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ORIGINAL RESEARCH

Virulence Gene Profiling and Molecular Typing of Clinical Isolates of *Listeria monocytogenes* from Wenzhou, Eastern China

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Objective: To investigate the serotypes, virulence genes, and molecular typing characteristics of *Listeria monocytogenes* strains isolated from a hospital in Wenzhou City.

Methods: Polymerase chain reaction (PCR) was employed to detect the serotypes and virulence genes of *Listeria monocytogenes*, as well as to perform multilocus sequence typing (MLST). The broth microdilution method was utilized for susceptibility testing. Additionally, epidemiological survey data were integrated to explore the relationship between the absence of key virulence genes and the serotypes of *Listeria monocytogenes*.

Results: All strains exhibited sensitivity to penicillin, erythromycin, meropenem, and co-trimoxazole. The strains were primarily categorized into three serotypes: 1/2a, 1/2b, and 4b, with serotype 1/2b being the most prevalent, accounting for 70.59% of the isolates. MLST analysis identified nine sequence types (ST) and eight clonal complexes (CC), with ST87 (CC87) being the most common. Virulence gene analysis revealed that the carriage rates for *inlA, inlC, plcB, prfA, mpl, iap, hly, inlB, plcA*, and *fbpA* were 100%. In contrast, the carriage rates for *actA, inlJ*, and *llsX* were 70.59%, 47.06%, and 35.29%, respectively. The 1/2a serotype strain carried all virulence genes except for *llsX*, and no statistically significant differences were observed in the carriage rates of the 13 virulence genes among serotypes 1/2a, 1/2b, and 4b ($P > 0.05$).

Conclusion: The clinical isolates of *Listeria monocytogenes* in Wenzhou City exhibit polymorphism in MLST typing, predominantly ST87 (CC87) and serotype 1/2b. All strains harbored the major virulence genes, indicating their potential pathogenicity. This underscores the necessity for enhanced surveillance and preventive measures regarding the virulence genes of *Listeria monocytogenes* and antibiotic resistance.

Keywords: serotype diversity, antibiotic susceptibility, epidemiological surveillance, gene carriage rate, public health

Introduction

Listeria monocytogenes (*L. monocytogenes*) is a facultative intracellular pathogen responsible for severe foodborne illnesses in both humans and animals.[1] This bacterium can breach the intestinal barrier, blood–brain barrier, and placental barrier, leading to conditions such as gastroenteritis, sepsis, meningitis, and miscarriages. It poses a significant risk, particularly to pregnant women, newborns, immunocompromised individuals, and the elderly. *L. monocytogenes* exhibits remarkable tolerance, allowing it to survive in high-salt, acidic, and alkaline environments, and it can proliferate at temperatures ranging from 0 to 45°C, earning it the moniker "refrigerator killer".[2] The World Health Organization has classified it as one of the four major foodborne pathogens. Although the incidence of human foodborne infections is relatively low, the mortality rate associated with listeriosis can reach as high as 15% to 21%, even with aggressive antibiotic treatment.[2] With the rising consumption of ready-to-eat foods, the risk of infection is also increasing, particularly due to potential cross-contamination during the processing of cooked foods. For instance, the *L. monocytogenes* outbreak in South Africa from 2017 to 2018

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resulted in 1060 reported cases, primarily linked to ready-to-eat meat products. In the United States, approximately 1,600 individuals are infected with this bacterium each year, resulting in about 260 deaths.[3]

The pathogenicity of *L. monocytogenes* is significantly influenced by several virulence genes, including *hly, iap, prfA, inlA*, and *actA*. Each of these genes encodes proteins that play critical roles in the bacterium's ability to invade host cells and evade the immune response.[4] The *hly* gene encodes listeriolysin O (LLO), a pore-forming toxin that is essential for the bacterium's escape from the phagosome of host cells, allowing it to survive and replicate within the cytoplasm.[5] The *iap* gene encodes the P60 protein, which is involved in the invasiveness of *L. monocytogenes*. This protein facilitates the bacterium's entry into host cells, enhancing its ability to establish infection. The *prfA* gene acts as a transcriptional regulator, controlling the expression of several virulence factors, including *hly* and *iap*. This regulation is crucial for the coordinated expression of virulence genes in response to environmental signals, allowing *L. monocytogenes* to adapt to different niches within the host.[6]

The *inlA* gene encodes an internal membrane protein that binds to the host cell receptor E-cadherin, which is essential for the adhesion and internalization of *L. monocytogenes* into epithelial cells. This interaction is critical for the bacterium's ability to invade host cells. Additionally, the *actA* gene encodes a protein that promotes the actin-based motility of *L. monocytogenes* within host cells, enabling the bacteria to spread from cell to cell and evade immune detection.[7] The regulatory expression of these genes enables the bacteria to effectively invade host tissues. Understanding their roles provides valuable insights into the pathogenicity of *L. monocytogenes*.

The standard treatment regimen for listeriosis caused by *L. monocytogenes* primarily involves β-lactam antibiotics, with ampicillin as the first-line choice. For severe invasive cases, combination therapy with gentamicin is often used to enhance efficacy.[8] Global surveillance indicates generally low resistance rates to first-line drugs (ampicillin 4.4%, gentamicin 2.7%).[9] However, regional resistance issues warrant attention: food-derived isolates in China exhibit multi-drug resistance (MDR), South African beef product isolates show alarmingly high resistance to cefotaxime (97.1%), and European clinical isolates demonstrate resistance to trimethoprim-sulfamethoxazole (29.23%).[10–12] Therefore, continuous monitoring of antimicrobial resistance in both food chain and clinical isolates is urgently needed.

L. monocytogenes is a highly heterogeneous microorganism, with its population structure divided into 13 serotypes and four phylogenetic lineages (I, II, III, and IV). These lineages are further subdivided into multiple clonal complexes (CCs) based on multilocus sequence typing (MLST).[1] Serotypes 1/2a and 1/2b are primarily associated with gastro-intestinal disease outbreaks, while serotype 4b is closely linked to listeriosis outbreaks that result in severe conditions such as sepsis, central nervous system damage, and fetal infections.[13] In Western countries, lineage I is the predominant type responsible for listeriosis outbreaks related to contaminated food. In contrast, outbreaks in China are less common, with sporadic cases being more frequent.[14] However, the incidence of listeriosis in China has increased over the past decade, mirroring trends observed in Western countries.[15] This underscores the importance of analyzing the distribution of serotypes and genotypes of *L. monocytogenes* as a critical method for monitoring and tracing its presence. Conducting dynamic epidemiological surveys is essential for effectively monitoring and controlling the spread of listeriosis.

This study primarily investigates the serological typing and multilocus sequence typing (MLST) of 17 clinical isolates of *L. monocytogenes* obtained from a hospital in Wenzhou City, alongside the detection of associated virulence genes. The significance of understanding the distribution of serotypes and pathogenic genes in these strains is underscored by its implications for population analysis and molecular epidemiological research regarding *L. monocytogenes* in Wenzhou City. This foundational data is crucial for tracing foodborne diseases linked to *L. monocytogenes*, which poses a significant public health risk due to its association with severe foodborne illnesses.

Materials and Methods

Bacterial Strains

Seventeen non-repetitive strains of *L. monocytogenes* were isolated from clinical samples at the First Affiliated Hospital of Wenzhou Medical University in Wenzhou City over a decade, from 2012 to 2022. The identification of these strains was initially performed through Gram staining, a standard microbiological technique that allows for the differentiation of bacterial species based on their cell wall composition. Subsequently, the identification was confirmed using the VITEK

MS automated rapid microbial mass spectrometry detection system developed by bioMérieux, France.[16] The standard strains used for comparison in this study included *L. monocytogenes* ATCC 19111, *Streptococcus pneumoniae* ATCC 49619, and *Staphylococcus aureus* ATCC 29213.

Bacterial DNA Extraction

The strains were inoculated into brain-heart infusion broth (BHIB) and incubated overnight at 37°C. Following incubation, the culture was processed according to the manufacturer's instructions using a bacterial genomic DNA extraction kit (Qiagen, Germany) to extract DNA.[16]

Serotyping

The serotyping of *L. monocytogenes* was conducted using the multiplex polymerase chain reaction (PCR) method established by Doumith et al.[17] The specific primers utilized for serotyping are detailed in Table 1. The multiplex PCR amplification system was prepared in a total volume of 50 μL, comprising 25 μL of 2 × TaqMaster Mix, 1 μL each of upstream and downstream primers for imo0737, orf2819, orf2110 (at a concentration of 10 μmol/L), 1.5 μL of the imo1118 primer (10 μmol/L), 0.5 μL of the prs primer (10 μmol/L), 2 μL of DNA template, and sterile water to complete the volume to 50 μL. The amplification conditions were as follows: an initial pre-denaturation step at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 53°C for 69 seconds, and extension at 72°C for 69 seconds. A final extension step was performed at 72°C for 7 minutes. After amplification, the Qsep100 fully automatic nucleic acid protein analysis system was employed for capillary electrophoresis to analyze the PCR products and determine the serotype of the isolates.

Detection of Virulence Genes

PCR amplification was conducted to detect various virulence genes, including *inlA, inlC, inlJ, plcB, prfA, mpl, iap, hly, actA, inlB, plcA, fbpA*, and *llsX*, with the specific primer sequences detailed in Table 2.[18–20] The PCR reaction mixture was composed of a total volume of 25 μL, which included 12.5 μL of Taq PCR Master Mix, 1 μL each of upstream and downstream primers (10 μmol/L), 1 μL of template DNA, and 9.5 μL of sterile water. The thermal cycling conditions were set as follows: an initial pre-denaturation step at 94°C for 4 minutes, followed by 32 cycles of denaturation at 94°C for 30 seconds, annealing for 30 seconds, and extension at 72°C for 1 minute, concluding with a final extension at 72°C for 10 minutes. The amplification results were analyzed using capillary gel electrophoresis.

MLST Typing

The MLST analysis of *L. monocytogenes* was conducted following the established protocols outlined in the *L. monocytogenes* MLST database maintained by the Pasteur Institute, France. This analysis focused on the partial sequences of seven housekeeping genes: *abcZ, blgA, cat, dapE, dat, ldh*, and *lhkA*, which were amplified using PCR.[21] The PCR conditions

Table 1 Primers for Serotyping of *Listeria monocytogenes*

Primer	Primer Sequence (5'-3')	Size of Amplified Fragment/bp	Serum Specificity
lmo0737	F: AGGGCTTCAAGGACTTACCC R: ACGATTTCTGCTTGCCATTC	691	1/2a, 1/2c, 3a, 3c
lmo1118	F: AGGGGTCTTAAATCCTGGAA R: CGGCTTGTTCGGCATACTTA	906	1/2c, 3c
orf2819	F: AGCAAAATGCCAAAACTCGT R: CATCACTAAAGCCTCCCATTG	471	1/2b, 3b, 4b, 4d, 4e
orf2110	F: AGTGGACAATTGATTGGTGAA R: CATCCATCCCTTACTTTGGAC	597	4b, 4d, 4e
prs	F: GCTGAAGAGATTGCGAAAGAAG R: CAAAGAAACCTTGGATTTGCGG	370	All strains of the genus Listeria

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Table 2 Primers for the 13 Major Virulence Genes of *Listeria monocytogenes*

Primer	Coding Protein	Primer Sequence (5'-3')	bp	Annealing Temperature
inlA	Endogenous A	F: AATCTAGCACCACTGTCGGG R: TGTGACCTTCTTTTACGGGC	733	58
hlyA	Listeriolysin O	F: GTTAATGAACCTACAAGHCCTTCC R: AACCGTTCTCCACCATTCCCA	702	57
iap	P60 protein	F: ACAAGCTGCACCTGTTGCAG R: TGACAGCGTGTGTAGTAGCA	131	56
prfA	PrfA protein	F: CCCAAGTAGCAGGACATGCTAA R: GGTATCACAAAGCTCACGAG	571	53
plcB	Phospholipase B	F: GATAACCCGACAAATACTGACGTAAATAC R: TCATCTGAGCAAAATCTTTTTGCTACCATGTC	503	57
inlB	Endogenous B	F: GATATTGTGCCACTTTCAGGT R: CCTCTTTCAGTGGTTGGGT	367	54
actA	ActA protein	F: CGCCGCGGAAATTAAAAAAAGA R: ACGAAGGAACCGGGCTGCTAG	839	56
plcA	Phospholipase A	F: CTGCTTGAGCGTTCATGTCTCATCCCCC R: CATGGGTTTCACTCTCCTTCTAC	1484	58
mpl	Mpl protein	F: ATAGCTTTTCAGGCTCATTTCA R: ATAGCTTTTCAGGCTCATTTCA	1184	58
inlJ	Endogenous J	F: TGTAACCCCGCTTACACAGTT R: AGCGGCTTGGCAGTCTAATA	238	58
inlC	Endogenous C	F: AATTCCCACAGGACACAACC R: CGGGAATGCAATTTTTCACTA	517	57
fbpA	Ferric Binding Protein A	F: AGCTCTTAGCTCCATGAGTT R: AGTAGCAGCACCTGTAGCAGT	420	60
llsX	Hemolysin S	F: TTATTGCATCAATTGTTCTAGGG R: CCCCTATAAACATCATGCTAGTG	200	52

were optimized as follows: an initial pre-denaturation step at 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 2 minutes, concluding with a final extension at 72°C for 10 minutes. After amplification, the PCR products were purified and sequenced bidirectionally. The resulting sequences were then compared against the *L. monocytogenes* MLST database to assign ST based on the serial numbers of each housekeeping gene. The combination of these seven serial numbers defines the ST type of the strain.

Antimicrobial Susceptibility Testing

Based on the study conducted by Silva et al, the method was adapted to implement the microbroth dilution technique for antimicrobial susceptibility testing of penicillin, erythromycin, meropenem, and trimethoprim-sulfamethoxazole.[22] The results were interpreted in accordance with the guidelines established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Statistical Analysis

Data analysis was conducted using SPSS version 23.0 statistical software, with a significance threshold set at $P < 0.05$. Furthermore, all visualizations and graphical representations were created using the R programming language, version 4.3.3.

Results

Basic Information of Strains and Susceptibility Results

A total of 17 strains were isolated from 2 males, 10 females, and 5 neonates, with 3 cases attributed to mother-to-child transmission. The age distribution of the patients ranged from 1 to 64 years. Blood samples constituted the primary

Table 3 Basic Information and Drug Susceptibility Results of *Listeria monocytogenes* Strains

NO	Gender	Age	Source	Sample Type	Penicillin	Erythromycin	Meropenem	Co-trimoxazole
1	Female	26	Inpatient	Blood	S	S	S	S
2	Female	30	Inpatient	Blood	S	S	S	S
3	Female	64	Outpatient	Blood	S	S	S	S
4	Female	33	Inpatient	Blood	S	S	S	S
5	Male	1	Inpatient	Blood	S	S	S	S
6	Female	1	Inpatient	Blood	S	S	S	S
7	Female	26	Inpatient	Blood	S	S	S	S
8	Female	64	Outpatient	Blood	S	S	S	S
9	Male	1	Inpatient	Blood	S	S	S	S
10	Female	22	Inpatient	Blood	S	S	S	S
11	Female	30	Inpatient	Amniotic fluid	S	S	S	S
12	Female	33	Inpatient	Blood	S	S	S	S
13	Female	26	Inpatient	Vaginal secretions	S	S	S	S
14	Male	57	Outpatient	Blood	S	S	S	S
15	Female	1	Inpatient	Blood	S	S	S	S
16	Male	57	Outpatient	Blood	S	S	S	S
17	Male	1	Inpatient	Cerebrospinal fluid	S	S	S	S

Notes: Penicillin (S ≤ 1 μg/mL, R > 1 μg/mL), Erythromycin (S ≤ 1 μg/mL, R > 1 μg/mL), Meropenem (S ≤ 0.25 μg/mL, R > 0.25 μg/mL), co-trimoxazole (S ≤ 0.06/1.14 μg/mL, R > 0.06/1.14 μg/mL).

sample type, accounting for 82.35% of the isolates, while cerebrospinal fluid, vaginal secretions, and amniotic fluid each contributing to one case. Notably, all strains demonstrated sensitivity to penicillin, erythromycin, meropenem, and co-trimoxazole (Table 3).

Genotyping Results

The 17 strains of *L. monocytogenes* were classified into three serotypes: 1/2a, 1/2b, and 4b. Among these, serotype 1/2b was the most prevalent, accounting for 70.59% (12 out of 17 strains), while serotypes 1/2a and 4b represented 17.64% and 11.76%, respectively. MLST analysis revealed nine distinct STs and eight clonal complexes (CCs), specifically: ST87 (5 strains), ST59 (3 strains), ST3 (2 strains), ST619 (2 strains), ST1 (1 strain), ST213 (1 strain), ST1534 (1 strain), ST91 (1 strain), and ST779 (1 strain). The isolates were categorized into two lineages, with lineage I comprising serotypes 1/2b (70.59%, n = 12) and 4b (11.76%, n = 2), while lineage II included serotype 1/2a (17.65%, n = 3).

Distribution of Virulence Genes

The carriage rates of virulence genes in *L. monocytogenes* were as follows: *inlA, inlC, plcB, prfA, mpl, iap, hly, inlB, plcA* and *fbpA* were present in 100% of the strains. In contrast, the carriage rates for *actA, inlJ*, and *llsX* were 70.59%, 47.06%, and 35.29%, respectively (Figure 1). The distribution of strains based on the number of virulence genes carried revealed that 17.65% (3 out of 17) of the strains carried 10 virulence genes, 11.76% (2 out of 17) 11 virulence genes, and the majority, 70.59% (12 out of 17), carried 12 virulence genes.

Distribution of Virulence Genes in Different Serotype Strains

Serotype 1/2a was found to carry all virulence genes except for llsX. The results of the statistical analysis are presented in Table 4, indicating no significant difference in the carriage rates of 13 virulence genes among serotypes 1/2a, 1/2b, and 4b ($P > 0.05$).

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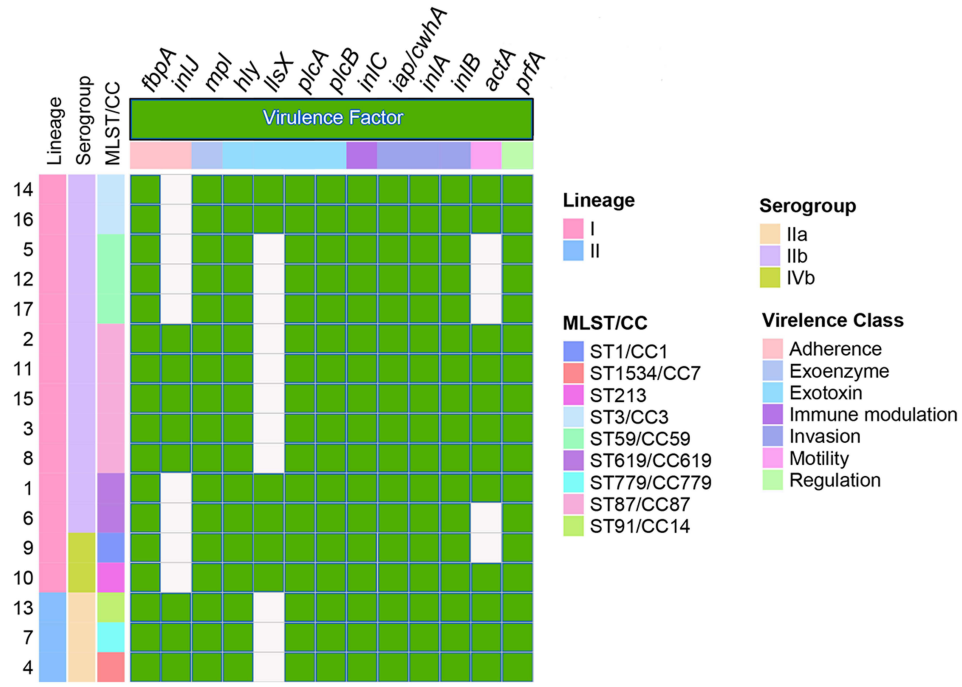

Figure 1 Genetic analysis of 17 clinical isolates of *Listeria monocytogenes* from the Wenzhou area of eastern China.

Discussion

L. monocytogenes is a significant foodborne pathogen with considerable public health implications globally. This bacterium is capable of invading various non-phagocytic cells, and its virulence potential varies among different serotypes. Understanding the carriage of virulence genes in *L. monocytogenes* is crucial for developing effective diagnostic strategies and mitigating the risk of human listeriosis.[23]

The distribution of virulence factors and their uniformity across different clonal complexes (CCs) and lineages of *L. monocytogenes* has garnered significant attention in recent studies, highlighting the pathogen's adaptability and transmission capabilities in diverse environments.[24] In the outbreaks of neurological and maternal–fetal listeriosis reported in Europe and the

Table 4 Distribution Virulence Genes of *Listeria monocytogenes* with Different Serotypes

Virulence Genes	1/2a (n = 3)		1/2b (n = 12)		4b (n = 2)		*P* - value
	Positive Strain Count	Carriage Rate (%)	Positive Strain Count	Carriage Rate (%)	Positive Strain Count	Carriage Rate (%)	
inlA	3	100.00	12	100.00	2	100.00	#
hlyA	3	100.00	12	100.00	2	100.00	#
iap	3	100.00	12	100.00	2	100.00	#
prfA	3	100.00	12	100.00	2	100.00	#
plcB	3	100.00	12	100.00	2	100.00	#
inlB	3	100.00	12	100.00	2	100.00	#
actA	3	100.00	8	66.67	1	50.00	0.547
plcA	3	100.00	12	100.00	2	100.00	#
mpl	3	100.00	12	100.00	2	100.00	#
inlJ	3	100.00	5	41.67	0	0.00	0.062
inlC	3	100.00	12	100.00	2	100.00	#
llxS	0	0.00	4	33.33	2	100.00	0.085

Notes: The *P* - value was calculated using Fisher's exact test. #*P* - value cannot be calculated.

United States, CC1, CC2, and CC6, which carry *Listeria* pathogenicity islands (LIPI) 1, as well as CC4, which harbors both LIPI-1 and LIPI-4, are the primary clonal groups associated with these occurrences.[25] Notably, CC4, which harbors LIPI-1 and 4, exhibits heightened pathogenicity by enhancing the invasion of the nervous system and placenta.[26,27] In contrast, the characteristics of sporadic listeriosis in China differ markedly from those observed in Western nations. Research indicates that CC87, CC8, CC9, CC121, CC155, and CC619 are prevalent among human clinical cases in China, with CC87 being particularly notable for carrying LIPI-1, −3, and −4, making it one of the most common high-pathogenicity clonal complexes in clinical isolates.[1] In this study conducted in the Wenzhou area, *L. monocytogenes* was predominantly represented by sequence type ST87 (CC87), aligning with national epidemiological trends. This sequence type has been recognized as an epidemic strain of Chinese origin, suggesting that its prevalence may be linked to its adaptability to specific environments and interactions with hosts.[28] It is noteworthy that Liu et al identified CC87-ST87 as one of the predominant sequence types in their study of *L. monocytogenes* isolates from Northern Taiwan during the period 2009–2019.[29] This observation not only underscores the high prevalence of CC87-ST87 in Taiwan but also reinforces evidence of its widespread distribution and potential high virulence across various regions in China.

L. monocytogenes is a significant intracellular pathogen, and its pathogenicity is intricately linked to its virulence genes. The absence or mutation of these virulence genes can lead to a marked decrease or complete loss of pathogenicity. For instance, the invasion-associated protein (*iap*) is essential for the bacterial invasion process.[30] Research has demonstrated that the expression of the *iap* gene is closely associated with the survival and proliferation of *L. monocytogenes* within host cells.[31] The *prfA* gene serves as the primary regulatory gene for virulence, orchestrating the expression of various virulence factors, including listeriolysin O (LLO), which is encoded by the *hlyA* gene, and internalin proteins such as *inlA* and *inlB*.[32] The activity of *PrfA* is crucial for the pathogenicity of *L. monocytogenes*; strains lacking this gene typically exhibit significantly reduced virulence.[33] LLO, encoded by *hlyA*, functions as a potent extracellular toxin that disrupts the host cell membrane, facilitating the escape of bacteria from the endosome into the cytoplasm, thereby promoting intracellular survival and replication.[34] Additionally, internalin A (*inlA*) acts as a key adhesion factor, binding to the E-cadherin receptor on host cells, which enhances bacterial internalization and invasion.[35] Furthermore, phospholipase C (*plcA*) plays a vital role in the intracellular survival and dissemination of *L. monocytogenes* by hydrolyzing phospholipids in the host cell membrane, aiding in the escape from the endosomal compartment.[34] In this study, all isolates from clinical samples in Wenzhou City were found to carry the five virulence genes: *iap, prfA, hlyA, inlA*, and *plcB*, indicating a potential pathogenicity of these *L. monocytogenes* strains. Notably, 52.94% of the specimens originated from obstetrics and neonatology, underscoring the potential threat this pathogen poses to pregnant women and newborns. Recent cases of listeriosis in pregnant women in Wenzhou City, resulting in sepsis and miscarriage, have drawn significant public and medical attention. Given the heightened vulnerability of pregnant women (due to immune dysfunctions during pregnancy, pre-existing risk factors like obesity, and susceptibility to vertical transmission) and newborns (with underdeveloped immune systems), health-care institutions must remain vigilant, promptly identifying and managing potential infection cases to reduce the incidence of listeriosis.[36–38]

In the investigation of antibiotic susceptibility among *L. monocytogenes* strains, the 17 strains analyzed in this study exhibited sensitivity to four commonly utilized antibiotics. This finding contrasts with existing literature, which has documented that *L. monocytogenes* possesses inherent resistance to several antibiotics, particularly nalidixic acid and certain cephalosporins. Notably, the resistance rate to co-trimoxazole has been reported to reach as high as 38.4% in some studies, indicating a significant challenge in the treatment of infections caused by this pathogen.[39] Therefore, China has implemented stringent policies for the control of *L. monocytogenes*: In clinical treatment, the use of first-line drugs such as ampicillin has been standardized; in agricultural environments, the abuse of antibiotics in livestock farming has been restricted.[40] These measures may help maintain bacterial susceptibility to antibiotics. The discrepancies between the findings of this study and existing literature may stem from: 1) the relatively small sample size of this study; or 2) the effective containment of antibiotic resistance gene dissemination by China's regulatory policies. Such discrepancies are not uncommon in antibiotic susceptibility studies, as insufficient sample sizes may lead to unrepresentative results, thereby affecting the accurate assessment of resistance patterns.

The natural resistance of *L. monocytogenes* to various antibiotics, including nalidixic acid and certain cephalosporins, is well-documented. For instance, Ndahi et al reported high frequencies of resistance to multiple antibiotics, including penicillin and co-trimoxazole, in *L. monocytogenes* strains isolated from raw meat.[41] Foodborne *L. monocytogenes* often

carries antibiotic resistance genes like *tetM* and *ermB*, which shape its resistance profile. *TetM* enables tetracycline resistance via a ribosomal protection protein, while *ermB* blocks macrolide and lincosamide action by methylating 23S rRNA. These genes commonly coexist in strains, causing multidrug resistance (MDR). Mobile genetic elements, such as plasmids and transposons, drive their rapid spread through horizontal transfer.[11,42–44] Under the One Health concept, resistance genes move across human, animal, and environmental boundaries via the food chain. This calls for integrated surveillance to monitor and control antibiotic resistance spread.

Further analysis revealed that the *L. monocytogenes* serotype 1/2a strain harbored all virulence genes except for *llsX*, suggesting that this serotype may possess a significant advantage in terms of pathogenicity. In clinical settings, infections caused by the 1/2a serotype are frequently associated with severe complications, such as sepsis and meningitis, particularly among vulnerable populations, including pregnant women and immunocompromised individuals.[45] For instance, studies have indicated that infections with the 1/2a serotype in pregnant women often result in fetal infections and miscarriages, highlighting the elevated pathogenic risk this serotype poses to specific demographics.[46,47] Moreover, statistical analysis showed no significant differences in the carriage rates of 13 virulence genes among the serotypes 1/2a, 1/2b, and 4b ($P > 0.05$). This observation implies a degree of conservatism in the virulence gene carriage across different serotypes of *L. monocytogenes*. Such conservatism may reflect the evolutionary pressures exerted on virulence genes, ensuring that *L. monocytogenes* maintains its pathogenic potential across various environments.[21] The presence of similar virulence gene profiles among these serotypes suggests that they may share common mechanisms of pathogenicity, which could be crucial for their survival and virulence in diverse ecological niches.[48]

Conclusion

In conclusion, *L. monocytogenes* strains in Wenzhou City, China, are predominantly of sequence type 87 (ST87, CC87) and exhibit multiple virulence genes, indicating significant pathogenic potential. The 1/2a serotype is known to be particularly concerning in terms of pathogenicity, especially for pregnant women and newborns. Additionally, different serotypes demonstrate a degree of conservatism in virulence gene carriage, reflecting evolutionary pressures on these genes. While the study indicates that *L. monocytogenes* in this region is generally sensitive to commonly used antibiotics, the small sample size may compromise the accuracy of resistance assessments. Overall, these findings provide critical insights into the virulence characteristics of *L. monocytogenes* and contribute valuable data for understanding its pathogenic mechanisms.

Ethics Statement

This study was approved by the Ethics Committee in Clinical Research of the First Affiliated Hospital of Wenzhou Medical University (Issuing No. KY2025-R045). No informed consent was required due to the observational nature of the study, which focused on bacteria and did no interventions on patients. All the patient information was anonymized and de-identified. Therefore, the Ethics Committee in Clinical Research of the First Affiliated Hospital of Wenzhou Medical University waived the need for consent. All experiments were performed in compliance with the relevant laws and institutional guidelines and in accordance with the ethical standards of the Declaration of Helsinki.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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