATCACTAAAGCCTCCCATTG	471	*Lm 1/2b*	Doumith et al., 2004
ORF 2110	F: AGTGGACAATTGATTGGTGAA R: CATCCATCCCTTACTTTGGAC	597	*Lm 4b*	Doumith et al., 2004
plcA	F:CTGCTTGAGCGTTCATGTCTCCATCCCCC R: CATGGGTTTCACTCTCCTTCTAC	1484	*plcA* gene	Momtaz & Yadollahi, 2013
hlyA	F: GCAGTTGCAAGCGCTTGGAGTGAA R: GCAACGTATCCTCCAGAGTGATCG	456	*hlyA* gene	Swetha et al., 2012
iap	F: ACAAGCTGCACCTGTTGCAG R: TGACAGCGTGTGTAGTAGCA	131	*iap* gene	Swetha et al., 2012

**Lm = L. monocytogenes.*

4°C. The isolates were also screened for the presence of virulence genes, *hylA* and *iap* by PCR, under the same conditions described under DNA amplification above.

RESULTS AND DISCUSSION

Out of a total of 75 *Fura* and 75 *Nunu* samples examined in this study, 23 (30.7%) and 41 (54.7%) samples, respectively, yielded *Listeria* spp., based on growth on PALCAM *Listeria* agar, *Listeria* chromogenic agar and biochemical characterization. These values indicate that both the cereal and milk used in preparation of the RTE *Fura-de-Nunu,* were contaminated with *Listeria* spp. The occurrence recorded in this study are very much higher than the values of about 5 to 7% reported by some authors for raw milk and dairy products (Jamali et al., 2013; Marnissi et al., 2013; Mansouri-Najand et al., 2015; Shamloo et al., 2016), but lower than 78% reported from a study in south western Nigeria on an RTE soft cheese product (Oyinloye, 2016). Six presumptive *Listeria* species were identified from the samples. The distribution of the presumptive *Listeria* spp. in the *Fura* was as follows: *L. ivanovii* (36.7%) > *L. monocytogenes* (30.0%) > *L. seeligeri* (20.0%) > *L. grayi* (10.0%) and *L. welshimeri* (3.3%), while for *Nunu*, it was *L. ivanovii* (46.4%) > *L. monocytogenes* (26.8%) > *L. seeligeri* (16.1%) > *L. innocua* (5.4%) > *L. grayi* (3.6%) and *L. welshimeri* (1.8%). Another study in Nigeria had also reported these species to be the most predominant *Listeria* species in raw milk and attributed their presence in milk to unhygienic milking practices (Yakubu et al., 2012). Among the *Listeria* species, *L. monocytogenes* is the most commonly associated with human listeriosis. Although the illness is relatively rare, it can have mortality rates as high as 30%, especially among vulnerable groups such as infants, pregnant women and the elderly (Mansouri-Najand et al., 2015). For this reason, most western countries have developed set-down policies and guidelines to monitor and control *L. monocytogenes* in foods. Unfortunately, the same cannot be said for Nigeria and some other African countries. The mean *Listeria* counts for the samples in this study were 7.7×10^4 CFU/g and 8.6×10^4 CFU/mL, for *Fura* and *Nunu*, respectively. Although these values represent total *Listeria* counts and not *L. monocytogenes* counts, they are still a cause for concern, considering that *L. monocytogenes* was the second most predominant species isolated from the food samples. According to international standards for acceptable levels of *L. monocytogenes* in foods, foods with a shelf life of less than five days or foods with the potential to support the growth of *L. monocytogenes* should not contain more than 100 CFU/g of food; and when the food is intended for infants, there should be complete absence of *L. monocytogenes* per 25 g of the food (EC 2073/2005; FSA UK, 2010; Health Canada, 2011; FOOD Standards Australia, 2014). The high frequency of *L. monocytogenes* in this study and the high total *Listeria* counts suggest a possibility that the *L. monocytogenes* counts in these products could well exceed the acceptable limit of 100 cfu/g of food. Moreover, as *Fura-de-Nunu* is a street-vended food,

Table 2. Intrinsic parameters of *Fura* and *Nunu* samples.

Sample	a_w	%LA	%NaCl	%Wc	pH
Fura	0.76±0.02	0.31±0.04	2.6±0.17	58.11±8.59	4.5±0.1
Nunu	0.94±0.01	1.17±0.02	3.3±0.12	91.13±1.87	3.6±0.1

Values represent means ± standard deviation of triplicate experiments; a_w = Water activity; LA = lactic acid; NaCl = Sodium chloride; Wc = Water content.

there is no specification for the groups of individuals that can consume the product. It is available to all groups of consumers including infants, pregnant women and the elderly; it is in fact considered a complete food, suitable for weaning babies and should therefore meet the standard of 'complete absence of *L. monocytogenes* / 25 g of food'. Other studies conducted in Nigeria have also reported high loads of *L. monocytogenes* from different foods, including RTE foods (Chukwu et al., 2006; Ikeh et al., 2010; Nwaiwu, 2015).

According to Food Safety guidelines, methods that have been recommended to control the growth of *Listeria* in foods including the use of antibacterial treatments, pasteurization, reduction of pH, refrigeration and consumption within safe periods (FSA UK, 2010; Health Canada, 2011; FOOD Standards Australia, 2014). However, for the RTE food studied here, none of these measures are usually applied. The products are made crudely by local Fulani women and then hawked in pans and containers on the streets, under conditions which would surely support growth of the *Listeria* organisms.

Evaluation of the physico-chemical properties of the samples showed that the pH and water activity (a_w) values of the *Fura* and *Nunu* samples were 4.5± 0.1, 0.76±0.0 and 3.6 ±0.1, 0.94±0.0, respectively (Table 2). Although these values appear to be within the safe ranges (pH < 4.4 and a_w <0.92), the lack of specified shelf life and the conditions under which the foods are vended still leave reasons for concern. According to the Canadian Bureau of Microbial Hazards policy on *L. monocytogenes* in RTE foods (2011), growth of *L. monocytogenes* is assumed to occur in RTE foods if the pH and a_w values fall outside the safe range, unless the RTE food processor is able to present data, to be reviewed by regulatory authorities which demonstrates that the growth of *L. monocytogenes* will not occur within the specified shelf life (Health Canada, 2011). Sadly, for the RTE *Fura de Nunu*, these criteria are neither considered nor met, before the street-hawking of the products.

Representative *L. monocytogenes* isolates (n=23) identified by phenotypic and biochemical methods were subjected to PCR, which has proved to be a very useful and rapid method for detection of *Listeria* (Mansouri-Najand et al., 2015). Screening of the presumptive *L. monocytogenes* isolates by PCR showed that only about 77% of the isolates could be confirmed as *L. monocytogenes*, by detection of the 370 bp amplicon and 456 bp amplicon of the *prs* and *hlyA* genes, for identification of genus *Listeria* and *L. monocytogenes*, respectively (Figures 1 and 2). This result suggests that non-molecular methods of detection of these organisms may not be very specific and may lead to artificially high prevalence values. Thus, more specific molecular methods such as PCR must be used when evaluating *L. monocytogenes* contamination of food samples. The value of molecular methods of identification and typing has also been emphasized by other authors. For instance, it has been suggested that molecular methods are useful not just for accurate identification, epidemiological and trace-back investigations, but also for understanding the diversity and evolution of the organism (Doumith et al., 2004; Laksanalamai et al., 2014). It has also been suggested that although the classical biochemical methods of identification are standard, they can sometimes give false positives (Nwaiwu, 2015).

L. monocytogenes has 13 serotypes (1/2a,1/2b,1/2c, 3a, 3b, 3c, 4a, 4b, 4ab, 4c, 4d, 4e, and 7), but serogroups 1/2a, 1/2b and 4b are believed to be responsible for about 95% of human listeriosis (Kathariou, 2002; Jamali et al., 2013). Out of 24 *L. monocytogenes* (23 isolates and reference strain) screened by PCR-serogrouping in this study, only seven (including the reference strain) could be placed in any of the three serogroups tested for; and of these, only 1/2a and 4b were identified (Table 3). The 1/2a isolates were positive for *lmo0737* gene alone while the 4b isolates showed the presence of *ORF 2110.* Therefore, even though it has been suggested that variant strains of one serotype can sometimes acquire the gene clusters of another as in the reports of Laksanalamai et al., (2014), no such variants were detected in this study. Due to the epidemiological and clinical relevance of serotypes of the organism, serotyping is usually recommended for suspected *L. monocytogenes* isolates, particularly since the development of a PCR-based grouping scheme by Doumith et al. (2004). This molecular serogroup-related PCR typing has been endorsed by other researchers (Huang et al., 2011; Laksanalamai et al., 2014).

It has been suggested that pathogenic *L. monocytogenes* may be identified based on detection of some virulence markers, including internalins (*inlA, inlC, inlJ*), listeriolysin O (*hlya*), actin polymerization protein

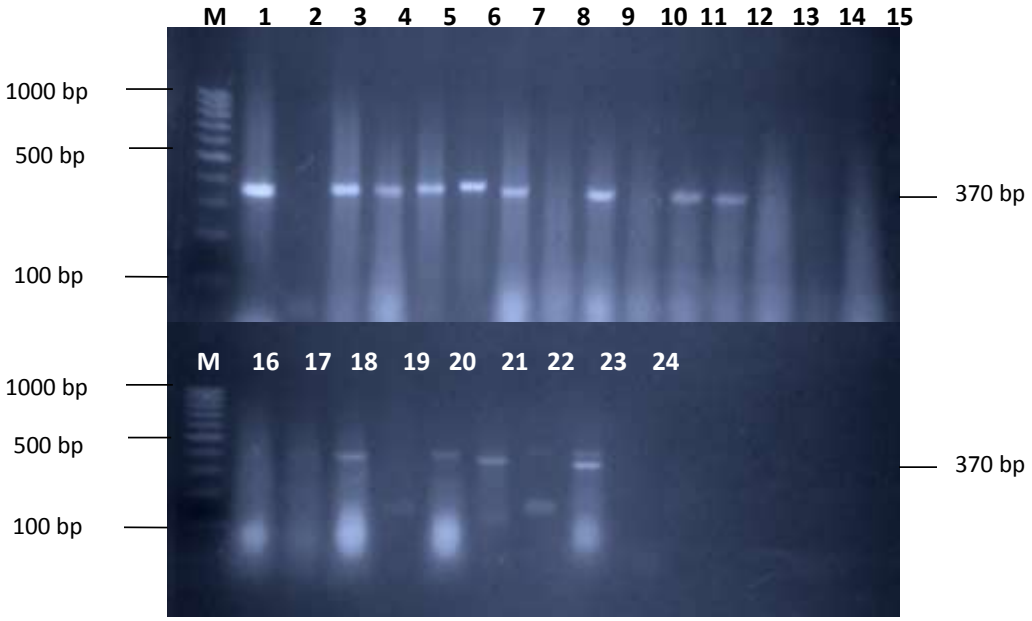

Figure 1. PCR detection of 370 bp amplicon of *prs* gene for *identification* of *Listeria* species. Lane M, 100 bp DNA ladder; lane 1, positive control (*L. monocytogenes* ATCC13932); lanes 2-23, presumptive *L. monocytogenes* isolates; lane 24 negative control. Lanes 3, 4, 5, 6, 7, 9, 11, 12, 18, 20, 21 and 23 are positive.

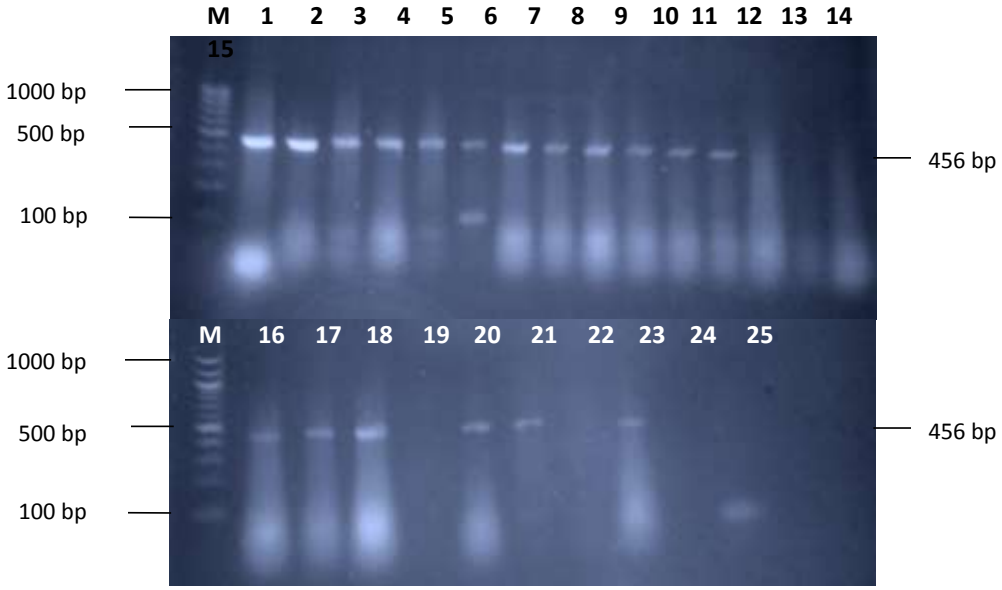

Figure 2. PCR detection of 456 bp amplicon of *hlyA* gene for identification of *L. monocytogenes*. Lane M, 100 bp DNA ladder; lane 1, positive control (*L. monocytogenes* ATCC13932); lanes 2-23, presumptive *L. monocytogenes* isolates; lane 25 negative control. Lanes 2-12, 16-18, 20, 21 and 23 are positive.

(*actA*), phosphatidyl-inositol-phospholipase C (*plcA*), invasive associated protein (*iap*) and virulence regulator (*prfA*), which are believed to be important in *L. monocytogenes* infection and pathogenesis (Liu et al., 2007; Di Ciccio et al., 2012). PCR-based methods have also been used to identify pathogenic *L. monocytogenes* strains possessing these genes (Huang et al., 2011; Laksanalamai et al., 2014; Swetha et al., 2015). The

Table 3. Genetic characteristics of some *L. monocytogenes* isolates from *Fura* and *Nunu.*

Isolate ID	Source	PCR amplification reaction						PCR serogroup
		prs	*hlyA*	*iap*	*Lmo 0737*	*ORF 2819*	*ORF 2110*	
N21	Nunu	+	+	+	-	-	+	4b
N54	Nunu	+	+	+	-	-	+	4b
N69	Nunu	+	+	+	-	-	+	4b
N71	Nunu	+	+	+	+	-	-	1/2a
N72	Nunu	+	+	+	+	-	-	1/2a
F3	Fura	+	+	+	+	-	-	1/2a
TC	ATCC 13932	+	+	+	-	-	+	4b

isolates in this study were screened for the presence of two virulence genes: *hlyA* and *iap* by PCR and both genes were detected in some of the isolates (Table 3). Detection of these virulence genes suggests that the *L. monocytogenes* isolates from this study are pathogenic. Further studies will aim to carry out full genome sequencing on these isolates, in order to further characterize and document the strains. The isolation of virulent *L. monocytogenes* from the RTE product, *Fura-de-Nunu,* in this study is a significant cause for concern. *Fura de Nunu* is considered to be a complete food and is relatively cheap. Therefore, in a country with a large population of low-income earners, it comes highly recommended as a means of reducing malnutrition and is widely consumed across demographic groups, including pregnant women, infants and the elderly, who are among the groups most at risk for listeriosis. The results from this study highlight a need for the development and implementation of food safety policies and standards to guide the production and distribution of RTE foods in Nigeria. Producers and consumers of RTE products should also be made aware of the need to observe the highest possible standards of hygiene during production of RTE and street-vended foods to reduce the risk of listeriosis and other food-borne diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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