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DETECTION AND CHARACTERIZATION OF *Listeria monocytogenes* FROM LOCALLY PROCESSED FERMENTED FOODS IN ETHIOPE WEST, DELTA STATE, NIGERIA

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ABSTRACT

Outbreaks linked with foodborne pathogens and associated diseases occur regularly and pose substantial health problems to consumer safety globally. Listeriosis is a leading foodborne disease initiated by the consumption of foods contaminated by *Listeria monocytogenes* (*L. monocytogenes*). This study evaluated the presence of *L. monocytogenes* in various food samples. Locally fermented food samples (n = 80), which included Garri (n = 30), Kunu (n = 25), and Zobo (n = 25), were obtained from five open local markets in Ethiope West, Delta State, Nigeria. Food samples were screened for *L. monocytogenes* using standard cultural techniques, biochemical tests including the Analytic Profile Index (API) *Listeria* kit. The isolates were screened for antimicrobial resistance using the disc diffusion method, and breakpoints were interpreted based on the guidelines of the Clinical Laboratory Standard Institute. Gelatinase and lipase activities, including β-haemolysis, were determined using standard methods. *L. monocytogenes* was identified in 3 (3.8%) and 4 (5%) samples from Zobo and Kunu, respectively. The Garri samples were negative for *L. monocytogenes,* and only 7 of the 80 (8.8%) samples were positive for *L. monocytogenes.* One positive sample each was obtained in the Oghara and Ogharefe markets, two from the Ugbenu market, and three from the Mosogar market. No sample was positive for *L. monocytogenes* in the Oghareki market. All the isolates were penicillin-resistant but susceptible to levofloxacin and tetracycline, whereas five were resistant to clindamycin. All isolates were resistant to at least two classes of antibiotics, and the multiple antibiotic resistance (MAR) index was ⩾ 0.22. Gelatinase activity was observed in one isolate, β-haemolytic activity in four isolates, and lipase activity in five isolates. This study revealed the importance of proper hygiene practices in food production and continuous surveillance of antibiotic resistance and virulence determinants in food. There is a need to adhere to standard hygiene measures in food processing environments.

Keywords: Foods, Markets, *Listeria*, Virulence, Prevalence, Antimicrobial resistance.

INTRODUCTION

Outbreaks associated with foodborne pathogens frequently occur worldwide with implications on public health. Globally, over 91 million people are affected by foodborne pathogens and other associated illnesses (Igbinosa *et al.*, 2021; Beshiru *et al.*, 2022). Illnesses from contaminated food are responsible for approximately 48 million cases, 128,000 hospitalizations, and 3,000 deaths annually, accounting for $72 billion in economic losses (Scallan *et al.*, 2011). Developed countries and economies with state-of-the-art infrastructure for monitoring food quality still grapple with foodborne illnesses and epidemics. Microbes can contaminate foods with severe economic and health implications. Listeriosis is an important foodborne disease in humans by foods contaminated by *Listeria monocytogenes* (Liao *et al.*, 2021). The global outbreak of listeriosis may be attributable to the increasing demand for food products that require inadequate treatment procedures before consumption (Igbinosa *et al.*, 2020). Numerous listeriosis outbreaks in humans are associated globally with the consumption of diverse foods, with relatively low incidences in the overall population. Nevertheless, it persists as a prominent and lethal foodborne menace with >95% hospitalization (Borena *et al.*, 2022). Listeriosis may result in life-threatening illnesses, such as meningitis, severe sepsis, and even death.

Listeria comprises several species, but only *L. monocytogenes* is pathogenic to humans, whereas *L. ivanovii* and *L. monocytogenes* cause various infections in animals (Orsi and Wiedmann, 2016). *L. monocytogenes* is a facultatively anaerobic, motile, Gram-positive, rod-shaped, non-spore-forming bacterium that flourishes from -0.4 °C to 50 °C. The ability of *L. monocytogenes* to survive in harsh environmental conditions, such as low water activity, high salt concentration, low pH, hypoxic conditions, and low temperature, favours it as a

foodborne pathogen (Radoshevich and Cossart, 2018). In addition, its ability to survive adverse environmental conditions is responsible for its capacity to thrive in a wide range of ecological spheres with an increased probability of re-introduction into the food chain. *L. monocytogenes* adapts well to the agricultural milieu, as it multiplies in rotting plant matter (Liao *et al.*, 2021). *L. monocytogenes* is often recovered from different food processing sites (Sullivan and Wiedmann, 2020). Contamination of the food processing environment and foods with pathogens such as *L. monocytogenes* is influenced by inadequate hygienic precautions during processing, handling, storage, and distribution (Abdeen *et al.*, 2021). The pervasiveness of *L. monocytogenes* in these environments poses a major concern to processors of minimally processed food products because they can be transmitted through processing and handling (Farber *et al.*, 2020).

Antibiotic resistance remains an obstacle in patient care and the management of infectious diseases. Recently, an increasing occurrence of antibiotic-resistant bacteria associated with food materials, resulting in life-threatening foodborne diseases, has been described (Olaimat *et al.*, 2018). However, it is worth noting that *L. monocytogenes* is susceptible to many classes of antibiotics. However, reports of multidrug resistance (MDR) among isolates recovered from food processing environments (Jorgensen *et al.*, 2021) have been documented. Detection of MDR *L. monocytogenes* from food materials is a public health challenge, particularly in immunocompromised individuals (Abdeens *et al.*, 2021). Therefore, proper antibiotic resistance monitoring in the healthcare system and the food industry enhances strategic prevention of its spread (Capita *et al.*, 2019). The proportion of resistance varies among strains and is influenced by antimicrobial misuse in animals and humans with geographical differences (Liao *et al.*, 2021). Therefore, the monitoring of L. *monocytogenes* associated with food materials and its association with antibiotic resistance is important. This study evaluated *L. monocytogenes* occurrence, distribution, and antibiotic susceptibility of isolates from locally processed fermented foods.

MATERIALS AND METHODS

Study Area and Sample Collection

This study area was Ethiope West Local Government Area (EWLGA), Delta State, Nigeria. EWLGA has a population of 203,592 people as of the 2006 census, with its headquarters in Oghara town. EWLGA has various educational and health institutions such as Western Delta University, Delta State Polytechnic, Oghareki Model Secondary School, Ogharefe Secondary School, Nigeria Naval Logistic Headquarters, and Delta State University Teaching Hospital. The major occupation of the local inhabitants is farming. The sample size for this study was determined using the sample size determination formula as follows:

$$\text{Sample (N)} = \frac{(Z_{1-\alpha/2})^2 P(1-P)}{d^2}$$

$Z_{1-\alpha/2}$ = Standard normal variant at 5% type I error ($P < 0.05$); P = Expected prevalence based on a previous study [2.85 – 28.28% (Ajayeoba *et al.*, 2015; Dufailu *et al.*, 2021; Borena *et al.*, 2022]; d = Absolute error or precision (which is 5%). The expected minimum number of samples collected was 43. However, the total number of samples examined in this study was 80 locally processed food samples, i.e., Garri (n=30), Kunu (n=25), and Zobo (n=25) from five local markets in EWLGA, Delta State.

During sampling, a tin milk cup, a measure used in the market (approximately 167 ml volume) of Garri, was bought from 10 different open market Garri traders, pooled together, and mixed as a single sample. In the case of Kunu and Zobo, five samples (1 L) were purchased and pooled as single samples. The study sites included Oghara, Ogharefe, Ugbenu, Mosogar, and the Oghareki market. The samples were immediately conveyed to the laboratory on ice for analysis in sterile sampling containers.

Isolation of *L. monocytogenes*

Twenty-five grams each of the pooled Garri and 25 mL of the Kunu and Zobo samples were added/mixed into 225 mL pre-enrichment half-Fraser broth (HFB) (Merck, Darmstadt, Germany), swirled using a shaker (V2 S000, IKA Vortex 2, Germany) for 30 s to mix the samples,

and incubated at 30°C for 24 h. Subsequently, 0.1 mL of the HFB cultured broth was inoculated into 10 mL of enrichment full-Fraser broth (FFB) (Merck, Darmstadt, Germany) and incubated at 37°C for 48 h. Then, a loopful of the FFB culture was inoculated onto Chromogenic *Listeria* Agar (CLA) (Oxoid, Basingstoke, United Kingdom) and incubated at 37°C for 24 h. The characteristic blue colonies with opaque halos on CLA were regarded as presumptive *L. monocytogenes* (AOAC, 2022). The isolates were subcultured on tryptone soya agar (Oxoid, Basingstoke, United Kingdom), incubated at 37°C for 24 h, and stored as stock cultures for further analysis. For long-term storage, isolates were prepared on glycerol stock and stored at freezing temperatures.

Identification of *L. monocytogenes*

Identification as *L. monocytogenes* was determined by Gram reaction and biochemical tests. Isolates, which were Gram-positive short rods that showed positive reactions to catalase, rhamnose haemolysis motility, and CAMP (Christie–Atkins–Munch–Peterson) and negative reactions to mannitol, xylose, and oxidase, were *presumptively identified as L. monocytogenes*. The isolates were further screened using Analytic Profile Index (API) *Listeria* kits (Biomerieux, France) following the manufacturer's guidelines. *L. monocytogenes* ATCC 35152 was used as a control in each experimental procedure.

Strip preparation: The incubation box (tray and lid) was prepared, and 5 mL of distilled water was added to the honeycomb wells in the tray to establish a humid environment. The strain reference number was written on the extended flap of the tray. Subsequently, the strip was removed from its packaging and placed in the incubation box.

Inoculum preparation: Using a sterile loop, a colony was transferred from an 18–24-h culture plate and introduced into an opened ampoule containing API NaCl medium under aseptic conditions. The suspension was then gently emulsified to obtain a uniform bacterial suspension.

Strip inoculation: The bacterial suspension was dispensed into the cupules of the strip. For citrate use (CIT), Voges–Proskauer (VP), and gelatine hydrolysis (GEL), both the tubes and cupules were filled with the bacterial suspension. For other tests, only the tubes were inoculated with the bacterial suspension. For the arginine dihydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), hydrogen sulphide (H_2S), and urease (URE) tests, mineral oil was added to overlay the contents, creating an anaerobic condition. Subsequently, the incubation box was sealed with its lid, and all boxes were incubated at 37°C for 18 to 24 h.

The various tests were investigated: arginine dihydrolase (ADH), orthro-nitrophenyl-beta-D-galactopyranosidase (ONPG), lysine decarboxylase (LDC), citrate (CIT), ornithine decarboxylase (ODC), hydrogen sulphide production (H_2S), tryptophan deaminase (TDA), urease (URE), indole production (IND), and gelatinase (GEL). Others include Voges Proskauer (VP), D-glucose (GLU), inositol (INO), D-mannitol (MAN), D-sorbitol (SOR), D-arabitol (DARL), D-xylose (XYL), saccharose (D-Sucrose) (SAC), L-rhamnose (RHA), D-melibiose (MEL), L-arabinose (ARA), amygdalin (AMY), cytochrome-oxidase (OX), MacConkey medium (McC), motility (MOB), oxidation exposed to air (OF-O), and fermentation under mineral oil (OF-F).

Strip reading: After the incubation period, specific reagents were added for certain strip tests. In the TDA test, one drop of the TDA reagent was introduced, and a reddish-brown colour signified a positive reaction. For the IND test (Indole), a drop of JAMES reagent was applied, and a pink colour indicated a positive reaction. In the VP test, one drop of each reagent was added, and colour (pink or red) was considered a positive reaction. For the NO_3–NO_2 test, one drop of NIT1 and NIT2 reagents was added to the GLU tube, and a red colour indicating a positive reaction. The motility (MOB) test involved inoculating an ampule of API medium. Growth on MacConkey agar medium (McC) was observed, and MacConkey agar plates were streaked and incubated at 36 °C ± 2 for 24 h. Positive reactions were documented on a recording sheet.

Interpretation: Identification was achieved using a numerical profile. The result sheet categorized the tests into three groups, with each tray summing up the values corresponding to positive reactions. Within each group, a 7-digit profile number was generated for the tests conducted on the API strips. These profile numbers were subsequently used for identification using APIweb identification software.

Antibacterial Susceptibility Testing (AST)

AST of the *L. monocytogenes* isolates was determined via the Kirby-Bauer disc diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute - CLSI (2020) using *Streptococcus pneumoniae* ATCC 49619 as the reference strain. An 18-24 h bacterial culture (100 µL) with turbidity adjusted to 0.5 McFarland standard was aseptically dispensed onto Mueller–Hinton agar plates (Lab M, Lancashire, United Kingdom) and spread all over the surface of the plate using a sterile glass rod. *L. monocytogenes* isolates were screened with the following antibiotics: macrolides - erythromycin (15 µg), glycopeptides - vancomycin (30 µg), penicillins - penicillin (10 units), tetracyclines - tetracycline (30 µg), fluoroquinolones - levofloxacin (5 µg), ansamycins - rifampicin (5 µg), folate pathway antagonists - trimethoprim-sulfamethoxazole (1.25/23.75 µg), phenicols - chloramphenicol (30 µg), and lincosamide - clindamycin (2 µg) (Oxoid, Hampshire, United Kingdom). Antibiotic classes were selected based on the recommendation of the CLSI. After overnight incubation at 37°C, the zone of inhibition was inferred according to the CLSI guidelines (2020).

Multiple Antibiotic Resistance Index

The multiple antibiotic resistance index (MARI) was determined by $\mathbf{MARI} = \frac{a}{b}$ where "a" = number of antibiotics the isolate was resistant to and "b" = total number of antibiotics tested with the isolate (Beshiru *et al.*, 2023). The multi-drug resistance (MDR) profile was determined when the isolate is resistant to at least one antibiotic in at least three classes of antibiotics (Beshiru and Igbinosa, 2023).

Determination of Phenotypic Virulence Factors

Virulence was determined using the protocol described by Beshiru *et al.* (2018). The isolates were cultured on tryptone soy agar (TSA) (Merck, Darmstadt, Germany). After that, colonies on TSA were inoculated into 3 mL of tryptic soy broth (TSB) (Merck, Darmstadt, Germany), and the turbidity of the bacterial suspension was adjusted to 0.5 McFarland standard. Lipase activity was determined by inoculating 5 mL of the sample suspension on TSA and incubating for 24 h at 37°C. Positive lipase activity was indicated by a clear halo surrounding the growth of the lipase-producing isolate. Gelatinase activity was determined by inoculating 5 mL of the bacterial culture in a gelatine medium and incubating it for 24 h at 37°C. The zone of clearance in the media indicated the proliferation of gelatin-liquefying microorganisms. Haemolytic activity was determined by inoculating 5 µL of the bacterial suspension on a sheep blood agar plate and incubating for 24 h at 37°C. Clear colourless zones around the colonies indicated haemolytic activity.

Data analysis

The occurrence of *L. monocytogenes* and resistance of the isolates were determined using Microsoft Excel version 2013.

RESULTS

***L. monocytogenes* occurrence in food based on sample type**

L. monocytogenes was identified in Kunu (16%; 4/25) and Zobo (12%; 3/25) based on cultural and API *Listeria* identification procedures. No *L. monocytogenes* was identified in the Garri samples. In total, 7 of 80 (8.8%) samples were positive for *L. monocytogenes*.

Occurrence of *Listeria monocytogenes* in food based on market location

The occurrence of *L. monocytogenes* in samples based on market location is described in Table 1. One isolate was recovered from the Oghara and Ugbenu markets, and two and three isolates were obtained from the Ugbenu and Mosogar markets, respectively. No isolate was recovered from the Oghareki market. Four isolates (AK3, BZ3, CK3, CK5) had the same API *Listeria* profile number

(2686510), whereas three isolates (DK1, DZ1, DZ2) had a different number (2686410). This observation occurred because the isolates (DK1, DZ1, DZ2) failed to acidify D-arabitol (DARL).

Table 1: Occurrence of *L. monocytogenes* based on market selected

Samples	Samples assessed	*Listeria monocytogenes* positive samples	Prevalence of *Listeria monocytogenes* (%)
Oghara junction Market	16	1/16	6.3
Ogharefe Market	16	1/16	6.3
Ugbenu Market	16	2/16	12.5
Mosogar Market	16	3/16	18.8
Oghareki Market	16	0/16	0.0
Total	**80**	**7 (8.8%)**	

Antimicrobial Resistance (AMR) Profile of *L. monocytogenes* isolates

Table 2 shows the antimicrobial resistance (AMR) profiles of *L. monocytogenes*. All the isolates (n=7) were resistant to penicillin, whereas five isolates were resistant to clindamycin, four to erythromycin and trimethoprim-sulfamethoxazole, and three to rifampicin and chloramphenicol. All isolates were susceptible to levofloxacin.

Table 2: Antibiotic susceptibility of *L. monocytogenes* isolates recovered from food samples.

Antimicrobial Class	Antibiotics	Total isolates (n=7)		
		R (%)	I (%)	S (%)
Macrolides	ERY	4 (57.1)	2 (28.6)	1 (14.3)
Penicillins	PEN	7 (100)	NA	0 (0)
Glycopeptides	VAN	1 (14.3)	NA	6 (85.7)
Tetracyclines	TET	0 (0)	1 (14.3)	6 (85.7)
Fluoroquinolones	LEV	0 (0)	0 (0)	7 (100)
Ansamycins	RIF	3 (42.9)	4 (57.1)	0 (0)
Folate Pathway Antagonists	SXT	4 (57.1)	0 (0)	3 (42.9)
Phenicols	CHL	3 (42.9)	NA	4 (57.1)
Lincosamide	CLI	5 (71.4%)	0 (0)	2 (28.6)

Legend: R: resistance, I: intermediate, S: sensitive, CHL: Chloramphenicol, LEV: Levofloxacin**,** ERY: Erythromycin, VAN: Vancomycin, PEN: Penicillin, TET: Tetracycline, RIF: Rifampicin, SXT: Trimethoprim-Sulfamethoxazole, CLI: Clindamycin, NA: Not applicable.

Multiple Antibiotic Resistance Index (MARI) of *L. monocytogenes*

Table 3 shows the MARI of *L. monocytogenes*. Two isolates were resistant to ⩾ 5 antibiotics, whereas six were resistant to ⩾ 3 antibiotics. In total, all isolates (n=7) were resistant to ⩾ 2 antibiotics. MARI of *L. monocytogenes* ranged from 0.22 to 0.55.

Table 3: Multiple antibiotic resistant (MAR) index of *L. monocytogenes* isolates tested against nine antibiotics.

Isolate code	Resistance phenotype (antibiotype)	MAR Index
CK3	ERY, PEN, RIF, SXT, CHL	0.55
CK5	PEN, RIF, SXT, CHL, CLI	0.55
DK1	ERY, PEN, VAN, CLI	0.44
AK3	ERY, PEN, RIF, CLI	0.44
DZ1	ERY, PEN, SXT, CLI	0.44
BZ3	PEN, SXT, CLI	0.33
DZ2	PEN, CHL	0.22

Legend: CHL: Chloramphenicol, ERY: Erythromycin, VAN: Vancomycin, PEN: Penicillin, TET: Tetracycline, RIF: Rifampicin, SXT: Trimethoprim-Sulfamethoxazole, and CLI: Clindamycin.

Phenotypic virulence factor formation potential of *Listeria monocytogenes*

The tests for the virulence factors showed that one isolate produced gelatinase, whereas four isolates were β-haemolytic and five were lipase-positive.

DISCUSSION

Listeria monocytogenes is associated with severe foodborne diseases. The occurrence of *L. monocytogenes* in food products reveals the risks that arise from ingesting poorly processed food products. *Listeria monocytogenes* was recovered from locally processed food (Kunu and Zobo). In agreement with this finding, *L. monocytogenes* reportedly could survive several stages of food processing and microbial control in the food industry, including standard disinfection and cleaning (Centorame *et al.*, 2017). *L. monocytogenes* is among the pathogenic organisms reported in traditional African fermented foods (Anyogu *et al.*, 2021). They can tolerate harsh food storage and processing conditions such as low temperature, low moisture content, and high acidity and salinity, which makes them a major concern for food safety (Makinde *et al.*, 2020). The recovery of microbes indicates a potential health concern to consumers of the products. Although the present study investigated traditionally processed food products of plant origin, previous studies have emphasized the possibility of plant products being contaminated with *Listeria* pathogens when contaminated water sources are used for irrigation (Kayode *et al.*, 2021).

The application of untreated water in food processing, storage of food crops in contaminated environments, and contaminated container usage can influence microbial contamination (Mritunjay and Kumar, 2015). Contaminated foods with microbes pose a public health threat to food safety (Beshiru and Igbinosa, 2023). Furthermore, the detection of *L. monocytogenes* might be due to poor hygiene practices and improper submission to the hazard analysis of critical control point (HACCP) principles during production (Kayode *et al.*, 2021). Similarly, the incidence of *Listeria* in food products can occur through cross-contamination from the environment, utensils, processing water, and packaging materials (Garedew *et al.*, 2015). The absence of *L. monocytogenes* in the Garri samples in our study could be due to the processing methods that involve heating to dryness and total elimination of moisture. A lower moisture content notably inhibits the proliferation of bacteria and delays the decomposition process. Unlike Kunu and Zobo, the processing of Garri involves a heat-drying protocol that eliminates moisture during production. David and Aderibigbe (2010) specified that higher moistness in food could enhance microbial contamination. In addition, Kunu and Zobo are locally processed fermented food products preserved via refrigeration. However, the ability of *L. monocytogenes* to grow at lower temperatures makes the pathogen difficult to control (Centorame *et al.*, 2017).

Varying exposure to environmental pollution, storage conditions, and different sanitary measures by retail sellers in various local markets could be responsible for the various recovery levels of *L. monocytogenes*. Because no definite selling and preservation standards for locally processed foods in Nigeria exist or are enforced, many street food sellers are available on street-side stalls. Street-vending food products are generally perceived by consumers as cheap and readily affordable, but they are major public health hazards in low- and middle-income countries. It can be due to poor infrastructural facilities to control microbial contamination (Beshiru *et al.*, 2021; 2023). Pathogenic bacteria recovery in street retailed or vented food from retail outlets poses a major risk to consumer safety, public health, and quality control measures (El-Demerdash and Raslan, 2019; Beshiru and Igbinosa, 2023). This trend is aggravated by inadequate data on the epidemiological importance of numerous locally processed foods, reduced sensitization of street vendors to rudimentary food safety processes, and poor public recognition of potential risks associated with the consumption of contaminated food products (Igbinosa and Beshiru, 2019; Beshiru *et al.*, 2020).

The challenges associated with potential pathogens such as *L. monocytogenes* are not restricted to only food product contamination but also their ability to resist the inhibitory action of several common antimicrobials used in therapeutics and infection control. Antimicrobial resistance (AMR) has increased globally, causing serious public health threats. In food production, antibiotic resistance could be linked to biofilm formation on instruments, utensils, and foods and the shortage of novel antimicrobials (Beshiru and Igbinosa, 2018). The high penicillin resistance of the isolates in our study agrees with the study of Borena *et al.* (2022). Ampicillin or penicillin G is often the first-line treatment for listeriosis. If the patient is allergic to penicillin, alternatives such as trimethoprim-sulfamethoxazole (Bactrim) may be considered. In severe cases, especially when listeriosis has spread to the central nervous system, a combination of ampicillin or penicillin G with gentamicin may be used (Rodríguez-Villodres *et al.*, 2020). Resistance to antibiotics, usually recommended for listeriosis treatment in humans, is a serious concern. Antibiotic resistance from local inhabitants could be linked to broad-spectrum drug availability, accessibility, and abuse (Garedew *et al.*, 2015). AMR is further heightened by extensive and indiscriminate antimicrobial usage.

While Borena *et al.* (2022) reported high resistance rates of *L. monocytogenes* to chloramphenicol (60%), tetracycline (55%), and vancomycin (81.82%), our study did not observe this trend. Nevertheless, the susceptibility of all isolates to levofloxacin is similar to a previous study (Borena *et al.*, 2022), where all isolates were susceptible to norfloxacin, a fluoroquinolone. This trend of resistance to tetracycline and chloramphenicol could be attributed to the frequent use of these drugs. Jorgensen *et al.* (2021) reported variable *L. monocytogenes* susceptibilities to antibiotics. The varying susceptibility patterns of *L. monocytogenes* may depend on the antibiotics commonly used in the geographical areas investigated. Resistance reservoirs may be disseminated indirectly or directly to humans via the ingestion of contaminated food. AMR bacteria can cause severe health menace directly or via the spread of AMR elements to pathogens, causing difficult-to-treat infections.

All the isolates in this study 7/7 (100%) demonstrated MARI $\geqslant$ 0.22. A high MARI could be detrimental to public health as MARI higher than the threshold index of 0.2 indicates an association with high-risk sources where antibiotics are abused (Sabaté *et al.*, 2008). This trend shows that the source of microbial contamination is associated with microorganisms from these high-risk areas. MARI of *L. monocytogenes* also revealed that all isolates, except one, demonstrated resistance to at least three antibiotics with a MAR index $\geqslant$ 0.33. This development indicates that most of the isolates are MDR because an isolate resistant to $\geqslant$ three classes of antibiotics is considered MDR. High percentages of MDR isolates in food products are largely dependent on inadequate knowledge of foodborne infections and a poor food safety governing system (Seyoum *et al.*, 2015). Furthermore, the peculiar features of *L. monocytogenes* enable it to persist in harsh conditions, including the activities of antibiotics.

Our findings corroborate a previous study that reported haemolytic activity and lipase-producing *L. monocytogenes* (Al-Mashhadany, 2019). *L. monocytogenes* has been reported to retain approximately 80% lipase activity upon incubation for 24 h in 30% (v/v) methanol (Priyanka *et al*., 2022). Olaniran *et al.* (2015) reported 25 (32%) *Listeria* isolates to have produced haemolytic enzymes. Nevertheless, all isolates were negative for protease and gelatinase production (Olaniran *et al*., 2015). *Listeria monocytogenes* is a foodborne β-haemolytic-producing pathogen. Conversely, *L. monocytogenes* with non-haemolytic potentials have been documented (Kawacka *et al*., 2022). The haemolytic activity of *L. monocytogenes* largely depends on *prfA* and *hly* genes (Kawacka *et al*., 2022). The *prfA* gene regulates virulence factor expression, whereas only the *hly* gene contributes to *the ability of L. monocytogenes* to invade and escape from host cells, making these genes critical for its pathogenicity. However, in most cases where haemolysis was not produced, it is ascribed to mutations within the *pfrA* gene, whereas *hly gene* mutations are detected less frequently (Kawacka *et al*., 2022). Production of extracellular lipase aids *L. monocytogenes* in the degradation of lipids. The link of *L. monocytogenes* to these virulence factors raises public health concerns because they are notably bacterial pathogenic features (Alzubaidy *et al*., 2013).

CONCLUSION

Recovery of *L. monocytogenes* from locally processed fermented foods is a public health concern. The findings of this study further emphasize the importance of proper food handling and adherence to proper hygiene practices to prevent cross-contamination and reduce consumer risks. The emergence of *L. monocytogenes* with MDR and virulence markers necessitates broad and continuous surveillance to curtail their roles as vehicles in the spread of these resistance genes and virulence traits along the food chain. Therefore, strict hygienic measures should be adopted during the processing of food products.

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CONFLICT OF INTEREST

The authors declare no competing interest.

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