

Genomic Characterization of a Clinical *Listeria monocytogenes* ST1 Isolate Recovered from the Blood Sample of a Woman with Third Trimester Stillbirth

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Background: *Listeria monocytogenes* is a foodborne gram-positive bacterium which causes adverse pregnancy outcomes. Here, the genomic and phylogenetic characteristics of a *L. monocytogenes* isolate obtained from blood sample of a third trimester pregnant woman with stillbirth are investigated.

Methods: Whole genome DNA of *L. monocytogenes* ST1 was sequenced with HiSeq X Ten platform. The NCBI Prokaryotic Genome Annotation Pipeline was used to annotate the genome sequence. The sequence type (ST) and antimicrobial resistance genes were then identified. The core genome multilocus sequence typing (cgMLST) analysis with other closely related *L. monocytogenes* stored in the NCBI GenBank database was performed using BacWGSTdb 2.0.

Results: The complete genome sequence of *L. monocytogenes* ST1 is made up of 20 contigs totaling 2,914,725 bp, with 2886 protein-coding sequences and a GC content of 37.9%. Fosfomycin [*fosX*], Lincosamide antibiotic [*lin*] and peptide antibiotic [*mprF*] were discovered as antimicrobial resistance genes. In silico serogroup typing prediction revealed that *L. monocytogenes* ST1 belonged to serotype IVb. The closest relative of *L. monocytogenes* ST1, obtained from Poland in 2015, differs by only 15 cgMLST alleles.

Conclusion: We identified a *L. monocytogenes* ST1 strain from blood sample of a woman with third trimester stillbirth in China. These discoveries would aid in our understanding of the genomic characteristics, mechanisms of antimicrobial resistance, and epidemiological features of this pathogen.

Keywords: whole genome sequencing, *Listeria monocytogenes*, stillbirth

Introduction

Listeria monocytogenes, a gram-positive pathogenic bacteria that can cause invasive human infection, was discovered for the first time in the 1980s.¹ Around 23,150 cases of listeriosis were estimated to have occurred globally per year in 2010, with a fatality rate of 20–30%.² Pregnant women, unborn babies, neonates, elderly people, and immunocompromised individuals are more vulnerable to *Listeria*, while *Listeria* infection during pregnancy is often associated with adverse pregnancy outcomes.³ The Foodborne Diseases Active Surveillance Network of the United States reported 104 confirmed cases, with an incidence of 0.2 per 100,000 population, 22 deaths (21.2%, highest fatality rate of all foodborne diseases) during 2020.⁴ At the same year, 1876 confirmed human cases of listeriosis and 167 deaths were reported by 27 European countries, the incidence rate was 0.42 cases per 100,000 population. The overall case fatality across the European Union was 13.0%, a minor decline from the previous years (13.6% in 2018 and 17.6% in 2019).⁵ A total of 211 listeriosis and 138 (65.4%) perinatal cases were reported with 26.1% fatality rate by China National Center for Food Safety Risk

Assessment (CFSA).⁶ In China mainland, a total of 562 patients with sporadic listeriosis was reported from 2011 to 2017; 227 non-perinatal listeriosis with a mortality rate of 23.78%, 231 perinatal listeriosis with perinatal fatality (abortion or neonatal death) of 32.68%.⁷ The reports of clinical perinatal listeriosis in China were mostly limited to the summary of clinical cases, and there were few related studies on whole-genome sequencing for serological prediction and phylogenetic analysis of globally disseminated *L. monocytogenes* isolates.^{7–11} In order to fill this gap, the genome of a *L. monocytogenes* isolate recovered from the blood specimen of a 30-week pregnant woman was sequenced, with the purpose of elucidating its genomic features and antibiotic resistance mechanisms.

Materials and Methods

The *L. monocytogenes* isolate was cultured in Mueller-Hinton broth overnight at 37°C (Oxoid Ltd., Basingstoke, UK). Using a MALDI Biotyper, the bacterial species was determined (Bruker Daltonics, Billerica, USA). The automated VITEK2 system (bioMérieux, Marcy-l'Étoile, France) was used to perform the antimicrobial susceptibility testing, which was then interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) 2018 standards (M100-S28). The Women's Hospital Ethics Committee at Zhejiang University School of Medicine in China approved this study and it was performed out in compliance with the Declaration of Helsinki. The patient provided written informed consent that was used to publish the case details.

A QIAamp DNA Mini Kit was used to extract the genomic DNA (QIAGEN, Valencia, USA). Nextera DNA Sample Preparation Kit (Illumina Inc., San Diego, USA) was used to create a DNA library, and the HiSeq X Ten platform (Illumina Inc.) was used to sequence the whole genome of *L. monocytogenes* using the 150-bp paired-end protocol. Low-quality or artefact bases were eliminated after sequencing. The raw sequence reads were *de novo* assembled using Unicycler v.0.4.8 with the default parameters.

The NCBI Prokaryotic Genomes Annotation Pipeline was used to annotate the genome sequence. The assembled genome was utilized to detect the antimicrobial resistance genes and virulence genes with the following online webserver: ResFinder 4.2, CARD 2021, and VFDB 2019. BacWGSTdb 2.0 server accomplished *in silico* multilocus sequence typing (MLST) and bacterial source tracing with core genome multilocus sequence typing (cgMLST).^{12–14} IslandViewer 4, ISfinder 1.0, PHASTER 2016, and antiSMASH 6.0 programs were utilized to perform additional bioinformatics analysis, such as the discovery of genomic islands, insertion sequence elements, prophage sequences, and secondary metabolite gene clusters, respectively. The minimal spanning tree created with the cgMLST allelic profile of all *L. monocytogenes* isolates obtained from the NCBI GenBank database was visualized using GrapeTree. LisSero (<https://github.com/MDU-PHL/LisSero>) was used for *in silico* serotyping.

Results and Discussion

Twenty contigs totaling 2,914,725 bases made up the draft genome sequence for *L. monocytogenes* ST1, serotype IVb, and the PGAP server annotated a total of 2886 protein-coding sequences. This strain's total G+C content was 37.9%. A total of 18 rRNA operons and 67 tRNA genes overall were found. The resistome of *L. monocytogenes* ST1 carrying genes that are responsible for resistance to Fosfomycin (*fosX*), Lincosamide (*lin*) and multiple peptide resistance factor (*mprF*). The genome also contains at least eight genomic islands and several IS elements, the majority belonging to the IS1182, Tn3, IS66 and IS3 families. Similarly, six prophage sequence were identified in the genome. Two secondary metabolite gene clusters, namely listeriolysin S and burkholderic acid can be predicted. The genome contained a number of virulence factors, such as *lapB*, *lap*, *clpE*, *llsD*, *hpt*, *oatA*, *inlK*, *iap/cwhA*, *llsG* and *llsB*, which are responsible for the entry into the host cells and the adaptation to an intracellular lifestyle. *In silico* serogroup typing prediction revealed that this isolate belonged to serotype IVb (Table S1).

The cgMLST analysis showed that the strain we isolated showed a close relationship as it had only 15 loci diversity with the strain collected in Poland in 2015 (Figure 1). The woman in this study, however, had not travelled recently or face-to-face interacted with foreigners. Thus, no epidemiological connection between this Chinese woman and the previously reported case could be found. One argument that has been floated is that the patient contracted this strain from the local population or the contaminated food, but this theory needs to be confirmed by more concrete evidence.

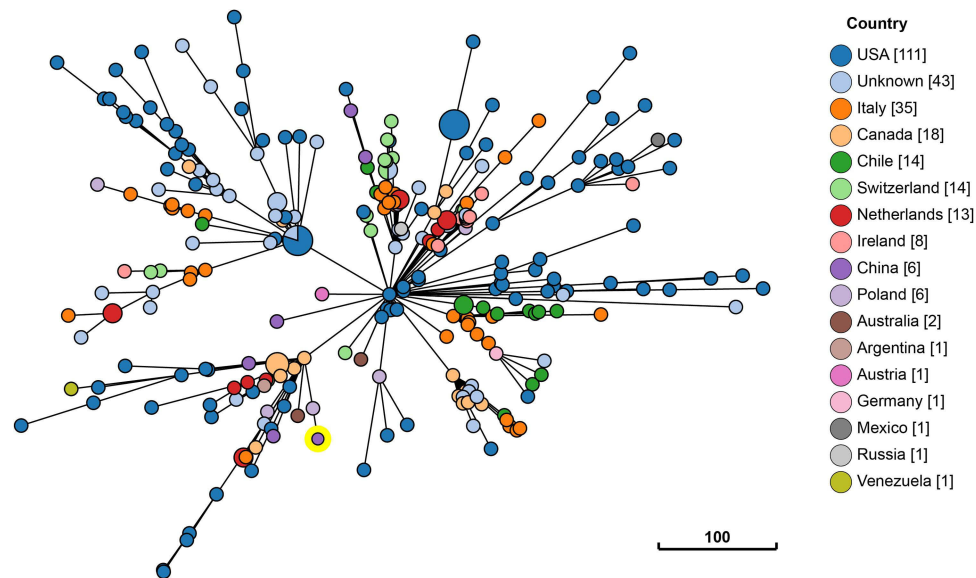


Figure 1 A minimum spanning tree based on cgMLST analysis of *L. monocytogenes* ST1 isolates retrieved from NCBI GenBank database. Each circle represents strains belong to the same complex type. The size of the nodes reflects the number of isolates contained within the clade. Clonal relationships between isolates are depicted by the line length connecting each circle. The number of isolates recovered from each country is given in square brackets.

We isolated *L. monocytogenes* from the blood sample of an infected pregnant woman, which caused the pregnant woman's Listeriosis infection and the fetal death. The patient was a 32-year-old G1P0 (gravida 1, parity 0) pregnant women with a 30-week stillbirth. A *L. monocytogenes* ST1 was cultured from the blood sample of the maternal within 24 h after admission. The vaginal swab, cervical swab, and urine samples cultured negative. *L. monocytogenes* was also confirmed in the placental swab. This strain was susceptible to penicillin, ampicillin, meropenem, and trimethoprim/sulfamethoxazole.

L. monocytogenes can be spread across continents through the international food trade, and different clonotypes are distributed globally.¹⁵ The strain of *L. monocytogenes* isolate in our study was ST1, which was not one of the predominant sequence types in China, suggesting that the possible source was non-indigenous. ST87 (15.7%, 18.4%) and ST8 (13.9%, 14.5%) were the most prevalent STs both in human invasive listeriosis and in perinatal listeriosis reported by CFSA.⁶ Higher prevalence of ST87 or ST8 was confirmed by other studies not only in China mainland^{7,10,16} but also in Taiwan,¹⁷ inconsistent with western countries.¹⁶ Mirroring to the result of previous studies in our hospital, the most common STs in 12 cases of perinatal *Listeria* infection were ST87 (25.0%), ST7 (25%), and ST2 (16.7%).⁹ One recent retrospective multi-center study highlighted ST87 (34.0%) was most prevalent in China, but the proportion of ST1 (12.5%) was not only abnormally second highest, but was also considered as a hypervirulent lineage, ie, increased invasiveness, intracellular replication and neurotropism of *L. monocytogenes* in ruminants.¹⁸

Perinatal listeriosis is a rare infection caused by *L. monocytogenes*, whereas it has extremely high perinatal fatality. We isolated one *L. monocytogenes* strain from a third-trimester maternal blood sample, which contains several virulence genes may lead to stillbirth. Whole genome sequencing of clinical *L. monocytogenes* can help to discover potential virulence genes in clinical practice and provide a basis for predicting the virulence potential of different clonal lineages of *L. monocytogenes*.

Conclusion

In conclusion, we present the genomic features of a clinical *L. monocytogenes* ST1 isolate obtained from a Chinese mother who had a stillbirth. Our results shed important light on the genetic diversity and evolution of *L. monocytogenes* infections associated with adverse pregnancy outcomes.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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