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Presence of *Listeria* spp., including pathogenic *Listeria monocytogenes* and *Listeria ivanovii*, on fresh vegetables in the markets of Abidjan (Côte d'Ivoire)

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Abstract

Listeria monocytogenes is a pathogenic bacterium that can contaminate various food products and is the causative agent of listeriosis, a severe and life-threatening foodborne illness. This study aimed to assess the occurrence of *Listeria* species in fresh vegetables and market garden produce from Côte d'Ivoire. Conducted over a 9-week period, this work analyzed 135 samples of fresh vegetables and market garden produce (including lettuces, carrots, cabbage, tomatoes, and cucumbers) collected from 3 major markets in 3 districts of Abidjan. Among the 135 samples, *Listeria* spp. were found in 11 (8.15%), including *Listeria innocua* in 8 (5.92%), *Listeria ivanovii* in 1 (0.74%), and *L. monocytogenes* in 2 (1.48%). Genetic analysis was performed using restriction fragment length polymorphism targeting the *16S rRNA* gene. Further characterization of genetic lineages, serotypes, and virulence genes was achieved through polymerase chain reaction. The two *L. monocytogenes* isolates belong to the genetic lineage I, associated with serogroups 1/2b, 3b, 4b, 4d, 4e, and 7. They harbored key virulence genes from *Listeria* pathogenicity island-1, such as *actA, hly, prfA,* and *pclB*, as well as internalin genes (*inlA, inlJ*, and *inlC*), indicating their potential pathogenicity. This study highlights the presence of *Listeria* spp., including *L. monocytogenes*, in fresh vegetables sold in Abidjan markets, underscoring a potential health risk for consumers.

Key words: fresh vegetables, *Listeria monocytogenes*, PCR-RFLP, serotype, virulence genes.

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Introduction

Listeria are bacteria belonging to the *Listeriaceae* family, which includes two Gram-positive pathogenic species. *Listeria ivanovii* primarily affects ruminants and rarely infects humans, while *Listeria monocytogenes* can infect both animals and humans (Bagatella *et al*., 2022). *L. ivanovii* can cause infections in ruminants with pathological manifestations similar to those caused by *L. monocytogenes*, except for meningoencephalitis, which is characteristic of *L. monocytogenes* infections (Vazquez-Boland *et al*., 2001; Guillet *et al*., 2010). In recent years, some sporadic cases of listeriosis caused by *L. ivanovii* have been reported in immunocompromised people (Guillet *et al*., 2010; Beye *et al*., 2016).

L. monocytogenes is widely distributed in natural and food-related environments and can thrive under diverse environmental conditions, including a pH range of 4.3 to 9.4, temperatures from 0 to 45°C, salt concentrations up to 14%, and water activity levels above 0.92 (Garrido-Maestu *et al*., 2018). These particular physico-chemical properties enable its survival and proliferation in a wide variety of foodstuffs, such as seafood, dairy products, meat, and vegetables (EFSA, 2017).

L. monocytogenes is a foodborne pathogen that primarily affects immunocompromised individuals and can cause meningoencephalitis, septicemia, and neonatal infections or miscarriage in pregnant women (Schlech, 2019). Maternal-neonatal infections account for 11.20% of invasive listeriosis cases in France and Spain. They cause approximately 3% of neonatal deaths and 6% of serious sequelae such as brain damage (Charlier *et al*., 2020; Charlier *et al*., 2022). In Africa, a major outbreak was reported in South Africa following the consumption of processed meats. In addition, Dufailu *et al*. (2021) demonstrated the prevalence and characteristics of *Listeria* species in several African countries. The mortality rate of *L. monocytogenes* is generally high compared to other foodborne infections. According to epidemiological data, it ranges between 20% and 30% in symptomatic individuals, and can reach up to 40% in certain high-risk populations, such as newborns, the elderly, immunocompromised individuals, or during severe outbreaks (WHO, 2018a). The pathogenicity of *L. monocytogenes* primarily depends on *Listeria* pathogenicity island (LIPI)-1, which carries core virulence genes essential for vacuole escape, intracellular survival, and cell-to-cell spread (Quereda *et al*., 2021). In addition, LIPI-3 and LIPI-4, two pathogenicity islands present only in specific *L. monocytogenes* lineages or clonal complexes (CC), are associated with increased virulence. LIPI-3 contributes to infection by modulating the intestinal microbiota through the action of listeriolysin S (Cotter *et al*., 2008; Quereda *et al*., 2016; Schiavano *et al*., 2023). LIPI-4, encoding a cellobiose-family phosphotransferase system, is associated with hypervirulent clones implicated in neuroinvasive and maternofetal infections (Maury *et al*., 2016; Schiavano *et al*., 2023). Listeriosis causes serious damage to humans, making it a public health problem.

Listeriosis has historically been linked to the consumption of high-risk food products, such as raw meat and fish, seafood, unpasteurized dairy products, and processed foods like soft cheeses and deli meats. Recently, fresh fruits and vegetables have become an increasingly recognized source of *Listeria* contamination (Buchanan *et al*., 2017). This shift is probably mainly due to greater consumption of raw vegetables and a growing demand for fresh-cut, ready-to-eat fruits and vegetables, which are often consumed without further processing, factors that have increased food safety risks. Consequently, an increasing number of studies worldwide have reported the presence of *L. monocytogenes* in fresh produce (Zhu *et al*., 2017). In Brazil, *L. monocytogenes* was detected in 3.03% of vegetable samples, which is a higher prevalence in ready-to-eat salads (de Vasconcelos Byrne *et al*., 2016). In the United States, contamination rates reached up to 66.7% of spinach and 50% of peas at frozen food processing facilities (Heisick *et al*., 1989). Similarly, a study in China reported that 5.49% of fresh vegetable samples were contaminated, with lettuce showing the highest rate (Chen *et al*., 2019). Although no major human listeriosis outbreaks have been reported in Africa, *L. monocytogenes* has already been isolated from foods such as carrot, lettuce, cucumber, and tomato collected from traditional markets in southwestern Nigeria (Ajayeoba *et al*., 2015).

Several outbreaks have been linked to fruits and vegetables contaminated with *Listeria*, showing the importance of monitoring this bacterium in non-animal food products (Buchanan *et al.*, 2017). One important example is the 2011 outbreak in the United States, where contaminated cantaloupes caused 147 cases and 33 deaths (Mccollum *et al*., 2013). In Europe, a multi-state listeriosis outbreak linked to sweetcorn occurred between 2015 and 2018, resulting in 47 cases and 9 deaths (Koopmans *et al*., 2023). These findings underscore the critical need to monitor *Listeria* spp. in fresh produce, especially as the consumption of raw and minimally processed vegetables continues to increase. Therefore, assessing the occurrence of *Listeria spp.* and *L. monocytogenes* in vegetables is essential for evaluating public health risks and implementing appropriate food safety measures. However, several studies have shown the prevalence of *L. monocytogenes* in raw and processed foods (Fallah *et al*., 2013; Skowron *et al*., 2018). Only a few studies focusing on the prevalence of *L. monocytogenes* in raw and processed foods in Côte d'Ivoire have been reported.

L. monocytogenes is classified into 13 serotypes based on O (somatic) and H (flagellar) antigens (Dongyou, 2008). Using polymerase chain reaction (PCR), these serotypes are grouped into 5 main serogroups: IIa (1/2a–3a), IIb (1/2b–3b–7), IIc (1/2c–3c), IVa (4a–4c), and IVb (4ab–4b–4d–4e) (Doumith *et al*., 2004). Among them, serotypes 1/2a, 1/2b, and 4b account for about 95% of human listeriosis cases: 1/2a and 1/2b are mostly found in food, while 4b is often linked to clinical cases (Orsi *et al*., 2011). To better distinguish strains, molecular typing methods have been developed, including multilocus sequence typing (MLST), introduced in 2003, which uses the comparison of 7 housekeeping genes (Salcedo *et al*., 2003). This study was carried out on 62 strains isolated from different sources in different regions of Spain between 1995 and 2001. The analysis identified 29 allelic profiles or 'sequence types' (STs). The most frequent, ST2 and ST6, grouped together 10 and 14 isolates, respectively (Salcedo *et al.* 2003). In 2008, a MLST method, based on the comparison of sequences from 7 housekeeping genes of 360 *L. monocytogenes* isolates, identified 126 distinct STs, each defined by a unique combination of alleles, and distributed into 23 CC. Seven major CC (CC1, CC2, CC3, CC4, CC5, CC7, and CC9) were highlighted, encompassing the majority of the analyzed isolates (Ragon *et al.*, 2008). The strains in each CC had a unique or dominant serotype (4b for CCs 1, 2, and 4; 1/2b for CCs 3 and 5; 1/2a for CC7 and 1/2c for CC9) (Ragon *et al.*, 2008). Recently, with the reduction in sequencing costs, comparative analysis of whole genomes has become the standard for molecular analysis of bacterial species. This method, based on gene-by-gene allelic profiling of the core genome, is often called 'core genome MLST' (Maury *et al.*, 2016). It was developed for *L. monocytogenes* with a core genome of 1748 genes and currently represents the most powerful diagnostic tool for strain typing (Ruppitsch *et al.*, 2015).

The aims of this study were: i) to assess the presence of *Listeria* spp. and *L. monocytogenes* in fresh vegetables and market garden samples collected from markets in Abidjan, Côte d'Ivoire; ii) to determine their genetic lineages and serotypes; and iii) to detect the presence of the major virulence genes in order to evaluate the potential pathogenicity of these strains.

Materials and Methods

Sample collection

Over 9 weeks, 135 fresh vegetables and market garden produce samples, including 27 samples for each of lettuce, cabbage, tomato, carrot, and cucumber, were collected from 3 major markets located in 3 communities of Abidjan (Abobo, Adjamé, Yopougon). Samples were placed in sterile Stomacker bags, stored in a cooler, and transported to the Biotechnology and Food Microbiology Laboratory at Nangui ABROGOUA University. Microbiological analyses were carried out on the same day as sampling.

Isolation and identification of strains of *Listeria* spp.

Strain identification was performed following ISO standard 11290-2 with minor modifications (ISO, 2017). Each sample (25 g) was aseptically homogenized in 225 mL of *Listeria* UVI-M enrichment broth in a Stomacher (Shaking incubator; BJPX-Kansas, Biobase, China) for 3 minutes at 260 rpm. The mixture was then incubated at 30°C for 24 hours. After incubation, 0.1 mL of the mixture was transferred into a tube containing 10 mL of *Listeria* UVI-M (Biokar, Beauvais, France) and incubated at 37°C for 48 hours. After this second incubation, a streak culture was performed on *Listeria* Agar according to Ottaviani & Agosti (ALOA, Biolife, Milan, Italy) and incubated at 37°C for 48 hours. From agar plates, we selected 3 to 5 isolated colonies with a typical *Listeria* spp. appearance to improve the chance of detecting the bacteria, as not all presumptive colonies are confirmed as *Listeria* after testing. Each selected colony was reisolated on Broth Heart Infusion Agar plates at 37°C for 24 h. To confirm the detection of *Listeria* spp., the presumptive isolates were further subjected to conventional biochemical tests (Gram staining, catalase, oxidase, and β-hemolysis tests). Isolates found to be Gram-positive, catalase-positive, β-hemolysis-positive, and had a blue-green appearance with an opaque halo around them on ALOA agar were considered *L. monocytogenes*. Isolates found to be blue-green without a halo on ALOA agar, Gram-positive, catalase-positive, and β-hemolysis-negative were considered to be *Listeria* spp. Positive samples were included in the final count only after confirmation by molecular tests.

Polymerase chain reaction and restriction fragment length polymorphism identification of *Listeria* spp.

DNA was extracted from isolates using the phenol chloroform method. A 1542-bp fragment (F1), corresponding to the full 16S rRNA gene, was amplified using the universal primers rD1 fD1 (5'-AGAGTTTGATCCTGGCTCAG-3′) and rD1 (5′-AAGGAGGTGATCCAGCC-3′) (Weisburg *et al*., 1991). In parallel, a 939-bp fragment (F2), covering a specific region of the 16S rRNA gene, was amplified using *Listeria*-specific primers 16sListF (5'-CAGCMGCCGCGGTAATWC-3') and 16sListR (5'-CTCCATAAAGGTGACCCT-3') (Border *et al.*, 1990). The PCR conditions included an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and elongation at 72°C for 1 minute. A final elongation was performed at 72°C for 5 minutes. F1 fragment was subsequently digested with the restriction enzymes *ApaI*, *XmaI*, *Tsp509I*, and *SacII* (Invitrogen, Carlsbad, USA), while the F2 fragment was digested with *AluI* and *AccII* (Chen et al., 2010). Digestion reactions were carried out in a final volume of 20 µL and incubated at 37°C for 4h in a dry bath (BDI-IV BIOBASE). *L. monocytogenes* EGDe, *L. ivanovii* ATCC 19119, *L. innocua* BUG 499, *L. seeligeri* 860478, and *L. welshimeri* CHUT 860477 were used as controls.

Sequencing of the 16S rRNA gene

Sequencing was carried out following the protocol described by Ouoba *et al.* (2012). A 1542-bp fragment of conserved regions of the 16S rRNA gene was amplified by PCR using the universal primers fD1 (5'-AGAGTTTGATCCTGGCTCAG-3′) and rD1 (5′-AAGGAGGTGATCCAGCC-3′) as described above. The PCR products were verified through agarose gel electrophoresis and subsequently sent to Eurofins (France) for sequencing.

Polymerase chain reaction-serogroups analysis and virulence genes determination in *Listeria monocytogenes* strains

DNA extraction from *L. monocytogenes* isolates was performed using the Fast DNA for Soil Extraction Kit (MP Biomedicals, California, United States), following the manufacturer's instructions. Serogroups were determined *via* multiplex PCR by targeting two genes (*ORF2819* and *lmo0737*) as described by Doumith *et al.* (2004) (Table 1). The *ORF2819* gene is present in *L. monocytogenes* of genetic lineage I, including serotypes (1/2b, 3b, 4b, 4d, 4e, and 7); on the other hand, the *lmo0737* gene is present in strains of genetic lineage II, including serotypes (1/2a, 1/2c, 3a, and 3c). Additionally, 7 virulence genes (*inlA*, *inlC*, *inlJ*, *actA*, *prfA*, *hly*, and *pclB*) were amplified using the primer pairs listed in Table 2 (Bubert *et al.*, 1999; Liu *et al.*, 2007; Lomonaco *et al.*, 2012; Coroneo *et al.*, 2016; Du *et al.*, 2017). Amplifications were carried out according to the following program: an initial denaturation of 5 minutes at 94°C, followed by 35 cycles comprising denaturation at 94°C for 35s, hybridization at 52°C for 30s, and elongation at 72°C for 1 minute. Final elongation at 72°C for 7 minutes. *L. monocytogenes* EGDe and *L. monocytogenes* CLIP 80459 were used as positive controls.

Results

Prevalence and biochemical characterization of presumptive *Listeria* spp. in fresh vegetables and market garden produce

Among the 135 fresh vegetable and market garden samples analyzed, 11 samples (8.15%) were found to be contaminated with *Listeria* spp. (Table 3). Lettuce showed the highest contamination rate (n=7, 25.92%), followed by cabbage (n=3, 7.41%) and tomato (n=1, 3.70%). In contrast, no *Listeria* spp. strains were detected in carrot or cucumber samples (Table 3). The 11 isolates were isolated on ALOA agar and showed a blue-green coloration; biochemical analysis showed that all 11 isolates were Gram-positive bacilli, catalase and oxidase positive. Only 3 of them were positive to the β-hemolysis test.

Polymerase chain reaction and restriction fragment length polymorphism identification of presumptive *Listeria* spp. species

Polymerase chain reaction and restriction fragment length polymorphism were initially performed using 6 reference strains from different *Listeria* species (*L. monocytogenes* EGDe, *L. monocytogenes* CLIP80459, *L. ivanovii* ATCC 19119, *L. innocua* BUG 499, *L. seeligeri* 860478, and *L. welshimeri* CHUT 860477), followed by testing on the 11 isolated strains. The results showed that restriction of fragment F1 with either *ApaI* or *XmaI* produced fragments of identical sizes across all *Listeria* species tested. Restriction with *SacII* allowed the distinction of *L. ivanovii*, *L. grayi,* and *L. welshimeri* from other species, producing fragments of 1013 bp, 291 bp, and 238 bp (Table 4). Digestion of fragment F2 with *AluI* allowed differentiation of *L. monocytogenes* from other species by specifically generating 5 fragments of 344, 206, 182, 160, and 47 bp. RFLP analysis of the restriction products from the two fragments suggested that, among the 11 samples, 2 were likely contaminated with *L. monocytogenes*, 8 with *L. innocua*, and 1 with *L. ivanovii* (Table 4).

16S rRNA gene sequencing and phylogeny

A specific region of the 16S rRNA gene was PCR-amplified

Table 1. Primers used to determine *Listeria monocytogenes* serotype.

Gene	5' – 3' sequence	Size (bp)	Serotype	Lineage	Reference
lmo0737	F: AGGGCTTCAAGGACTTACCC R: ACGATTTCTGCTTGCCATTC	691	1/2a, 1/2c, 3a and 3c	II	Doumith *et al.*, 2004
ORF2819	F: AGCAAAATGCCAAAACTCGT R :CATCACTAAAGCCTCCCATTG	471	1/2b, 3b, 4b, 4d,4e and 7	I	Doumith *et al.*, 2004

and sequenced. The obtained DNA sequences were compared with the available sequences of the 16S rRNA gene from reference strains (*L. monocytogenes*: EGDe, ATCC7644, ATCC19113, ATCC19115, ATCC1772, ATCC19117 and ATCC15313; *L. ivanovii*: ATCC19119, ATCC BAA-139, ATCC49954; *L. innocua*: ATCC33090, ATCC33091, ATCC51742, CLIP11262; *L. welshimeri*: ATCC35897, SLCC5354; *L. grayi:* ATCC19120 and *L. seeligeri* ATCC35967). This analysis confirmed that the 11 strains isolated from fresh vegetables and market garden produce in Abidjan indeed belong to the genus *Listeria*. While the sequences of the two *L. monocytogenes* strains were 100% identical to the reference strains, the sequences of *L. innocua* and *L. ivanovii* exhibited identities ranging from 99.6 to 99.9%. The accession numbers of the *Listeria* strains isolated from vegetables and market garden produce from Côte d'Ivoire, along with the BLAST results, are listed in Table 5. A phylogenetic tree was constructed based on these results. The study strains are marked in red on the tree (Figure 1).

Serotyping and virulence genes in *Listeria monocytogenes* isolates

PCR analysis revealed a 471-bp fragment for strains *L208* and *L238* corresponding to the *ORF2819* gene present in *L. monocytogenes* species belonging to lineage I (serotypes 1/2b, 3b, 4b, 4d, 4e, and 7) (Figure 2, see lanes 4 and 5). A 691-bp fragment (*lmo0737*) is present in *L. monocytogenes* species of genetic lineage II (see

Table 2. Primers used for amplification of *L. monocytogenes* virulence genes.

Gene	5' – 3' sequence	Size (bp)	Reference
actA	F-CCAAGCGAGGTAAATACGGGA R-GTCCGAAGCATTTACCTCTTC	650	Lomonaco *et al*., 2012
hly	F-ATCATCGACGGCAACCTCGGAGAC R-CACCATTCCCAAGCTAAACCAGTGC	404	Du *et al*, 2016
inlA	F-CCTAGCAGGTCTAACCGCAC R-TCGCTAATTTGGTTATGCCC	256	Coroneo *et al*., 2016
inlC	F-AATTCCCACAGGACACAACC R-CGGGAATGCAATTTTTCACTA	517	Liu *et al*., 2007
inlJ	F-TGTAACCCCGCTTACACAGTT R-AGCGGCTTGGCAGTCTAATA	238	Liu *et al*., 2007
prfA	F-ACCAATGGGATCCACAAGA R-CAGCTGAGCTATGTGCGAT	467	Bubert *et al*., 1999
plcB	F-AATATTTCAATCAATCGGTGGCTGA R-GGGTAGTCCGCTTTCGCTCTCTT	289	Du *et al*, 2016

Table 3. Prevalence of *Listeria s*pecies in fresh vegetable and market garden samples.

Type of food	Number of samples	Number of positive samples (%) *Listeria* spp.
Lettuce	27	7 (25.92)
Tomato	27	1 (3.70)
Cabbage	27	3 (11.11)
Carrot	27	0 (0)
Cucumber	27	0 (0)
Total	135	11 (8.15)

Table 4. Restriction profile of Listeria spp.

Profile	F1 restriction fragments				F2 restriction fragments		Species reference strain	Tested strains
	ApaI	*XmaI*	*Tsp509I*	*SacII*	*AluI*	*AccII*		
I	698+609+235	1382+160	675+523+244+60+40	840+291+238+173	–	–	*L. ivanovii*	L135
II	698+609+235	1382+160	675+523+244+60+40	1013+291+238	344+206 +182+160+47	–	*L. monocytogenes*	L208, L238
III	698+609+235	1382+160	675+523+244+60+40	1013+291+238	389+343+160+47	285+209+192 +184+50+9+8+2	*L. innocua*	L136, L191, L209,L213, L218, L224, L233, L234
IV	698+609+235	1382+160	675+364+244+159+60+40	–	–	–	*L. welshimeri*	–
V	698+609+235	1382+160	561+523+244+117+60+40	–	–	–	*L. grayi*	–
VI	698+609+235	1382+160	675+523+244+60+40	1013+291 +238	389+343+160+47	293+209+192+ 184+50+9+2	*L. seeligeri*	–

F1, fragment (1542bp) frompolymerase chain reaction (PCR) with primers fD1 and rD1; F2, fragment (939bp) from PCR with primers 16SListF and 16SListR.

lane 1) (serotypes 1/2a, 1/2c, 3a, and 3c). The reference strains *L. monocytogenes* EGDe (Glaser *et al.*, 2001) and *L. monocytogenes* CLIP80459 (Hain *et al.*, 2012) are used as controls (lanes 1 and 2) and are serotype 1/2a and 4b, respectively. The presence of 7 virulence genes in *L. monocytogenes* whose expression enables infection of the host cell was confirmed in *L. monocytogenes* strains *L208* and *L238*, namely *actA*, *hly*, and *inlJ* (Figure 3A), and *inlC*, *inlA*, *prfA*, and *pclB* (Figure 3B).

Discussion

The objective of this study was to identify bacteria belonging to the *Listeria* genus in fresh vegetables and market garden produce (lettuce, cabbage, tomato, carrot, and cucumber) sold in 3 communities in Abidjan (Côte d'Ivoire). The results of the various analyses revealed the presence of *Listeria* in these vegetables. The rate of occurrence of *Listeria* spp. was 8.14%, and the prevalence

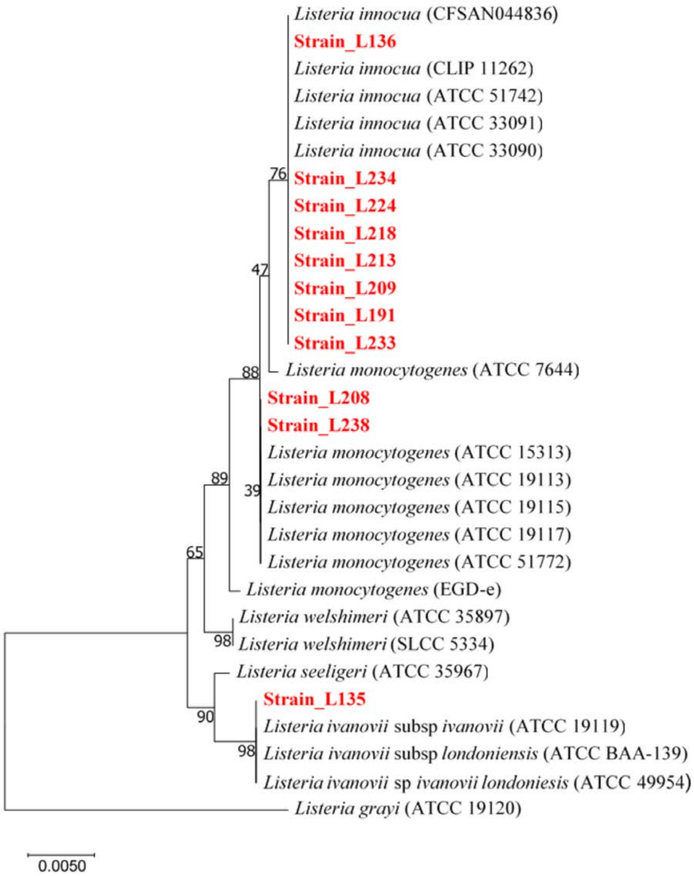

Figure 1. Neighbor-joining phylogenetic tree based on the analysis of 16S rRNA gene sequences (1542 bp) of all *Listeria* isolates obtained from fresh vegetables and market garden produce in Côte d'Ivoire (shown in red) and their homologous species obtained from National Center for Biotechnology Information (NCBI) GenBank. Numbers at the nodes indicate the bootstrap values.

Table 5. BLAST results (at NCBI) with the partially sequenced 16S rRNA genes from the 11 isolated strains with their accession number and percentage of identity.

Isolates	NCBI Blast results	Accession number	% identity
L135	*Listeria ivanovii*	PQ473711	99.89
L136	*Listeria innocua*	PQ473721	99.91
L191	*Listeria innocua*	PQ473715	99.80
L208	*Listeria monocytogenes*	PQ473712	100
L209	*Listeria innocua*	PQ473716	99.73
L213	*Listeria innocua*	PQ473717	99.77
L218	*Listeria innocua*	PQ473718	99.81
L224	*Listeria innocua*	PQ473719	99.64
L233	*Listeria innocua*	PQ473713	100
L234	*Listeria innocua*	PQ473720	99.91
L238	*Listeria monocytogenes*	PQ4737143	100

NCBI, National Center for Biotechnology Information.

of *L. monocytogenes* was 1.48%. These results are in agreement with those reported in the city of Tétouan, Morocco, where *Listeria* spp. and *L. monocytogenes* were found at rates of 7.3% and 1.5%, respectively (Amajoud *et al.*, 2018), and in Japan, where *L. monocytogenes* was found at a rate of 1.7% (Shimojima *et al.*, 2016). However, the prevalence of *L. monocytogenes* remains lower than that reported in Nigeria where the percentage distribution of the *L. monocytogenes* isolates in the Ready-to-eat vegetables was 28.28, 9.02, 23.36, 19.67, and 19.67% for cabbage, carrot, cucumber, lettuce, and tomatoes, respectively (Ajayeoba, 2015), in the Eastern Cape Province, South Africa where the highest prevalence was recorded in tomato (65.52%) followed by spinach (56.67%), cabbage (38.10%), apple (36.84%), mushroom (29.41%) and carrot (10%) (Kayode and Okoh, 2022). Furthermore, lettuces were the foods most contaminated (5.7%), followed by cabbages (3.8%), and tomatoes (1.9%) by *Listeria* spp. *L. monocytogenes* was isolated only from lettuce. The presence of *L. monocytogenes* in lettuces can be attributed to factors such as geographical variations, sanitary and hygienic conditions during harvesting, storage, and marketing. In fact, temperature, humidity, and precipitation can directly influence the survival and growth of *Listeria*. For example, temperate and humid climates favor the persistence of the bacteria in

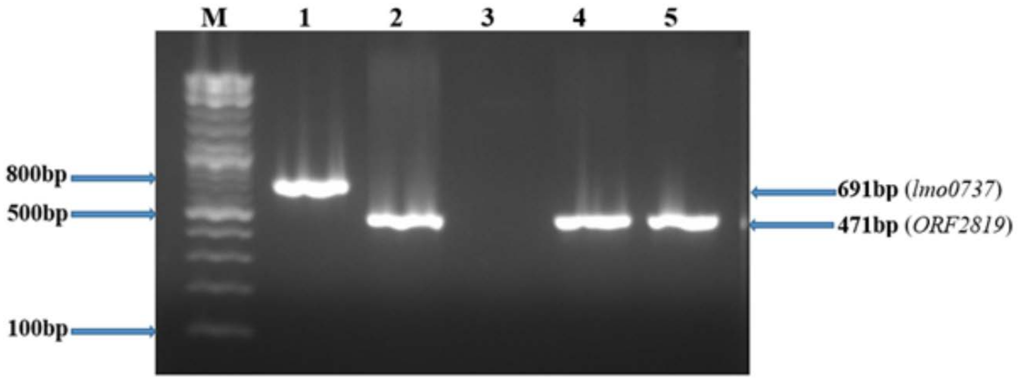

Figure 2. Serotyping by multiplex PCR targeting two genes (*ORF2819* and *lmo0737*). Lane 1: *L. monocytogenes* serotype 1/2a EGDe (positive control), Lane 2: *L. monocytogenes* serotype 4b CLIP80459 (positive control), Lane 4: strain *L208*, Lane 5: strain *L238*. Lane M: 1kb DNA Ladder plus. Selected molecular size markers in bp are noted on the left. Genes corresponding to the amplified fragments are indicated on the right.

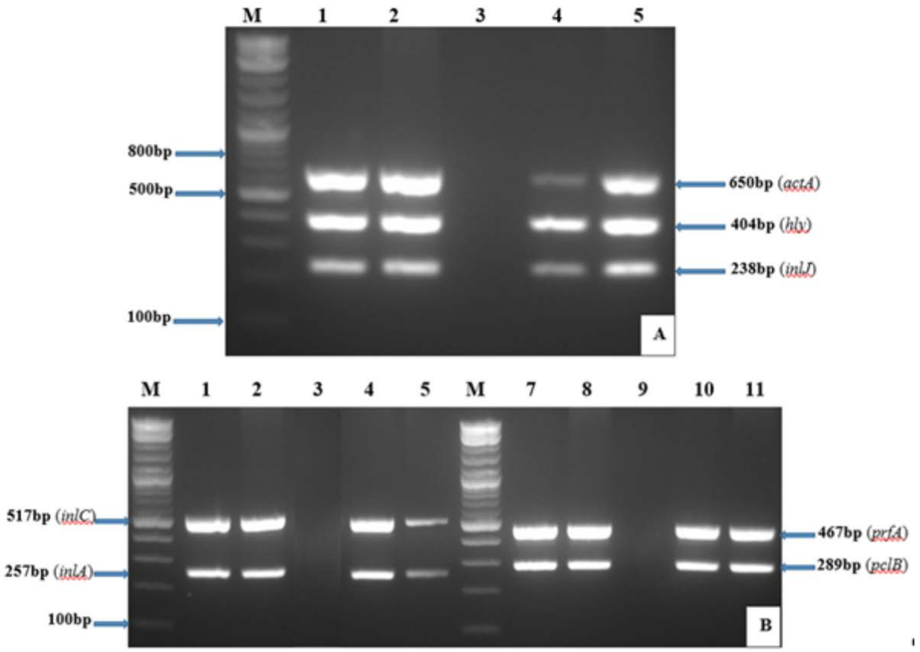

Figure 3. Amplicons of virulence genes in L. monocytogenes. 3A- Lane 1: *L. monocytogenes* EGDe (positive control); Lane 2: *L. monocytogenes* CLIP80459; (positive control) Lane 4: Strain *L208*; Lane 5: Strain *L238*); 3B- Lanes 1 and 7: *L. monocytogenes* EGDe (positive control); Lanes 2 and 8: *L. monocytogenes* CLIP80459 (positive control); Lanes 4 and 10: Strain *L208*; Lanes 5 and 11: Strain *L238*). Lane M: 1kb DNA Ladder plus. **A**) Targeted genes *actA, hly, inlJ*; **B**) targeted genes *prfA, pclB, inlA, inlC.*

soil and water, thereby increasing the risk of crop contamination (Linke *et al*., 2014). The use of organic fertilizers (manure), irrigation practices (using wastewater or surface water), and proximity to livestock farms vary between regions and can influence contamination levels (Islam *et al*., 2004). The presence of pathogenic bacteria on these fresh products can cause food-borne epidemics (Settanni *et al.*, 2012; Buchanan *et al.*, 2017). From a food safety point of view, these products are known to be foods at risk of transmitting pathogenic microorganisms (Callejón *et al.*, 2015). Cases of listeriosis infection due to the ingestion of different types of vegetables are often reported in certain countries around the world. An outbreak of *L. monocytogenes* infection associated with the consumption of watermelons (cantaloupe) in Australia was reported to the World Health Organization in 2018, over a period from 17 January to 16 April 2018, and 20 cases of listeriosis (19 confirmed and 1 suspected) were reported. All patients were hospitalized, and 7 deaths and one miscarriage associated with the outbreak were reported (WHO, 2018b). In recent years, melon and other fruits and vegetables have been implicated in outbreaks of listeriosis. In April-May 2025, a multistate outbreak of *L. monocytogenes* infections was linked to ready-to-eat products manufactured by Fresh & Ready Foods LLC, distributed in California, Nevada, and other states. The outbreak resulted in at least 10 hospitalizations and one death, prompting a large-scale recall of sandwiches, wraps, and snack boxes (CDC, 2025). Environmental sampling revealed the persistence of the outbreak strain within the production facility, indicating deficiencies in sanitation and control measures. Earlier in 2025, Marketside-branded broccoli florets sold at Walmart were recalled due to potential contamination with *L. monocytogenes*. Although no cases were reported, the recall underscored the ongoing risk of contamination in minimally processed vegetables, even when refrigerated (FDA, 2024). Similarly, Wegmans recalled multiple varieties of soft cheeses, including Camembert, due to concerns over possible *Listeria* contamination (FDA, 2025). While no illnesses were directly associated with the cheese recall, these preventive actions demonstrate the vigilance required in monitoring high-risk food categories. To reduce the risk, it is important to thoroughly wash all fruits and vegetables using clean, potable water, as the use of contaminated water may lead to the reintroduction or spread of *Listeria* and other pathogens, thereby increasing the risk of contamination (FAO and WHO, 2008). The world's largest known listeriosis outbreak occurred in South Africa, with over 1060 confirmed cases and 216 deaths, primarily linked to contaminated ready-to-eat polony (processed meat) products. It was traced to the ST6 strain at an Enterprise Foods facility in Polokwane. The outbreak led to widespread product recalls in South Africa and 15 other African countries (Dufailu *et al*., 2021). Among confirmed cases, 42% were neonates infected during pregnancy or delivery, and the case-fatality rate reached 27% (WHO, 2018a). The WHO issued alerts and initiated response efforts across 15-16 African countries – including Angola, Botswana, Ghana, Nigeria, Uganda, and Zimbabwe – to enhance surveillance, recall contaminated products, and improve outbreak preparedness. Numerous studies have indicated that various foods promote the growth of *L. monocytogenes* due to their nutritional content and physicochemical characteristics (Jacob *et al.*, 2014; Poimenidou *et al.*, 2016). Our study established that the isolated *L. monocytogenes* strains L208 and L238 belong to the serogroups (1/2b, 3b, 4b, 4d, 4e, and 7) corresponding to the genetic lineage I. We did not perform classical serotyping with antisera. Serotype 1/2a dominates in fresh produce, suggesting environmental persistence and adaptation. Serotype 4b, though less frequent in vegetables, is of great clinical concern due to its strong association with severe outbreaks (including meningitis and pregnancy-related listeriosis). In root vegetables (*e.g*., carrots, radishes, beets), the presence of *L. monocytogenes* serotypes 1/2a and 1/2c is more common. A study carried out in Ireland showed that *L. monocytogenes* strains isolated from vegetables, seafood, dairy products, and meat were serogroup (1/2a, 3a, 1/2b, 3b, 7, 1/2c, 3c, 4b, 4d, 4e) (Leong *et al.*, 2017). Another study conducted by Skowron *et al.* (2018) in Poland showed that the *L. monocytogenes* strains isolated in a fish processing plant were serogroups 1/2a-3a (38.6%) and 1/2b, 3b (32.8%). In China, Su *et al.* (2016) reported the occurrence of serogroups 1/2c, 3c (39.1%), 1/2a, 3a (36.7%), and 1/2b, 3b, 7 (24.2%) in *L. monocytogenes* strains isolated from food and human samples. Studies have reported that serotypes 1/2a, 1/2b, 1/2c, and 4b are involved in about 95% of all described cases of human listeriosis worldwide (Pontello *et al.*, 2012). The presence of these serotypes in food products, particularly ready-to-eat foods, poses a potential health risk to consumers and can lead to severe cases of human listeriosis. The presence of virulence genes in *L. monocytogenes* strains has been detected. The results obtained in this study are in line with those previously described for the *actA*, *hly*, *inlA*, *inlC*, and *inlJ* genes in *L. monocytogenes* isolated from raw retail foods (Wu *et al.*, 2015) from ready-to-eat foods in Malaysia and China (Jamali *et al.*, 2014; Wu *et al.*, 2016). Maury *et al*. (2016) showed that some clones of *L. monocytogenes* isolated from food (CC9, CC121) carry attenuating mutations in the *inlA* gene and are better adapted to persistence in the environment, while others, considered hypervirulent (CC1, CC2, CC4, CC6), harbor additional pathogenicity islands such as LIPI-3 and LIPI-4, which enhance their ability to cause infections in humans. The USDA's Economic Research Service estimates that the annual cost of *L. monocytogenes* in the U.S. is approximately $2.8 billion, mainly due to medical expenses, lost productivity, and premature deaths (USDA, 2014). Consequently, the detection of *L. monocytogenes* in fresh vegetables results from a failure either during cultivation and harvesting or along the production chain and represents a public health hazard. The consumption of fresh vegetables contaminated with these strains poses a significant risk to human health. Additionally, there is an economic risk linked to the presence of *L. monocytogenes* in foodstuffs, as contaminated products must be recalled from the market, resulting in significant financial losses.

Conclusions

The present study provided data on *Listeria* spp. prevalence, serotype, and virulence genes of *L. monocytogenes* isolated from fresh vegetables and market garden produce in the city of Abidjan, Côte d'Ivoire. This study revealed a fairly low presence rate of *Listeria* spp. and *L. monocytogenes* strains in the samples collected. Three *Listeria* species (*L. innocua*, *L. ivanovii*, and *L. monocytogenes*) were identified. The *L. monocytogenes* isolates belong to serogroups (1/2b, 3b, 4b, 4d, 4e and 7) corresponding to genetic lineage I and possessing virulence genes (*inlA*, *inlC*, *inlJ*, *prfA*, *plcB*, *hly*, and *actA*). However, the presence of these strains in food products poses a considerable threat to consumer safety. This study could be improved by using a larger sample size, taking seasonal variations into account, and conducting sampling over time to observe the distribution of Listeria. Despite these limitations, it provides valuable data on the presence of *L. monocytogenes* in fresh vegetables in Côte d'Ivoire. These results contribute to food risk assessment, highlight the important role of raw vegetables in the transmission of listeriosis, and support the need for enhanced surveillance and appropriate food safety policies.

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