

Maternal–Fetal Listeriosis in China: Clinical and Genomic Characteristics From an ST8 *Listeria monocytogenes* Case

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Background: Listeriosis, a severe foodborne infection caused by *Listeria monocytogenes*, poses significant risks during pregnancy, including maternal–neonatal transmission. This study describes the clinical and genomic characteristics of an sequence type 8 (ST8) *L. monocytogenes* strain involved in maternal–neonatal transmission during pregnancy.

Methods: Clinical presentation, diagnostic process, and treatment outcomes of the case were documented in detail. Whole-genome sequencing (WGS) and subsequent genomic analyses were performed on *L. monocytogenes* isolates obtained from the maternal and neonatal blood cultures.

Results: A 33-week pregnant woman presented with decreased fetal movements and underwent an emergency cesarean delivery. Postpartum, she developed a high fever, and blood cultures from both the mother and the neonate the day after caesarean delivery confirmed *L. monocytogenes* infection. WGS revealed that the isolates belonged to serotype 1/2a, ST8, clonal complex (CC) 8, and lineage II. Both isolates exhibited susceptibility to first-line antibiotics, including penicillin and ampicillin, and contained virulence and stress adaptation genes such as *LIP1-1* and *SSI-1*. Phylogenetic analysis based on cg-SNP linked the clinical isolates to foodborne ST8 strains from Huzhou and Shanghai, suggesting potential contamination routes.

Conclusion: This case highlights the importance of timely diagnosis and effective antibiotic management in preventing adverse pregnancy outcomes. It also underscores the need for enhanced food safety surveillance and genomic monitoring of *L. monocytogenes* to better understand the transmission dynamics and to avoid the extension of a foodborne infection.

Keywords: *Listeria monocytogenes*, maternal–fetal transmission, whole-genome sequencing, ST8, antimicrobial susceptibility

Introduction

Listeria monocytogenes (*L. monocytogenes*) is a Gram-positive bacterium that infect people mainly via consumption of *L. monocytogenes*-contaminated food and cause human listeriosis.^{1,2} The clinical manifestations of listeriosis vary among individuals, from asymptomatic forms, non-invasive febrile gastroenteritis to severe invasive listeriosis. Invasive listeriosis occurs mainly in children, elderly people, immunocompromised individuals and pregnant women and can lead to septicemia, meningitis, endocarditis, and resulting in abortion or complications to pregnancy.^{3,4}

Compared with other foodborne pathogens, although the number of infections caused by *L. monocytogenes* is moderately low (three to six patients per one million population per year globally), the mortality among infected individuals is very high (20–30%).^{5,6} Fan et al reviewed the cases of listeriosis patients reported in China from 2011 to 2017 and found that there were 562 sporadic cases, with mortality rates of 23.78% in non-perinatal listeriosis patients and of the 231 perinatal listeriosis patients, 32.68% resulted in abortion and/or newborn death.⁷ During 2013–2017,

a total of 211 cases of listeriosis were reported by sentinel hospitals of China National Center for Food Safety Risk Assessment (CFSA).⁸ The average case-fatality rate was 31.2% for perinatal cases and 16.4% for non-perinatal cases.

Globally, Listeriosis is more common in pregnancy. Epidemiologic data suggest that about 11.0–17.7% of *L. monocytogenes* infections occur in pregnant women.^{9–11} Moreover, recent studies performed in China showed that 41.1–65.4% of listeriosis cases were pregnancy-associated infections, highlighting the nationwide pressure of this disease.^{7,8,12}

As a common foodborne pathogen, the prevalence and genomic analyses of foodborne *L. monocytogenes* isolates have been extensively reported in China.^{13–16} However, comprehensive investigations of clinical isolates, particularly those associated with pregnancy-related cases, remain limited.^{12,17–19} Numerous studies have reported varying predominant sequence types (STs) of *L. monocytogenes* across different regions of China. Among the foodborne isolates, ST9, ST8, ST121, and ST87 have consistently been identified as the major STs.^{16,20,21} Meanwhile, ST8, ST87, ST5, and ST3 are the most commonly associated STs with human infections.^{22–24} Notably, ST8 is one of the most prevalent *L. monocytogenes* types found in both food contamination and human infections. In this study, we report the clinical characteristics and outcomes of a maternal–fetal transmission case of listeriosis caused by ST8 *L. monocytogenes*, which occurred in 2021 in Huzhou, China. Additionally, we retrospectively performed serotyping, antimicrobial susceptibility testing, and molecular characterization based on whole-genome sequencing (WGS) of the clinical ST8 *L. monocytogenes* isolates (one from the neonate and one from the mother) involved in this case.

Methods

The Patient and Isolates

A 32-year-old pregnant woman was admitted to the inpatient unit at Huzhou Maternity and Child Health Care Hospital at 33+4 weeks of gestation due to “decreased fetal movements for one day”. Upon admission, fetal heart monitoring was abnormal, raising concerns for intrauterine fetal distress. An emergency cesarean section was performed on the same day, delivering a female infant. The neonate was afebrile at birth, while the mother developed a high fever (with a peak temperature of 39.5°C) postoperatively. Peripheral venous blood from both the mother and the neonate were collected for further bacterial culture and identification. Briefly, the blood was injected into aerobic and facultative anaerobic microbial culture bottles (BacT/ALERT[®] PF Plus, bioMérieux, Lyons, France) and then placed in an automated blood culture system (BacT/ALERT[®] 3D Microbial Detection Systems, bioMérieux, Lyons, France) for cultivation. After a positive alarm from the blood culture bottles, subcultures were performed on Columbia blood agar plates and incubated at 37°C for 24 to 48 hours. Suspected *L. monocytogenes* colonies on the blood agar plate appeared as moist, smooth, grayish-white colonies, accompanied by a narrow zone of β -hemolysis. Bacterial identification was performed using the VITEK[®] 2 Compact System (bioMérieux, Lyons, France). Briefly, the suspected *L. monocytogenes* colony suspension is inoculated onto a VITEK[®] 2 test card, which contains a variety of substrates (such as amino acids, sugars, esters, etc.) to assess the bacterial metabolic reactions. The VITEK[®] 2 Compact System then analyzes these biochemical reactions and compares them with its internal database. If the reaction patterns on the test card align with those characteristic of *L. monocytogenes*, the system will ultimately identify the strain as *L. monocytogenes*. Subsequently, based on the clinical presentation of the mother and neonate, as well as a series of laboratory results, appropriate clinical treatments were administered. Two *L. monocytogenes* strains isolated from the blood of the mother and neonate were sent to the Huzhou Center for Disease Control and Prevention for further antibiotic susceptibility testing, serotyping, and WGS.

Serotyping

L. monocytogenes strains were serotyped using multiplex PCR and slide agglutination method as described previously.¹³ Briefly, serogroups of the *L. monocytogenes* isolates were first determined via a multiplex PCR method described by Doumith et al,²⁵ then O antigen was detected using the slide agglutination method (Denka Seiken, Tokyo, Japan), combined with PCR serological typing results to determine the serotype of *L. monocytogenes*.

Antimicrobial Susceptibility Testing (AST)

Susceptibility to a panel of 12 antibiotics was performed using broth dilution method as described previously. The antibiotics tested included β -Lactam/ β -Lactamase inhibitor combinants: penicillin (PEN), ampicillin (AMP), oxacillin (OXA); macrolides: erythromycin (ERY); lincosamides: clindamycin (CLI); quinolones and fluoroquinolones: daptomycin (DAP) and ciprofloxacin (CIP); antifolate: trimethoprim-sulfamethoxazole (SXT); glycopeptides: vancomycin (VAN); tetracyclines: tetracycline (TET); aminoglycosides: gentamicin (GEN); phenylpropanol: chloramphenicol (CHL). The minimum inhibitory concentrations (MICs) of PEN, AMP, and SXT were interpreted using the guidelines of the CLSI (Third Edition: M45), while the remaining nine antibiotics, lacking CLSI standards for *L. monocytogenes*, were interpreted based on breakpoints for *Staphylococcus* species. *Staphylococcus aureus* ATCC29213 served as quality control strains.

WGS and in silico Analysis

Genomic DNA from pure cultures was extracted using a Bacterial Genomic DNA Purification Kit (Magen Biotech Inc., Guangzhou, China) according to the manufacturer's instructions. Purified DNA was used for library preparation, and WGS was performed on Illumina NovaSeq PE150 platform (with a coverage rate of more than 100-fold) and Nanopore GridION platform (BioGerm Medical Technology Co., Ltd., Shanghai, China), respectively. Low-quality reads (minimum quality of Q20), ambiguous sequences and adapter sequences were filtered using FASTP software. The Nanopore reads were assembled and merged into one original sequence and assembled with Illumina MiSeq with Unicycler. Then, the complete genome sequence of *L. monocytogenes* isolate S2021551 (from the mother's blood culture) and S2021552 (from the neonate's blood culture) was assembled. The annotation of the whole-genome sequence was performed using Prokka (v1.14.6).

The assembled sequences were uploaded individually to the Bacterial Isolate Genome Sequence Database (BIGSdb) platform (<https://bigsdb.pasteur.fr/listeria/>) for analysis to determine the lineage, clonal complex (CC) and ST for each isolate. The same method was used to assess the presence of virulence factors, stress resistance and antibiotic resistance genes of each strain, with the minimum of 80% coverage and 80% identity.

Core genomic SNPs (cgSNPs) were generated using Snippy pipeline v4.6.0 by mapping genome sequences to ST8 reference genome sequences Lm N1546 (GCF_001483445.1). Recombination was removed by Gubbins pipeline, whole genome phylogeny of strains based on cgSNPs was inferred by FastTree. The maximum likelihood phylogenetic tree based on cgSNPs was constructed by Raxml. The cgSNPs phylogenetic tree was visualized and edited using the online software Interactive Tree of Life (iTOL).

Sequence Data Accession Numbers

All WGS data have been deposited at the National Center for Biotechnology Information under BioProject PRJNA1188664.

Results

Clinical Characteristics and Treatment

On August 12, 2021, a 32-year-old pregnant woman at 33+4 weeks of gestation was admitted to the inpatient department of Huzhou Maternity and Child Health Hospital due to reduced fetal movements for one day. Upon admission, the patient denied significant abdominal pain, diarrhea, nausea, or vomiting, and her body temperature was recorded at 36.6°C. Considering suspected fetal distress, an emergency cesarean section was performed on the same day, resulting in the delivery of a female neonate. Postoperatively, the patient was administered cefuroxime for infection prophylaxis. Approximately 12 hours post-surgery, on August 13, the patient developed a high fever of 38.9°C. Laboratory investigations revealed a significantly elevated hypersensitive C-reactive protein (hsCRP) level (>200 mg/L) and leukocytosis (white blood cell count: $18.3 \times 10^9/L$). Blood cultures were obtained the same day. The patient continued to experience abnormal febrile episodes postoperatively, with a peak temperature of 39.5°C. On August 15 (postoperative day 3), blood culture results confirmed *L. monocytogenes* infection. Antibiotic therapy was switched to

meropenem via intravenous infusion. However, by August 17 (postoperative day 5), the patient continued to exhibit persistent high fever accompanied by chills, significant nausea, vomiting, epigastric pain, and watery diarrhea. These symptoms were suspected to be gastrointestinal adverse effects related to meropenem. Consequently, the antibiotic regimen was changed to penicillin G (4 million units every 4 hours). By August 19 (postoperative day 7), the patient’s body temperature normalized, and she was subsequently transferred to the Infectious Diseases Department at Huzhou First People’s Hospital for further management.

At birth, the neonate did not present with fever but was admitted to the neonatal intensive care unit (NICU) due to prematurity and respiratory distress. On the first day of admission (August 12), laboratory results showed an elevated hypersensitive C-reactive protein (hsCRP) level of 11 mg/L (above the normal range) and a white blood cell (WBC) count of $9 \times 10^9/L$ (below the normal range). Blood cultures were obtained on the same day. Empiric antibiotic therapy was initiated with piperacillin-tazobactam 0.23 g via slow intravenous push every 12 hours. On day 4, blood culture results confirmed *L. monocytogenes* infection. A lumbar puncture was performed on the same day for cerebrospinal fluid (CSF) culture, which remained negative for bacterial growth after 5 days of incubation. On day 6, laboratory results showed a significant improvement in the hsCRP level (1.5 mg/L, within normal range); however, the WBC count remained elevated at $33.4 \times 10^9/L$, above the normal range. A repeat blood culture was performed, which again showed no bacterial growth after 5 days. On day 7, the antibiotic regimen was switched to meropenem (20 mg/kg every 8 hours) for continued infection management. On day 16 of hospitalization, the WBC count decreased to $18.2 \times 10^9/L$ (within normal limits), and the neonate was discharged on day 18.

The timeline of the clinical presentations, assessment, and treatment of the pregnant woman and the neonate are summarized in Figure 1.

Bacterial Identification, Serotyping and AST

The first blood cultures drawn by both the mother and neonate reported the growth of *L.monocytogenes*, designated as S2021551 and S2021552, respectively. Based on the results of slide agglutination tests and PCR serological typing, both strains were determined to belong to serotype 1/2a. AST for isolates S2021551 and S2021552 showed consistent results, as detailed in Table 1. Both strains were sensitive to PEN, AMP, ERY, CIP, SXT, VAN, TET, GEN, and CHL. They exhibited intermediate resistance to CLI and were resistant to OXA and DAP.

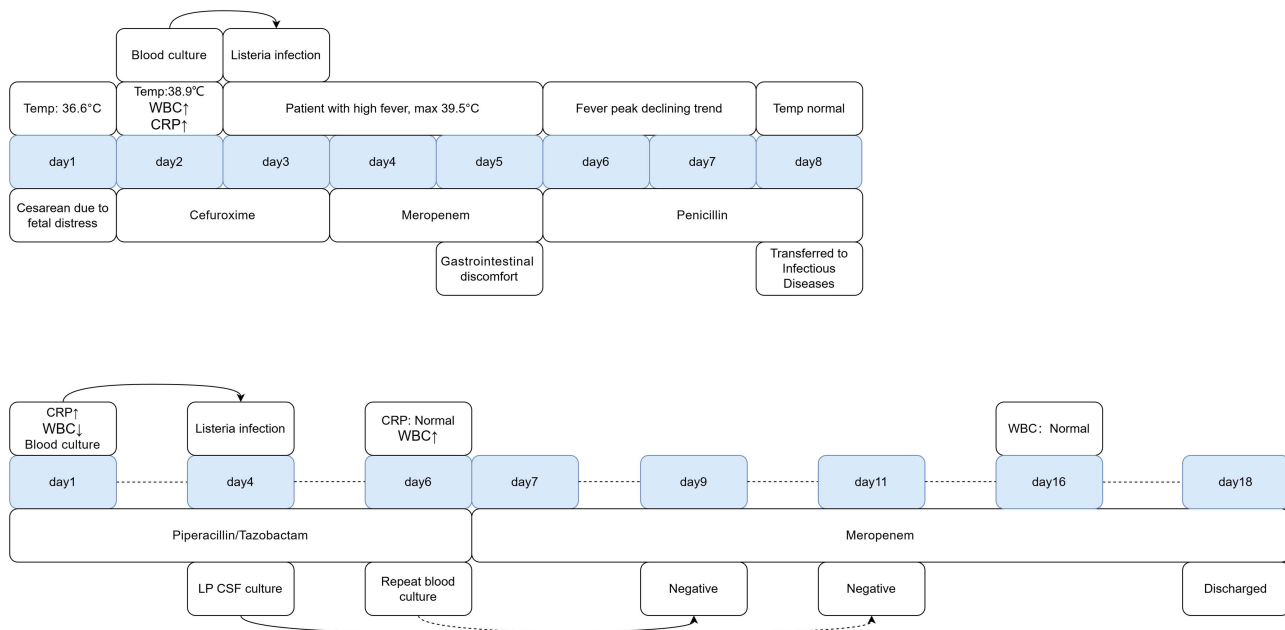


Figure 1 The timeline of the clinical presentations, assessment, and treatment of the maternal–neonatal listeriosis.

Table 1 Antibiotic Susceptibility of S2021551 and S2021552 in This Study

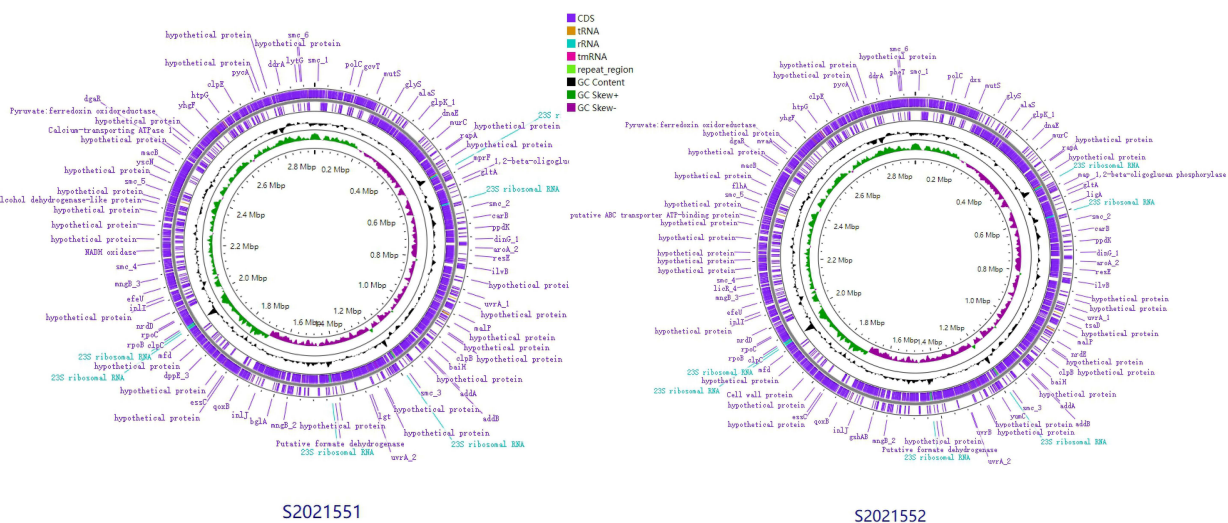
Antimicrobial Class	Antimicrobial Agents	MIC ($\mu\text{g/mL}$) Interpretive Criteria			MIC Values of Isolates in this Study	
		Susceptible	Intermediate	Resistant	S2021551	S2021552
β -Lactam/ β -Lactamase Inhibitor Combinants	PEN	≤ 2	–	–	0.25	0.25
	AMP	≤ 2	–	–	0.25	0.25
	OXA	≤ 2	–	≥ 4	4	4
Macrolides	ERY	≤ 0.5	1–4	≥ 8	≤ 0.25	≤ 0.25
Lincosamide	CLI	≤ 0.5	1–2	≥ 4	2	2
Quinolones	CIP	≤ 1	2	≥ 4	≤ 1	≤ 1
	DAP	≤ 1	–	–	4	4
Antifolate	SXT	$\leq 0.5/9.5$	–	–	≤ 0.5	≤ 0.5
Glycopeptides	VAN	≤ 2	4–8	≥ 16	1	1
Tetracyclines	TET	≤ 4	8	≥ 16	≤ 2	≤ 2
Phenylpropanol	CHL	≤ 8	16	≥ 32	4	4
Aminoglycosides	GEN	≤ 4	8	≥ 16	≤ 2	≤ 2

The General Genomic Features

The isolates S2021551 and S2021552 have a 2,953,263 bp single chromosome with a GC content of 37.96% (Figure 2). The chromosome genome contained 2903 protein-coding sequences, 18 rRNA operons, 68 tRNA genes, and 1 tmRNA. In addition, both S2021551 and S2021552 carried a 86632 bp plasmid, named pLMS21551 and pLMS21552, respectively.

In Silico Analysis

Sequencing data confirmed serogroup IIa for the isolates and MLST analyses indicated that they all belonged to ST8, CC8, lineageII. The presence of antibiotic resistance, virulence factors and stress resistance genes were the same for each strain and were reported in Table S1. The isolates S21551 and S21552 were found to harbor antibiotic resistance genes for lincomycin (lin), quinolone (norB), sulfonamides (sul), fosfomycin (fosX) and cationic antimicrobial peptides (mprF). A total of 55 virulence factor-related genes were identified, including the well-known virulence factors *L. monocytogenes*-specific pathogenicity islands (LPI)-I (prfA, hly, mpl, actA and plcB) and multiple internalins (internalin A, B, C, E, F, G, H, J, and K). Genes or gene clusters related to stress adaptation, including stress survival islet 1 (SSI-1) and those from *Listeria* genomic islands LGI2 and LGI3, were also identified in S21551 and S21552.

**Figure 2** The representation of the isolate S2021551 and S2021552 chromosome.

cgSNPs-Based Phylogenetic Analysis

To determine the relationship between the clinical isolates in this study and those ST8 *L. monocytogenes* isolates from different cities of China, a total of 75 publicly accessible genomes of ST8 *L. monocytogenes*, including 4 isolates from food in Huzhou, were selected for comparison analysis (Table S2). A maximum-likelihood phylogenetic tree was constructed based on the alignment of 777 cgSNPs with Lm N1546 as the reference that were identified by Snippy (Figure 3). With the sequence of Lm N1546 as the reference, the neonate-blood-source isolate S21552 was identical with mother-blood-source isolate S21551. The ST8 clinical isolates in this study formed a cluster with ST8 food isolates from Huzhou (2021–2022) and Shanghai (2015–2019), with SNP differences ranging from 0 to 18 in core genome (Figure 4). Compared to the 12–15 SNP differences with the Huzhou food isolates from 2021 to 2022 (S2021427, S2021666, S2022162), S21551 and S21552 showed a closer genetic relationship to one Shanghai food isolate from 2019 (GCA_016597675), with only 9 SNP differences in core genome.

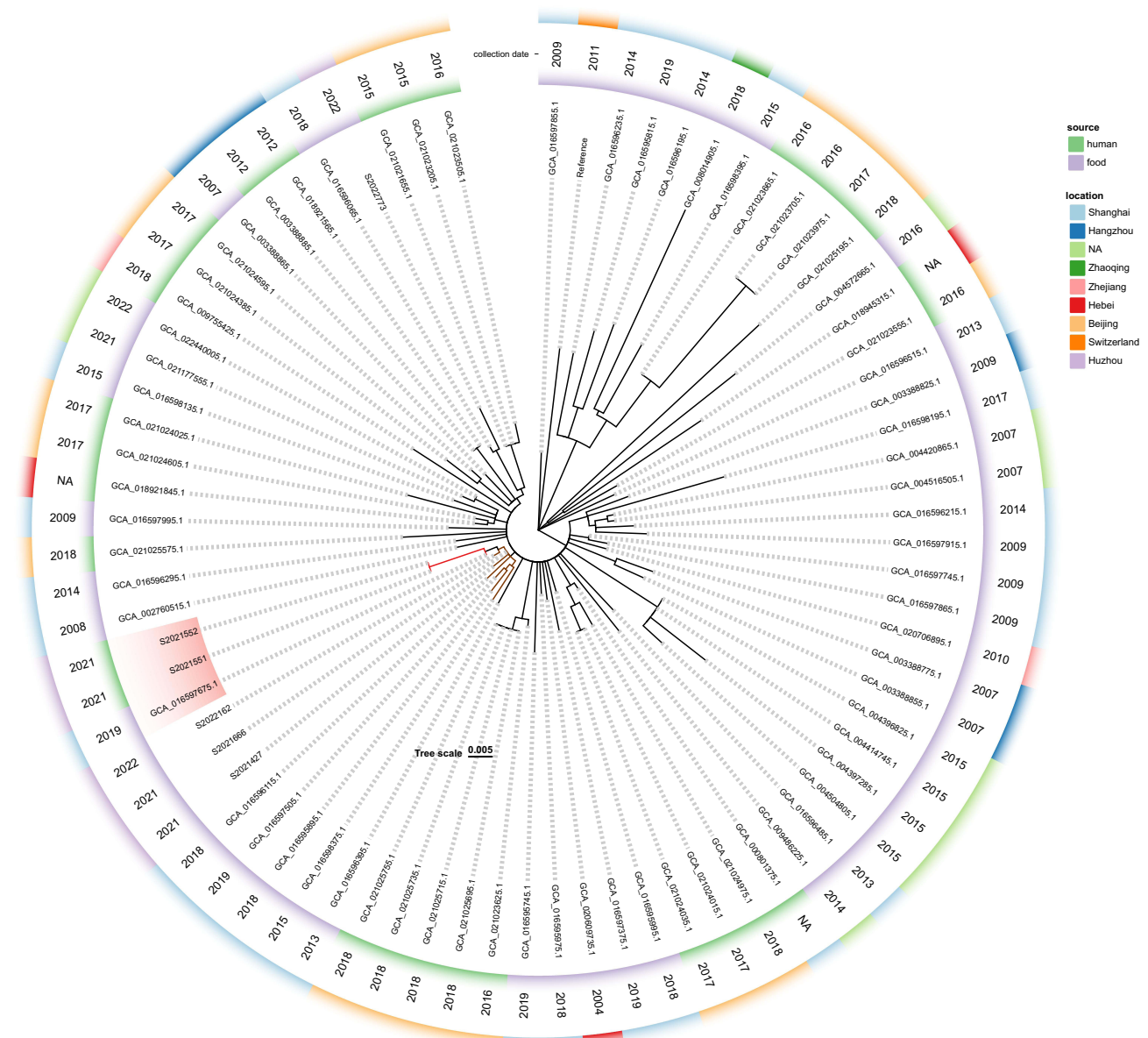


Figure 3 The core-SNP phylogenetic tree of 75 ST8 *L. monocytogenes* isolates. The geographic location, collection date, and isolation source are shown on the tree (from outer to inner circles).

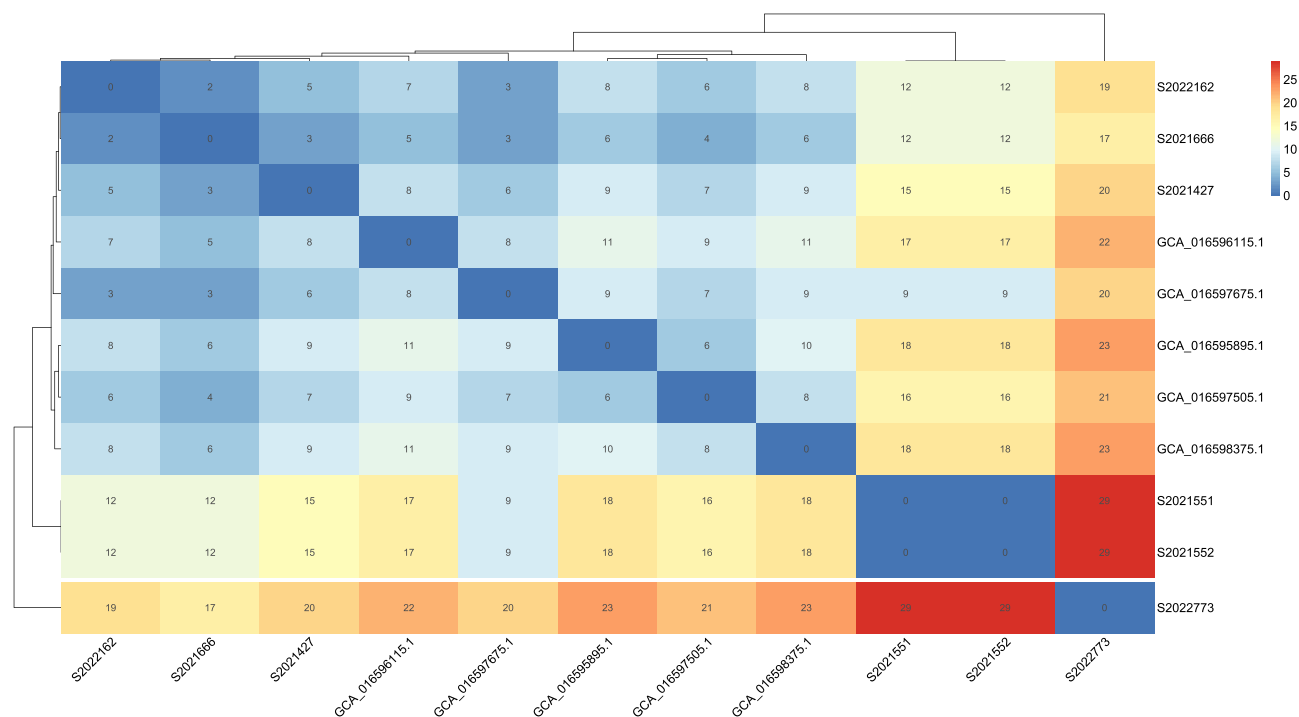


Figure 4 SNPs' matrix-based heatmap between clinical isolates in this study and ST8 food isolates from Huzhou (2021–2022) and Shanghai (2015–2019), which formed a distinct cluster in the phylogenetic tree based on cgSNPs analysis.

Discussion

L. monocytogenes is a widespread, Gram-positive intracellular pathogen and the etiological agent of listeriosis, a rare but severe foodborne infection. This pathogen typically enters the body through contaminated food, initially infecting the intestinal mucosa, then spreading to other organs via the bloodstream and lymphatic system. One key feature of *L. monocytogenes* intracellular parasitic lifestyle is its ability to spread directly from cell to cell,²⁶ avoiding the extracellular environment. Since extracellular host defenses, such as antibodies, complement, and neutrophils (which are highly listericidal), cannot access the intracellularly spreading bacteria, infection clearance relies primarily on cytosolic innate immunity and an effective cell-mediated immune response.^{27,28} During pregnancy, cellular immunity is suppressed due to elevated progesterone levels, which increases susceptibility to invasive *L. monocytogenes* infection.^{29,30} Pregnant women are at a 10 to 18-fold greater risk for listeriosis compared to the general population^{31,32} and over 100 times more likely to be infected than non-pregnant women of reproductive age.³³ In most documented cases, maternal–fetal listeriosis cases more frequently occur in the third trimester when cellular immunity is at its lowest.⁴ A review of listeriosis sentinel surveillance data from 2013 to 2017 in China showed that of 138 perinatal infections, the median gestational age was 32 weeks.⁸ A separate three-year surveillance (2016–2018) conducted at a women's hospital in Zhejiang province found that 66.67% of perinatal listeriosis cases occurred in the third trimester (between 28 and 41 weeks of gestation).³⁴ The perinatal listeriosis case reported in this study also occurred in the third trimester of pregnancy (33+4 weeks gestation).

Unlike other foodborne infections, the incubation period for invasive listeriosis can be significantly longer. According to the reported data, the median incubation period for pregnancy-associated listeriosis is 27.5 days, with a range of 17 to 67 days.³⁵ Pregnant women infected with *L. monocytogenes* are often asymptomatic or present with nonspecific symptoms, such as mild malaise, fever, gastrointestinal disturbances, or flu-like symptoms.³ Due to the nonspecific nature of the clinical symptoms and the prolonged incubation period, maternal listeriosis may be overlooked until the fetus has developed listeriosis or the mother presents with obstetric symptoms. In some cases, the mother may experience few symptoms, with the only manifestation being early labor, which may be associated with severe fetal distress.^{36,37} In this case, the pregnant woman presented with reduced fetal movement and suspected fetal distress, but no other specific

clinical signs. If the diagnosis and treatment had been delayed, the condition could have progressed to a poor outcome. Postpartum, the mother exhibited a high fever, accompanied by elevated levels of both CRP and WBC count. Although the neonate did not present with fever, laboratory results revealed an increased CRP and a reduced WBC count. Subsequent positive blood cultures from both the mother and the neonate confirmed a diagnosis of maternal–neonatal listeriosis. Clinically, listeriosis can be diagnosed through positive cultures from sterile samples, such as maternal or neonatal blood, CSF, amniotic fluid, intrauterine mucosa, or placenta, with blood cultures being the most commonly used diagnostic method.³ According to existing literature, blood cultures positive for *L. monocytogenes* in mothers have been reported in 33% to 68% of cases.⁴ Additionally, uterine swabs or cervical smears can also be suitable for cultural detection of *L. monocytogenes*. In the French MONALISA study, 24% of vaginal smears were positive for the pathogen.³⁸ A retrospective Chinese study conducted between 2008 and 2017 found that 89% of pregnant women with listeriosis had intrauterine infection, as confirmed by cervical swabs.³⁹ Therefore, for pregnant women with fever or suspected *L. monocytogenes* infection, it is recommended to perform both blood cultures and non-invasive cervical/genital swabs to improve diagnostic accuracy.

The gestational age at the time of infection plays a crucial role in determining the prognosis for the neonate. The earlier the infection occurs during pregnancy, the worse the chances of survival for the fetus, and survival is more likely if the infection occurs during the third trimester. Consistent with previous studies,^{12,40,41} in this case, we promptly terminated the pregnancy and, based on the blood culture results, initiated treatment with a more sensitive antibiotic regimen. After a series of supportive and antimicrobial treatments, the neonate, despite being a preterm infant at 33 weeks, successfully recovered and was discharged after 17 days of hospitalization.

Regarding antibiotic therapy, β -lactam antibiotics, such as ampicillin or amoxicillin, are recommended as first-line treatment for *L. monocytogenes* infections in many countries.^{3,37,42} Erythromycin is suggested as a second-line option for pregnant women with penicillin allergies.⁴³ In China, however, there are currently no national guidelines for the treatment of listeriosis. Empirical treatment for obstetric infections with nonspecific symptoms often involves the use of cephalosporins,^{12,17,18} which are generally ineffective against *L. monocytogenes* due to its natural resistance to this class of antibiotics.⁴⁴ For the empirical treatment of neonatal sepsis, broad-spectrum semi-synthetic penicillins such as ampicillin or piperacillin, in combination with β -lactamase inhibitors like ampicillin-sulbactam or piperacillin-tazobactam, are commonly used. Third-generation cephalosporins are also frequently employed. As observed in this study and other studies conducted in China,^{12,17} maternal–neonatal listeriosis cases often do not promptly transition to the recommended first-line antimicrobial treatments upon confirmation of *L. monocytogenes* infection. In this case, once the diagnosis of *L. monocytogenes* infection was confirmed in the mother and neonate, the empirical therapy was switched to the broad-spectrum carbapenem meropenem, a β -lactam antibiotic. Subsequently, due to gastrointestinal side effects experienced by the mother while on meropenem, treatment was further adjusted to penicillin. In general, the rate of antibiotic resistance in *L. monocytogenes* isolates causing human listeriosis is low. Retrospective antimicrobial susceptibility testing in our study confirmed that the isolates from both the mother and neonate remained sensitive to first-line antibiotics such as penicillin (PEN) and ampicillin (AMP). While meropenem is effective against *L. monocytogenes*,⁴⁵ its broad-spectrum activity and potential for adverse effects highlight the importance of early administration of classic first-line antibiotics, such as penicillin. This approach not only minimizes the risk of side effects but also reduces the use of broad-spectrum antibiotics, thereby mitigating the emergence of antibiotic-resistant strains.

Whole genome single nucleotide polymorphism (SNP) analyses reveal that *L. monocytogenes* comprises at least four evolutionary lineages (I, II, III, and IV). These lineages are further subdivided into 13 serotype-associated sublineages (1/2a, 1/2b, 1/2c, 4b, 3a, 3b, 3c, 4a, 4c, 4e, 4ab, 4d, and 7)⁴⁶ and over 1,500 registered STs, which are grouped into CCs.⁴⁷ Epidemiological data indicate heterogeneity in virulence within the species, with three serotypes (1/2b and 4b from lineage I, and 1/2a from lineage II) accounting for over 95% of human listeriosis cases. Data from China's listeriosis sentinel surveillance (2013–2019) show that CC87 (lineage I), CC5 (lineage I), and CC8 (lineage I) are the most prevalent clonal complexes associated with pregnancy-related listeriosis cases.⁴⁸ In this study, isolates from the mother (S2021551) and neonate (S2021552) were confirmed to be identical through slide agglutination tests and WGS. These isolates were identified as serotype 1/2a, ST8 (CC8, lineage II), a ST commonly associated with pregnancy-related cases in China.⁸ Both isolates harbored known virulence factors, including *LIP1* and multiple internalins, which are essential

for host infection, mediating bacterial invasion and intracellular replication.^{49,50} Additionally, genes related to stress adaptation, such as SSI-1 and cadmium resistance genes located on LGI3 (specifically *cadC* and *cadA*),⁵¹ were identified in the isolates. SSI-1 has been linked to biofilm formation⁵² and the ability of *L. monocytogenes* to survive under low pH and high salt concentration conditions.⁵³ The presence of these genes enhances the environmental adaptability of the strains, making them more likely to persist in food processing environments, thereby increasing the risk of contamination. The ST8 isolates in this case did not carry *LIPI-3* or *LIPI-4*, consistent with prior observations that these pathogenicity islands are primarily found in certain lineage I isolates.^{15,21} *LIPI-3* is associated with the production of Listeriolysin S (LLS), which enhances bacterial virulence.⁵⁴ *LIPI-4*, a recently identified gene cluster, encodes a cellobiose-family phosphotransferase system (PTS) involved in neural and placental infections and serves as a marker for distinguishing hypervirulent clones from hypovirulent ones.⁵⁵ In this case, the neonate's CSF culture was negative for *L. monocytogenes*, and no symptoms of central nervous system infection were observed. This outcome may be attributed to the lower virulence of the ST8 strain involved in the infection.

In China, surveillance of *L. monocytogenes* in food products was initiated in 2000, and specialized human listeriosis surveillance through sentinel hospitals was integrated into the National Foodborne Disease Surveillance Plan in 2013.⁸ Unfortunately, the hospital involved in this case was not part of the human listeriosis sentinel surveillance system, which delayed reporting to the Centers for Disease Control and Prevention (CDC) and precluded further epidemiological investigations, including tracing the potential foodborne source of infection. Based on our 2020–2022 surveillance of *L. monocytogenes* in retail food products in Huzhou, the predominant STs identified were ST9, ST3, and ST121, followed by ST8, ST87, and ST5, with ST8 predominantly isolated from raw meat products.¹³ In recent years, cgSNP analysis has become a highly effective tool for comparing bacterial isolates in outbreak detection and investigation due to its high discriminatory power.^{56–58} However, precise SNP thresholds for defining epidemiologically linked groups are influenced by several factors, including the organism, reference genome selection, SNP calling parameters, and the nature of the outbreak. As demonstrated in a national WGS-based surveillance of *L. monocytogenes* conducted in Australia, the majority of mother-baby paired isolates differed by fewer than 10 cgSNPs, while isolates within epidemiologically linked outbreak groups showed differences of fewer than 15 cgSNPs.⁵⁸ To try explore the relationship between the clinical isolates in this study and ST8 isolates from Huzhou and other cities in China, we conducted cgSNP phylogenetic analysis. The results revealed that the isolates from the mother (S2021551) and neonate (S2021552) in this case differed by 12–15 cgSNPs from food isolates obtained in Huzhou during 2021–2022, all of which were recovered from seasoned meat products. However, these clinical isolates were more closely related to a Shanghai food isolate from 2019 (GCA_016597675), with only 9 SNP differences in the core genome. One of the limitations of this study was the failure to promptly trace the potential foodborne source of infection after the case was reported. In the absence of epidemiological investigations, we can only speculate that a shared contamination source of ST8 *L. monocytogenes* may exist between Huzhou and Shanghai. Seasoned meat products, which are often quickly stir-fried on high heat before consumption, pose a significant food safety risk to local consumers due to the potential for *L. monocytogenes* contamination.

Conclusion

Listeriosis caused by the foodborne pathogen *L. monocytogenes* during pregnancy is a rare but severe condition that can result in fetal loss and neonatal infection through vertical transmission. *L. monocytogenes* is commonly found in contaminated processed meats, dairy products, pre-packaged sandwiches, prepared vegetables and fruits, and is known for its ability to withstand significant temperature fluctuations, even surviving for extended periods in refrigerated environments. Therefore, improving awareness of food safety, particularly among high-risk populations, such as pregnant women and immuno-compromised individuals, along with strengthening health education on proper dietary practices, can significantly aid in prevention. Early detection of *L. monocytogenes* infections, particularly during pregnancy, remains a significant challenge. The development of standardized treatment guidelines would greatly contribute to the early diagnosis and standardized management of the disease. For pregnant women presenting with nonspecific clinical symptoms such as fever, flu-like symptoms, and decreased fetal movement, it is crucial to consider the possibility of *L. monocytogenes* infection, particularly when dietary habits suggest increased risk. Timely non-invasive diagnostic

approaches, such as cervical/genital swabs or blood cultures, should be employed to enhance early diagnosis. Upon diagnosis, prompt initiation of appropriate antibiotic therapy is necessary to reduce the risk of adverse outcomes. Finally, our study underscores the importance of continuous surveillance of foodborne diseases, conducted by public health authorities in collaboration with clinicians and laboratorians. This coordinated effort—from case notification to molecular epidemiological investigations based on WGS—plays a crucial role in tracing the sources of contamination, thereby preventing the further spread of foodborne infections. It also provides in-depth insights into the genetic determinants of stress response and virulence factors that facilitate human infections. These findings are essential for developing more effective prevention and control strategies for foodborne diseases.

Ethical Approval

The protocol was approved by the Ethics Committee of Huzhou Center for Disease Control and Prevention (approval number: HZ2022016) and Huzhou Maternity & Child Health Care Hospital (approval number: 2023-R-011).

Consent for Publication

Written informed consent was obtained from the patient to publish this article and clinical information. Additionally, approval for case publication was granted by the Ethics Committee of Huzhou Maternity & Child Health Care Hospital.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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