

## Occurrence of *Listeria* Species in Nigerian Street-Prepared Foods and Association with their Environment and Antibiotic Resistance Status

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### Abstract

The presence and detection frequency of *Listeria* species in some Nigerian street-prepared foods were investigated along with association with their sales locations and antibiotic resistance. Multi-stage sampling technique was adopted with towns, street locations (earth/tarred roads, market and Motor Park areas, and dumpsites' vicinity), and sales points as primary, secondary, and tertiary stages, respectively. The selected popular foods were roasted plantain and yam, akara, and fried fish. Detection and enumeration of *Listeria* in 192 food samples were undertaken using buffered peptone broth (resuscitation) and *Listeria* selective medium. Identification was by API system while identities of 3 most frequently detected species were confirmed by molecular methods. Susceptibility of the 3 species to 10 commonly used antibiotics was determined by agar diffusion method. Detected species with frequencies (%) were: *L. monocytogenes*, 29.6; *L. innocua*, 23.9; *L. grayi* 21.8; *L. ivanovii* 5.7; *L. seeligeri* 3.6; and *L. welshimeri* 3.1. The presence of *L. monocytogenes* and *L. grayi* in food samples was significantly associated with all locations ( $X^2=5.16-17.42$ ;  $P=0.000-0.02$ ) while only Motor Park and dumpsite environments were associated with *L. innocua* ( $X^2=12.08$ ;  $P=0.001$ ). The 3 species were MDR and amoxicillin, co-trimoxazol and erythromycin were the commonly resisted antibiotics. MAR index of *L. monocytogenes* was higher ( $0.44\pm0.08$  vs  $0.40\pm0.05$  and  $0.31\pm0.06$ ;  $F=45.03$ ;  $P=0.003-0.006$ ) than those of *L. innocua* and *L. grayi*. The vulnerability of street foods to contamination by *L. monocytogenes*, the causative agent of listeriosis, was therefore indicated. Health agencies can intervene at sales environment to limit street food contamination by *Listeria* species.

**Keywords:** *Listeria* species, street foods, sales environment, antibiotics resistance.

### Резюме

Изследвани са наличието и честотата на откриване на видове *Listeria* в някои нигерийски улично приготвени храни, както и връзката им с местата на продажба и антибиотичната резистентност. Възприета е многостепена техника за вземане на проби, като градовете, уличните местоположения (земни/асфалтирани пътища, пазарни зони и автопаркове, както и близостта до сметища) и пунктовете за продажба са съответно първични, вторични и третични етапи. Избраните популярни храни са печени живовляк и ямс, акара и пържена риба. Откриването и преброяването на *Listeria* в 192 хранителни проби са извършени с помощта на буфериран пептонов бульон (реанимация) и специфична селективна среда. Идентификацията е извършена чрез API система, докато идентичността на 3-те най-често откривани вида е потвърдена чрез молекулярни методи. Чувствителността на 3-те вида към 10 често използвани антибиотика е определена чрез метод на агар-дифузия. Откритите видове със съответната честота (%) са: *L. monocytogenes* - 29.6; *L. innocua* - 23.9; *L. grayi* - 21.8; *L. ivanovii* - 5.7; *L. seeligeri* - 3.6; и *L. welshimeri* 3.1. Наличието на *L. monocytogenes* и *L. grayi* в хранителни проби е свързано в значителна степен с всички местоположения ( $X^2=5.16-17.42$ ;  $P=0.000-0.02$ ), докато със средата на автопаркове и сметища е свързана с *L. innocua* ( $X^2=12.08$ ;  $P=0.001$ ). Трите вида са MDR, като най-често са те проявяват резистентност към антибиотичите амоксицилин, ко-тримоксазол и еритромицин. MAR индексът на *L. monocytogenes* е по-висок ( $0.44\pm0.08$  спрямо  $0.40\pm0.05$  и  $0.31\pm0.06$ ;  $F=45.03$ ;  $P=0.003-0.006$ ) от този на *L. innocua* и *L. grayi*. Посочена е уязвимостта на уличните храни към замърсяване с *L. monocytogenes*, причинителят на листериоза. Здравните агенции могат да се намесят в средата на продажбите, за да ограничат замърсяването на уличната храна с видове *Listeria*.

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Acta Microbiol. Bulg. 2025; 41(02). <https://doi.org/10.59393/amb25410212>

## Introduction

Members of the genus *Listeria* are Gram-positive bacteria that are non-spore-forming rods, but may sometimes look coccoid or occur in short chains (Holt *et al.*, 2000). They are catalase-positive organisms that can grow at low temperatures and are also capable of survival for a considerable length of time in the environment (Dufailu *et al.*, 2021). The identified species of *Listeria* are 17 (Dufailu *et al.*, 2021) although 20 were reported by Nwaiwu (2020). *Listeria monocytogenes* is known to be the major cause of the food-borne disease known as listeriosis which arises mainly from ingestion of contaminated food, especially ready-to-eat (RTE) foods. However, the report by Orsi and Wiedmann (2016) suggests that *L. innocua* can be pathogenic in humans. While there are reports that *L. ivanovii* is pathogenic in animals (Dufailu *et al.*, 2021; Sarr *et al.*, 2023), species such as *L. grayi*, *L. seeligeri* and *L. welshimeri* that have been isolated from milk, fish and meat (Rahimi *et al.*, 2012; Nayak *et al.*, 2015) are not known to be pathogenic.

*Listeria* species are ubiquitous having been isolated from soil, water, vegetation, and foods (Sarr *et al.*, 2023). The genus *Listeria* is important in public health because of listeriosis caused by *L. monocytogenes*. Isolation of several species of *Listeria* from raw and processed milk, raw meat and meat products, vegetables, and RTEs has been documented in several studies across the world (e.g. Ikeh *et al.*, 2010; Orsi and Wiedmann, 2016; Koskar, 2019; Gebremedhin *et al.*, 2021; Sotohy *et al.*, 2024). Investigations by El-Shenawy *et al.* (2011), Ajayeoba *et al.* (2016), Gebremedhin *et al.* (2021), and the review report by Dufailu *et al.* (2021) showed that studies on *Listeria* focused more on the prevalence of *L. monocytogenes* isolated mainly from RTEs such as meat, fish, dairy products and vegetables, and their antibiotics susceptibility profiles. Other potential sources of *Listeria* like the environment and typical traditional street foods in Africa are under-investigated.

Several studies on the occurrence of *Listeria* species in Nigeria focused more on meat (raw pork, beef, chicken), fish, seafood, poultry, dairy products and vegetables (e.g. Amusa *et al.*, 2017; Amusan *et al.*, 2019; Okorie-Kanu *et al.*, 2020; Chukwu *et al.*, 2021; Uzeh *et al.*, 2021; Agboola and Bisi-Johnson, 2023; Kester *et al.*, 2024). Though limited, some reports focused on *Listeria* species in humans, animals, and the environment (Nwaiwu, 2015). However, investigations on the occurrence

of *Listeria* spp in Nigeria rarely include typical traditional foods prepared along the streets such as akara, roasted plantain, roasted yam, and fried fish, or those prepared at home and sold along the streets with pushcarts or in stalls (e.g. rice, beans, moimoin, and garri). These street foods attract very good patronage in the mornings and evenings hence their vulnerability to contamination by *Listeria* species should be of public health concern. Studies on bacterial contamination of street foods in Nigeria (e.g. Ossai, 2012; Oluwafemi *et al.*, 2013; Madueke *et al.*, 2014; Garba, 2023) rarely report the presence of *Listeria* species.

It will be of public health interest if *Listeria* species especially *L. monocytogenes*, are also found in street-prepared foods because listeriosis has a 20-30% mortality rate across the world (Buchanan *et al.*, 2017). The investigation was therefore, aimed at ascertaining: 1, the presence of *Listeria* species in 4 selected street-prepared foods (akara, fried fish, roasted yam, and roasted plantain); 2, frequently detected *Listeria* species; 3, their association with preparation and sales environment; and 4, their antibiotics resistance status.

## Materials and Methods

### Collection of samples

The focus of the investigation was on foods prepared on street-sides, hence, roasted plantain (*Musa parasidisiaca*), roasted yam (*Dioscorea* sp.), fried fish, and akara (made from cowpea *Vigna unguiculata*), was selected for the study. While akara is mostly sold in the mornings and evenings, roasted yam and plantain are virtually sold throughout the day. Akara is prepared from de-hulled and wet-milled cowpea paste mixed with pepper, onions, and salt, and fried directly with vegetable oil.

A multi-stage technique was adopted for sample collections with 3 major towns (Asaba, Ughelli, and Warri) in the 3 districts of Delta State, Nigeria as primary stages. Thereafter, 4 environmental-based locations (road, market, Motor Park, and vicinity of waste dumpsites) were selected as the secondary stage. These 4 locations were further split into 8 as follows: earth roads; tarred roads; within markets; outside markets (50-100m); within Motor Parks; outside Motor Parks (50-100m) and within ( $\leq 50$ m) and outside ( $> 50$ -100m) vicinity of dumpsites. The third stage was the random selection of 2 sales points at each location in the 3 towns for each of the 4 street foods (2 x 3 x 4). This brings the total number of sales-point food samples to be analyzed to 24/locations and a total of 192

for all locations. The details of the distribution are presented in Appendix A. The samples were placed in ice packs and transported to the laboratory for analysis.

#### *Isolation and enumeration of Listeria species*

The Biological Analytical Manual's direct plating method (Hitchins *et al.*, 2022) was adopted for the detection and enumeration of *Listeria* spp. Food samples weighing 25 g each were blended in 225 ml of buffered peptone water (Sheridan *et al.*, 1994) and kept for 4h to resuscitate *Listeria* cells. Triplicate plates of *Listeria* selective agar (Lifesave Biotech, San Diego USA) were subsequently inoculated with 1ml of the homogenate and incubated for up to 72 h at room temperature (30±2°C). The colonies that appeared were sub-cultured for purification and identification by biochemical tests using the analytical profile index (API). The identity of the most frequently detected 3 *Listeria* species was subsequently confirmed by molecular method and tested for antibiotic resistance.

#### *API protocol*

API *Listeria* 10 test kits (Bio Merieux, France) are for determining the presence or absence of acrylamidase known as differentiation inocula monocytogenes (DIM) test, hydrolysis of esculin (ESC), detection of  $\alpha$ -mannosidase (MAN) and fermentation of D-arabitol (DARL), D-xylose (XYL), L-rhamnose (RHA),  $\alpha$ -methyl-D-glucoside (MDG), D-ribose (RIB), glucose-1-phosphate (GIP), and D-tagatose (TAG). In order to create a humid environment, 3 ml of sterile distilled water or de-mineralized water was distributed into honeycomb wells of the incubation box (tray and lid). Thereafter the reaction test strip was removed from the package and placed in the incubation box. Ampule of API suspension medium (2ml) was opened and *Listeria* colonies were taken from 18-24 hours old cultures and transferred into 2ml of the API suspension medium to attain a turbidity equivalent to 1.0 McFarland standard. The bacterial suspension of each isolate was distributed into each of the cupules (100µl for the DIM test and 50 µl for other tests). With regards to the DIM test, a drop of ZYMB reagent was added and the reactions were read within 3 minutes. The color reactions given by the manufacturers and the API web identification software were used for identifying the species of *Listeria*.

#### *Molecular identification*

Based on the outcome of the API tests, the most frequently detected 3 *Listeria* species were

subjected to molecular identification tests. DNA was extracted using the protocol stated by (Trinidad *et al.*, 2007). Succinctly, the pure culture of the *Listeria* that was propagated on nutrient agar was transferred to 1.5 ml of liquid medium, and grown on a shaker for 48 h at 28°C before centrifugation at 4600 g for 5 min. The pellets were re-suspended in 520 µl of TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) and thereafter 15 µl of 20% SDS and 3 µl of proteinase K (20 mg/ml) were added. The reaction mixture was incubated at 37 °C for 1 h after which 100 µl of 5 M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and vortexed. The mixture was subsequently incubated at 65 °C for 10 mins and set aside on ice for 15 mins. Chloroform: isoamyl alcohol (24:1) was added, and incubated on ice for 5 mins. This was followed by centrifugation at 7200 g for 20 mins. DNA was precipitated at -20°C for 16 h following the transfer of the aqueous phase to another tube where isopropanol (1: 0.6) was added. The DNA was recovered by centrifugation at 13000g for 10 mins and washed with 500 µl of 70% ethanol before air-drying for approximately 3 h at room temperature. It was thereafter dissolved in 50 µl of TE buffer.

The combination of reagents used for the PCR process was: 10 µl of 5x Go Taq colorless reaction; 3 µl of 25 mM MgCl<sub>2</sub>; 1 µl of 10 mM of dNTPs mix; 1 µl of 10 pmol each 27F 5' AGA GTT TGA TCM TGG CTC AG3' and 1525R, AAG-GAGGTGATCCAGCC3' primers; and 0.3 units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water, 8 µl DNA template. Gene Amp 9700 PCR System Thermal cycler (Applied Biosystem Inc., USA) was used for the PCR in accordance with the manufacturer's guidelines..

Agarose gel (1.5%) was used to confirm amplification. Electrophoresis of the gel was carried out at 120 V for 45 mins and visualized by ultraviolet transillumination and photographed. The amplified fragments were purified with ethanol for the purpose of removing the PCR reagents. The fragment was checked on a 1.5% agarose gel run on a voltage of 110V for about 1 h to confirm the presence of the purified products which were subsequently quantified using a nano drop of model 2000 from Thermo Scientific.

Genetic Analyzer 3130 xl (Applied Biosystems) was used for sequencing as guided by the manufacturer's manual. Big Dye terminator v3.1 cycle sequencing kit was used and Bio Edit soft-

ware was used for all sequence editing and cluster alignment, while all genetic analyses were by MEGA 6.

#### Antibiotics susceptibility profile

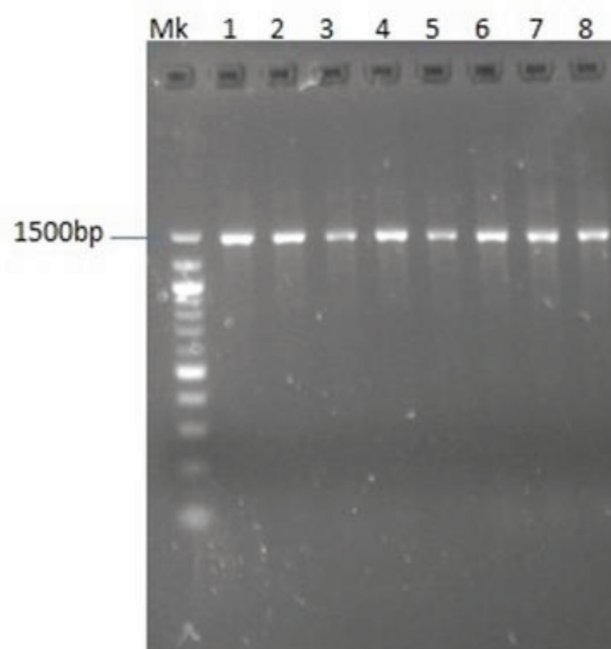
The most frequently isolated 3 *Listeria* species were tested for antibiotic resistance on Mueller-Hinton agar using the agar diffusion method by the protocol of the Clinical and Laboratory Standards Institute (CLSI, 2018). The number of randomly selected isolates used for the tests was 120 based on 10/species/food type; (10 x 3 x 4=120). The antibiotics used were: Amoxicillin (10 µg); Co-trimoxazole (25 µg), ceftriazone (30 µg), tetracycline (10 µg), levofloxacin (5 µg), perfloxacin (30 µg); ofloxacin (5 µg), Erythromycin (15 µg), Azithromycin (15 µg) and Ciprofloxacin (10 µg).

#### Data analyses

The frequency of occurrence of the *Listeria* species was calculated on the basis of the number of food samples where they were detected. The relationship between the sales points' environment and the number of food samples where the species were detected was analyzed by Chi-square statistics. The multiple antibiotics resistance (MAR) index was calculated by dividing the number of antibiotics resisted by the total number of antibiotics used. MAR indexes of the *Listeria* spp isolated from each of the 4 street foods were compared by ANOVA.

## Results

The identity of the *Listeria* species isolated from the 4 street foods is presented in Table 1. The identity of the most frequently isolated 3 species (*L. monocytogenes*, *L. innocua*, and *L. grayi*) was confirmed following DNA extraction and amplification (Fig. 1), sequencing, and NCBI BLAST analyses (Table 2).



**Fig. 1.** Agarose gel electrophoresis of the PCR products 16S rRNA amplified from selected bacteria isolates. (Band size approximately 1500bp). Gel image indicates a positive amplification in all samples

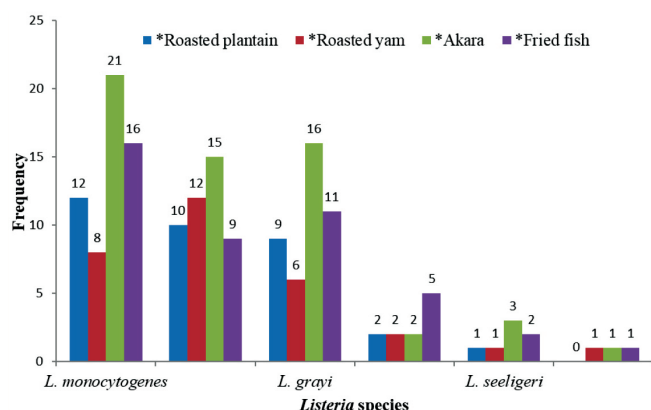
**Table 1.** Identity of isolates by API system

| Tests               | Identified <i>Listeria</i> species |                   |                 |                    |                     |                      |
|---------------------|------------------------------------|-------------------|-----------------|--------------------|---------------------|----------------------|
|                     | <i>L. monocytogenes</i>            | <i>L. innocua</i> | <i>L. grayi</i> | <i>L. ivanovii</i> | <i>L. seeligeri</i> | <i>L. welshimeri</i> |
| DIM                 | -                                  | +                 | +               | +                  | +                   | +                    |
| Esculin             | +                                  | +                 | +               | +                  | +                   | +                    |
| α-Mannose           | +                                  | +                 | +               | -                  | -                   | +                    |
| D-Arabitol          | +                                  | +                 | +               | +                  | +                   | +                    |
| D-Xylose            | -                                  | -                 | -               | +                  | +                   | +                    |
| L-Rhamnose          | +                                  | +                 | -               | -                  | -                   | +                    |
| α-Methyl-D-glucose  | +                                  | +                 | +               | +                  | +                   | +                    |
| D-Ribose            | -                                  | -                 | +               | +                  | -                   | -                    |
| Glucose-1-phosphate | -                                  | -                 | -               | +                  | -                   | -                    |
| D-Tagatose          | -                                  | -                 | -               | -                  | -                   | +                    |

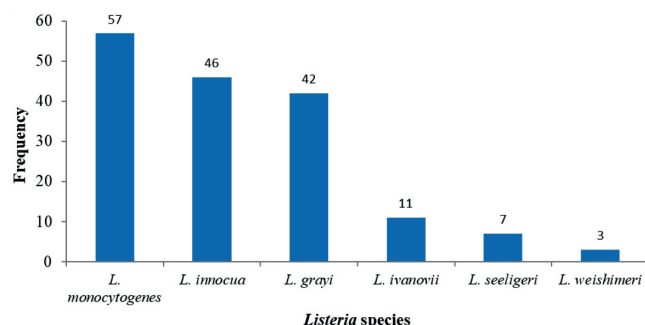
**Table 2.** Molecular identification: NCBI Blast showing the sequence identity of the isolate

| Scientific Name         | Max Score | Total Score | Query Cover | E value | Per. Ident | Accession |
|-------------------------|-----------|-------------|-------------|---------|------------|-----------|
| <i>L. innocua</i>       | 2037      | 12216       | 99%         | 0       | 99.82%     | PQ796058  |
| <i>L. innocua</i>       | 2013      | 12072       | 99%         | 0       | 99.37%     | PQ796059  |
| <i>L. grayi</i>         | 1829      | 10966       | 99%         | 0       | 99.80%     | PQ796061  |
| <i>L. grayi</i>         | 2049      | 2049        | 99%         | 0       | 99.82%     | PQ796062  |
| <i>L. monocytogenes</i> | 2041      | 12250       | 99%         | 0       | 100.00%    | PQ796063  |
| <i>L. monocytogenes</i> | 2034      | 12205       | 99%         | 0       | 99.91%     | PQ796064  |

Figure 2 presents the frequency of occurrence of the API system-identified *Listeria* species which showed *L. monocytogenes*, *L. innocua*, and *L. grayi* as the most frequently isolated. *L. monocytogenes* “took the lead” in all the food types except in roasted yam where *L. innocua* was the most frequently detected (Fig. 2). The occurrence of *Listeria* species in the 4 street foods tended to be more in akara and fried fish (Fig. 1). Although, by overall assessment, *L. monocytogenes* remained the most frequently encountered, it was only detected in 57 food samples (Fig. 3). This translates to 29.68% of the 192 food samples tested.



**Fig. 2.** Frequency of detection of *Listeria* spp. based on food type (\*n=48/food type, see Appendix A)



**Fig. 3.** Frequency of detection of *Listeria* spp in the street foods by overall assessment (N=192, see Appendix A)

The results of the Chi-square analyses of the association between the presence of *Listeria* species on the tested street foods and the environment of the sales points are presented in Table 3. The presence of *L. monocytogenes* and *L. grayi* was significantly associated with the earth road and market environment while the presence of *L. innocua* was not associated with both environments (Table 3). However, significant associations were found between the presence of the 3 *Listeria* species, and the Motor Park and dumpsite environments (Table 3).

Table 4 presents the antibiotic resistance profile of *Listeria* species isolated from each of the

4 tested street foods. A high prevalence of resistance to cotrimoxazol, azythromycin, and erythromycin was observed with *L. monocytogenes* irrespective of food source while a high prevalence of resistance to amoxicillin occurred only with the strains isolated from roasted plantain and yam.

In addition, *L. monocytogenes* strains from akara and fried fish were highly resistant to tetracycline and ofloxacin. Concerning *L. innocua*, a high prevalence of resistance to amoxicillin, cotrimoxazol, tetracycline, and erythromycin was encountered. On the other hand, *L. grayi* was highly resistant to only 3 antibiotics (amoxicillin, cotrimoxazol, erythromycin) irrespective of food source. Generally, the commonly resisted antibiotics were amoxicillin, cotrimoxazol, and erythromycin. As can be seen in Table 5, the mean MAR indexes were generally above 0.3 with *L. monocytogenes* having a significantly higher index than those of the other two species. The MAR index of *L. innocua* was similarly found to be significantly higher than that of *L. grayi* isolates. These differences occurred irrespective of the food source and it remained when the food sources were combined (Table 5).

## Discussion

The results have shown that street foods are also vulnerable to contamination by *Listeria* species; hence, the potential for an outbreak of listeriosis arising from street foods exists. Traditional foods mainly constitute street foods and they are yet to receive adequate attention in respect of the occurrence of *Listeria* spp. Although El-Shenawy *et al.* (2011) reported the presence of *L. monocytogenes* and *L. innocua* in street-vended Egyptian traditional foods, follow-up studies were scarce. The review by Dufailu *et al.* (2021) did not report any case of *Listeria* spp. from typical street foods in Nigeria or other African countries. This substantiates the paucity of reports on the occurrence of *Listeria* spp. in street foods. This paucity may be attributed to methods of isolation that often fail to consider the slow growth habit of *Listeria* amongst other pathogenic bacteria present in street foods. Rejuvenation or resuscitation processes via the use of a pre-enrichment medium are generally used in the scheme for the detection and isolation of *Listeria*.

The *Listeria* species identified were 6 and except *L. monocytogenes*, the other 5 species have been inconsistently isolated from non-street foods in some countries. These are: pelagic fish, sea foods, smoked fish, raw pork meat, beef and chicken meat in Nigeria (Amusan *et al.*, 2017; Amusan

**Table 3.** Chi square analyses of the association between location/environment and the presence of *Listeria* species in the foods

| Location          | <i>Listeria</i> species |         | No of sales points |                          | X <sup>2</sup> (P) |
|-------------------|-------------------------|---------|--------------------|--------------------------|--------------------|
|                   |                         |         | Earth roads (n=24) | Tarred roads (n=24)      |                    |
| Roads             | <i>L. monocytogenes</i> | Present | 16                 | 6                        | 8.892(0.004)       |
|                   |                         | Absent  | 18                 | 18                       |                    |
|                   | <i>L. innocua</i>       | Present | 14                 | 9                        | 2.087(0.124)       |
|                   |                         | Absent  | 10                 | 15                       |                    |
|                   | <i>L. grayi</i>         | Present | 15                 | 7                        | 5.371(0.021)       |
|                   |                         | Absent  | 9                  | 17                       |                    |
| Market            | <i>L. monocytogenes</i> | Present | Within<br>22       | Outside (50-100m)<br>12  | 10.084(0.002)      |
|                   |                         | Absent  | 2                  | 12                       |                    |
|                   | <i>L. innocua</i>       | Present | 17                 | 11                       | 3.086(0.071)       |
|                   |                         | Absent  | 7                  | 13                       |                    |
|                   | <i>L. grayi</i>         | Present | 14                 | 6                        | 5.480(0.021)       |
|                   |                         | Absent  | 10                 | 18                       |                    |
| Motor Park        | <i>L. monocytogenes</i> | Present | Within<br>21       | Outside (50-100 m)<br>14 | 5.169(0.024)       |
|                   |                         | Absent  | 3                  | 10                       |                    |
|                   | <i>L. innocua</i>       | Present | 19                 | 7                        | 12.084(0.001)      |
|                   |                         | Absent  | 5                  | 17                       |                    |
|                   | <i>L. grayi</i>         | Present | 18                 | 4                        | 16.448(0.000)      |
|                   |                         | Absent  | 6                  | 20                       |                    |
| Dumpsite vicinity | <i>L. monocytogenes</i> | Present | ≤50m<br>22         | >50m<br>8                | 17.422(0.000)      |
|                   |                         | Absent  | 2                  | 16                       |                    |
|                   | <i>L. innocua</i>       | Present | 19                 | 7                        | 12.084(0.001)      |
|                   |                         | Absent  | 5                  | 17                       |                    |
|                   | <i>L. grayi</i>         | Present | 19                 | 9                        | 8.571(0.004)       |
|                   |                         | Absent  | 5                  | 15                       |                    |

**Table 4.** Antibiotics resistance patterns of strains of *Listeria* species isolated from the street foods

| Antibiotics   | Resistance (%)          |      |      |      |                   |       |       |      |                 |      |      |      |
|---------------|-------------------------|------|------|------|-------------------|-------|-------|------|-----------------|------|------|------|
|               | <i>L. monocytogenes</i> |      |      |      | <i>L. innocua</i> |       |       |      | <i>L. grayi</i> |      |      |      |
|               | RPL*                    | RY*  | AK*  | FF*  | RPL*              | RY*   | AK*   | FF*  | RPL*            | RY*  | AK*  | FF*  |
| Amoxicillin   | 70.0                    | 60.0 | 10.0 | 20.0 | 80.0              | 70.0  | 90.0  | 90.0 | 90.0            | 100  | 80.0 | 100  |
| Cetirizone    | 0.00                    | 0.00 | 0.00 | 0.00 | 0.00              | 0.00  | 0.00  | 0.00 | 10.0            | 10.  | 10.0 | 20.0 |
| Cotrimoxazol  | 80.0                    | 80.0 | 90.0 | 100  | 60.0              | 70.0  | 80.0  | 70.0 | 80.0            | 90.0 | 100  | 100  |
| Tetracycline  | 10.0                    | 0.00 | 70.0 | 100  | 60.0              | 60.0  | 70.0  | 70.0 | 20.0            | 0.00 | 10.0 | 20.0 |
| Levofloxacin  | 0.00                    | 0.00 | 10.0 | 20.0 | 0.00              | 0.00  | 10.0  | 10.0 | 0.00            | 0.00 | 0.00 | 0.00 |
| Pefloxacin    | 0.00                    | 0.00 | 0.00 | 10.0 | 0.00              | 0.00  | 0.00  | 0.00 | 0.00            | 0.00 | 0.00 | 0.00 |
| Azythromycin  | 100                     | 100  | 100  | 100  | 10.0              | 10.0  | 20.0  | 0.00 | 0.00            | 0.00 | 10.0 | 10.0 |
| Ofloxacin     | 20.0                    | 20.0 | 90.0 | 90.0 | 0.00              | 0.00  | 0.00  | 0.00 | 0.00            | 0.00 | 0.00 | 0.00 |
| Ciprofloxacin | 0.00                    | 0.00 | 0.00 | 10.0 | 0.00              | 20.00 | 20.00 | 0.00 | 0.00            | 0.00 | 0.00 | 0.00 |
| Erythromycin  | 100.                    | 100  | 100  | 100  | 100               | 100   | 90.0  | 100  | 100             | 100  | 80.0 | 90.0 |

Street foods: RPL, roasted plantain; RY, roasted yam; AK, akara; FF, fried fish. \*n=10

**Table 5.** Comparison of MAR index of *Listeria* species isolated from the four street foods

| Street food source of <i>Listeria</i> spp. | Mean MAR index±SD of:    |                          |                          | F(P)         |
|--------------------------------------------|--------------------------|--------------------------|--------------------------|--------------|
|                                            | <i>L. monocytogenes</i>  | <i>L. innocua</i>        | <i>L. grayi</i>          |              |
| Roasted plantain                           | 0.40±0.047 <sup>a</sup>  | 0.4±0.066 <sup>b</sup>   | 0.30±0.066 <sup>ab</sup> | 9.00(0.001)  |
| Roasted yam                                | 0.39±0.056 <sup>c</sup>  | 0.39±0.056 <sup>d</sup>  | 0.31±0.073 <sup>cd</sup> | 5.38(0.011)  |
| Akara                                      | 0.51±0.087 <sup>ef</sup> | 0.4±0.047 <sup>eg</sup>  | 0.31±0.056 <sup>fg</sup> | 22.95(0.000) |
| Fried fish                                 | 0.49±0.056 <sup>hj</sup> | 0.41±0.056 <sup>hk</sup> | 0.30±0.066 <sup>jk</sup> | 25.07(0.000) |
| All foods combined                         | 0.44±0.081 <sup>mn</sup> | 0.40±0.055 <sup>mp</sup> | 0.31±0.063 <sup>np</sup> | 45.73(0.00)  |

Values with the same superscripts are significantly different across rows.  $P^{a,b,c,n}=0.003$ ;  $P^{c,d}=0.022$ ;  $P^{f,i,p}=0.000$ ;  $P^g=0.014$ ;  $P^h=0.017$ ;  $P^k=0.001$ ;  $P^m=0.006$ .

*et al.*, 2019; Okorie-Kanu *et al.*, 2020; Chuku *et al.*, 2021; Uzeh *et al.*, 2021; Kester *et al.*, 2024); chicken, fish and camel milk in Egypt (Yehia *et al.*, 2016); meat from abattoirs and butchers in Ethiopia (Gebremedhin *et al.*, 2021), and raw meat in Iran (Tahimi *et al.*, 2012). The frequency of occurrence of these species reported in these studies varied with *L. monocytogenes* and *L. innocua* being the most frequently isolated. This tends to be consistent with the findings here because *L. monocytogenes* and *L. innocua* were the most frequently detected species in the 4 street foods investigated. *Listeria grayi* followed *L. innocua* marginally in the third position. The frequencies of the remaining 3 species were markedly lower; hence, the focus of attention in subsequent tests was on *L. monocytogenes*, *L. innocua*, and *L. grayi*.

It is known from literature sources that *Listeria* species are ubiquitous having been isolated from foods, soil, and water bodies including sewage (Kayode *et al.*, 2020). The locations of the foods investigated for the presence of *Listeria* in the reports cited in the preceding paragraph, were markets, streets, abattoirs, and butchers' environments. Human traffic and wind can aerosolise bacteria from soil and nearby waste dumpsites which may eventually settle on food items. It was, therefore, not a surprise that the occurrence of the *Listeria* species in the street foods investigated was associated with the environment of the sales points as indicated by the chi-square analysis. A similar association with environments was found concerning makeshift eateries (Ejechi *et al.*, 2023) and pushcart street foods (Ejechi and Ejechi, 2024) in a Nigerian region. *Listeria* can easily be eliminated at cooking temperatures (Chan and Wiedmann, 2008); hence, the source of the *Listeria* spp. found in foods cooked and sold in the open can only be the environment.

The frequencies of detection of the *Listeria* spp. tended to be higher in akara and fried fish than in roasted plantain and yam. Both akara and fried fish are protein-based foods while plantain and yam are mainly carbohydrates. This tends to suggest that the protein content made akara and fried fish better supporters of growth than plantain and yam. Literature sources indicate that protein-rich foods such as, raw and processed forms of milk, fish, chicken, beef, and pork meat are main sources of *Listeria* spp. although fruits and vegetables have also been reported as sources (FSAI, 2011, Kayode *et al.*, 2020).

The antibiotics resistance profiles of the most frequently detected 3 *Listeria* species were as-

certained and they can all be classified as multi-drug resistant because the MAR indexes were  $\geq 0.3$ . This finding is a reflection of the increasing trend of antibiotic resistance in the world (Antimicrobial Resistance Collaborators, 2024). The differences in the antibiotic resistance or susceptibility patterns of *L. monocytogenes* isolates indicated strain variation with strains from akara and fried fish being resistant to more antibiotics. These variations have been observed globally hence it is difficult to make conclusive comparisons of the antibiotics resisted across the world. For example, the compilation of reports on antibiotic-resistant patterns of *Listeria* spp. in Africa by Dufailu *et al.* (2021) showed differences in resistance and susceptibility patterns within countries, between countries, and by sources of *Listeria* spp. This pattern is again substantiated by the finding in this study that significant differences in MAR index occurred based on the type of food (roasted plantain and yam, akara, or fried fish) where *Listeria* spp. was isolated. This is understandable because the frequency of application and abuse of antibiotics usage varies across the world resulting in varying patterns of antibiotic resistance. In this study, amoxicillin, co-trimoxazol, and erythromycin were the most commonly resisted which differed from the outcome of some studies in Africa (Dufatlu *et al.*, 2021). The MAR index of *L. monocytogenes* isolates was found to be significantly higher than those of *L. ivanovii* and *L. innocua*. This was not unexpected because the organism is a well-known agent of food-borne disease (listeriosis). It has been receiving greater attention in studies and greater exposure to antibiotics in the course of treatment and control of listeriosis.

The presence and frequency of detection of *L. monocytogenes*, *L. grayi*, and *L. innocua* in these inexpensive and widely consumed street foods should be a source of concern to public health authorities. While listeriosis caused by *L. monocytogenes* has been the focus of extensive investigation, the pathogenicity of *L. grayi* and *L. innocua* as it concerns humans has not been given commensurate attention. Occasional reports of the involvement of *L. innocua* in human infection have been documented (e.g. Perrin *et al.*, 2003) and *L. grayi* has also been reported to be occasionally involved in the disease of immune-compromised patients (e.g. Salimnia *et al.*, 2010). The habitats of *L. grayi* and *L. innocua* are soil, vegetation, water, and food environment, hence, they have the potential to serve as indicators of the presence of the pathogenic *L. monocytogenes* and thus prompt control and preventive measures.

Generally, the fact that the 3 *Listeria* species were multi-drug resistant is worrisome in the context of public health.

## Conclusion

The *Listeria* species detected in the four foods prepared on the streets were six out of which *L. monocytogenes*, *L. innocua*, and *L. grayi* were the most frequently detected in that order. The *Listeria* species tended to occur more in akara and fried fish which have greater levels of protein than roasted plantain and yam. The presence of the three most frequently detected *Listeria* species was significantly associated with the environment of the street foods and this information should be useful to public health agencies. Although the three species were found to be MDR, the MAR index of *L. monocytogenes* was significantly higher than those of *L. innocua* and *L. grayi*. The dominant presence of *L. monocytogenes* a well-known causative agent of listeriosis, should be of public health concern because the four street foods are inexpensive and widely consumed in Nigeria. On the other hand *L. innocua* and *L. grayi* are usually considered non-pathogenic, but their presence can be considered indicative of the presence of *L. monocytogenes* and subsequently prompt preventive and control measures. In conclusion, the findings indicate that typical Nigerian street foods hitherto under-investigated for *Listeria* species, can be associated with listeriosis; and the presence of MDR strains can limit therapeutic options.

## Appendix A. Sources of street food samples and the distributions

| Street food      | Sources<br>(towns) | No of sales points/location for sample collection |        |             |         |            |         |                       |     | Total |
|------------------|--------------------|---------------------------------------------------|--------|-------------|---------|------------|---------|-----------------------|-----|-------|
|                  |                    | Road                                              |        | Market area |         | Motor Park |         | Dumpsite vicinity (m) |     |       |
|                  |                    | Earth                                             | Tarred | Within      | Outside | Within     | Outside | <50                   | >50 |       |
| Akara            | Asaba              | 2                                                 | 2      | 2           | 2       | 2          | 2       | 2                     | 2   | 16    |
|                  | Ughelli            | 2                                                 | 2      | 2           | 2       | 2          | 2       | 2                     | 2   | 16    |
|                  | Warri              | 2                                                 | 2      | 2           | 2       | 2          | 2       | 2                     | 2   | 16    |
| Sub-total        |                    | 6                                                 | 6      | 6           | 6       | 6          | 6       | 6                     | 6   | 48    |
| Fried fish       | Asaba              | 2                                                 | 2      | 2           | 2       | 2          | 2       | 2                     | 2   | 16    |
|                  | Ughelli            | 2                                                 | 2      | 2           | 2       | 2          | 2       | 2                     | 2   | 16    |
|                  | Warri              | 2                                                 | 2      | 2           | 2       | 2          | 2       | 2                     | 2   | 16    |
| Sub-total        |                    | 6                                                 | 6      | 6           | 6       | 6          | 6       | 6                     | 6   | 48    |
| Roasted Plantain | Asaba              | 2                                                 | 2      | 2           | 2       | 2          | 2       | 2                     | 2   | 16    |
|                  | Ughelli            | 2                                                 | 2      | 2           | 2       | 2          | 2       | 2                     | 2   | 16    |
| Sub-total        | Warri              | 2                                                 | 2      | 2           | 2       | 2          | 2       | 2                     | 2   | 16    |
| Roasted yam      |                    | 6                                                 | 6      | 6           | 6       | 6          | 6       | 6                     | 6   | 48    |
|                  | Asaba              | 2                                                 | 2      | 2           | 2       | 2          | 2       | 2                     | 2   | 16    |
|                  | Ughelli            | 2                                                 | 2      | 2           | 2       | 2          | 2       | 2                     | 2   | 16    |
| Sub-total        | Warri              | 2                                                 | 2      | 2           | 2       | 2          | 2       | 2                     | 2   | 16    |
| Grand-total      |                    | 6                                                 | 6      | 6           | 6       | 6          | 6       | 6                     | 6   | 48    |
|                  |                    | 24                                                | 24     | 24          | 24      | 24         | 24      | 24                    | 24  | 192   |

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