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ORIGINAL RESEARCH

Genomic and Phenotypic Diversity of *Listeria monocytogenes* Causing Pregnancy-Associated Listeriosis from Zhejiang Province, China, 2016–2018

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Introduction: There are few investigations describing the pregnancy-associated listeriosis in China, and the molecular characteristics of *Listeria monocytogenes* causing such infections remain largely unknown. We aim to investigate the phenotypic and genomic profiles of pregnancy-associated *L. monocytogenes* isolates and their association with isolates recovered from human and non-human in China.

Materials and Methods: In this study, we conducted a 3-year surveillance of listeriosis in a women's hospital in Zhejiang province, using whole genome sequencing and bioinformatics tools.

Results: From 2016 to 2018, we identified 13 clinical *L. monocytogenes* isolates. Among these pregnancy-associated isolates, we found seven sequence types (STs), with the prevalent STs of ST87 and ST7. Serotyping divided the strains into four serotypes, including serotype 1/2a, 1/2b, 3a, and 4b. Antimicrobial resistance testing showed that all the isolates were susceptible to 10 antibiotics. Comparative genomics analysis clearly classified our genome collection into four distinct evolutionary lineages with most isolates grouping into lineages I and II. Interestingly, we found three pairs of isolates with high identity, although no evident epidemiological association was observed.

Conclusion: This study reports for the first time the surveillance of pregnancy-associated listeriosis in Zhejiang province, China, which indicates that the infection rate is low in this region. Our findings provide insight into the evolution and genetic diversity of pregnancy-associated *L. monocytogenes* from Zhejiang province. Additional investigations involving more human and non-human isolates with a "one health" strategy are needed for prediction of the listeriosis risk associated with a typical prevalent clone in Zhejiang province, such as ST87.

Keywords: *Listeria monocytogenses*, pregnancy-associated, ST87, whole genome sequencing, comparative genomics analysis

Background

Listeria monocytogenes is a Gram-positive bacterium first identified in the 1980s as a food-borne pathogen causing human disease posing a serious threat to public health worldwide.[1] It is well documented that epidemic and sporadic *L. monocytogenes* infections are usually associated with contaminated food.[2–7] Human invasive infection by *L. monocytogenes* leads to relatively rare but serious food-borne diseases mainly affecting elderly people, immunocompromised individuals and pregnant women.[8]

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Clinical manifestations include sepsis and infection of the central nervous system, which can lead to lifelong sequelae or even high mortality.[1] Pregnancy-associated listeriosis can result in preterm birth, miscarriage or stillbirth.[9]

Listeriosis is particularly worrisome since it has a low incidence but its fatality rate is high.[10] In the United States, a previous study reported an outbreak of 147 human cases and 33 deaths.[11] In the European Union, a total of 2224 human cases of invasive listeriosis were reported in 2015, with an overall case mortality rate of 18.8%.[9] In China, listeriosis is a rare disease and it has not yet been regulated as a notifiable disease. Previous studies documented the contribution of food-borne and environmental *L. monocytogenes* isolates causing human invasive infection in China.[12–15] Thus far there was no report of human outbreaks in China.[13] Therefore, information on this infection has been largely scarce among the Chinese population, which partly due to a lack of surveillance of clinical listeriosis, especially in pregnancy-associated cases.

Materials and Methods

Study Design and Study Site

In this study, we described a 3-year surveillance of listeriosis in a women's hospital in Zhejiang province, China, using whole genome sequencing (WGS) and bioinformatics tools. We aim to investigate the phenotypic and genomic profiles of pregnancy-associated *L. monocytogenes* isolates, and their association with isolates recovered in human and non-human in China. These data should provide insight into the evolution and genetic diversity of *L. monocytogenes* from China. The surveillance of listeriosis was carried out at The Women's Affiliated Hospital, College of Medicine, Zhejiang University (WAHZU), the largest women's hospital in Zhejiang province, China, from January 2016 to December 2018. WAHZU is a 1100 beds tertiary medical facility located in Hangzhou, the capital of Zhejiang province, providing health care for 57,000,000 residents. The hospital attends to an average of 1,600,000 outpatient cases and 80,000 inpatient cases per year.

Strains and Molecular Analysis

Bacterial identification was performed using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) (Bruker, Leipzig, Germany), and further checked by PCR and sequencing of *L. monocytogenes* species-specific *hly* gene.[16] Serotyping was performed based on the multiplex PCR as described previously.[17]

Antimicrobial Susceptibility Testing (AST)

AST of the *L. monocytogenes* isolates was performed using broth dilution method. The minimum inhibitory concentrations (MICs) of 19 antimicrobials were tested as described previously.[18] The MIC results were interpreted using the guidelines of the CLSI (Third Edition: M45).

Whole Genome Sequencing (WGS)

Genomic DNA from 13 sub-cultured frozen stocks of isolates was extracted using a commercial kit (OMEGA, Norcross, USA) according to the instructions of the manufacturer. WGS was performed with the HiSeq 4000-PE150 platform (Illumina, San Diego, CA, USA). Genome assembly was performed as described previously.[3] Multilocus sequencing typing (MLST) and antimicrobial resistance genes analysis were performed using online tools (http://www.genomicepidemiology.org/).

Comparative Genomic Analysis

In addition to the 13 genomes generated in this work, 82 publicly available *L. monocytogenes* genomes from China were selected to determine the evolutionary relationship among *L. monocytogenes* (Table S1). The isolate collection includes strains from humans (n = 15), food (n = 43) and the environment (n = 24) that were widely distributed over time and geographical locations. All collection genomes were annotated using Prokka.[19] Roary (https://sanger-pathogens.github.io/Roary/) was used to calculate the pan-genome for the genome dataset. The resulting consensus tree was visualized and edited using the Interactive Tree of Life (iTOL).[20]

Results

Between January 1, 2016 and December 31, 2018, a total of 13 clinical samples from 12 patients were positive for *L. monocytogenes* (Table 1). Of note, isolates Lmo9017 and Lmo2001 were recovered from the blood culture and vaginal swab, respectively, from the same patient. Eight cases peaked in 2018, another four cases were observed in 2016 (n = 2) and 2017 (n = 2). Among 13 pregnancy-associated isolates, we found 7 sequence types (STs). Briefly, the two most prevalent STs were ST87 (n = 3) and ST7 (n = 3), followed by ST2 (n = 2). The other four STs contained one isolate, respectively. Serotyping divided the human isolates into four serotypes, including serotype 1/2a, 1/2b, 3a, and 4b. There are three main serotypes: 1/2a, 1/2b, and 4b with four isolates. Serotype 3a was represented by a single isolate.

Table 1 Characteristics of 12 Cases of Pregnancy-Associated Listeriosis

Isolate No.	Age	Gestation (wks)	Presentation	Culture Ste	STs	Serotype	Treatment	Maternal Outcome	Fetal Outcome
Lmo5004	32y	29	Fever (Tmax 38.4°C), WBC: 11.6 × 10^9/L	Blood	5	1/2b	Ampicillin + cefuroxime	Recovered	Survived
Lmo 5148	28y	20	Fever (Tmax 39.6°C), WBC: 16.3 × 10^9/L	Blood	1	4b	Cefoperazone/ sulbactam	Recovered	Fetal death
Lmo 5214	37y	27	Fever (Tmax 38.3°C), WBC: 19.8 × 10^9/L	Blood	14	1/2a	Meropenem	Recovered	Fetal death
Lmo 6079	28y	24	Fever (Tmax 39.4°C), WBC: 21.5 × 10^9/L	Blood	429	4b	Cefoperazone/ sulbactam	Recovered	Fetal death
Lmo 9017	23y	38	Fever (Tmax 39.3°C), WBC: 26.6 × 10^9/L	Blood	7	1/2a	Penicillin G	Recovered	Survived
Lmo 2001	23y	38	Fever (Tmax 39.3°C), WBC: 26.6 × 10^9/L	Vaginal swab	7	1/2a	Penicillin G	Recovered	Survived
Lmo 2050	21y	8	36.4 °C	Vaginal swab	87	1/2b	Penicillin G	Recovered	Fetal death
Lmo 5011	1d	33	Apgar score (1–6)	Blood	7	1/2a	Penicillin G	Recovered	Fetal death
Lmo 5272	1d	39	Fetal distress, WBC:2.00 × 10^6/L	Blood	8	3a	Meropenem + penicillin G	Recovered	Survived
Lmo 5304	1d	32	Fetal distress, Apgar score (8–10)	Blood	87	1/2b	Penicillin G	Recovered	Survived
Lmo 5414	1d	31	Fetal distress, Apgar score 8, WBC:12.31 × 10^9/L	Blood	2	4b	Meropenem + penicillin G	Recovered	Fetal death
Lmo 13	1d	31	Fetal distress, Apgar score 8, WBC:12.31 × 10^9/L	Blood	2	4b	Meropenem + penicillin G	Recovered	fFtal death
Lmo 5965	1d	NA	Fetal distress, Apgar score 8	Blood	87	1/2b	Ampicillin + penicillin G	Recovered	Survived

AST results of 13 *L. monocytogenes* isolates were detailed in Table S2. The full resistance rate was observed for cefoxitin (100%), followed by cefuroxime (92.3%), ceftazidime (84.6%), and ceftriaxone (76.9%). All the isolates were susceptible to amoxicillin-clavulanicinic acid, benzylpenicillin, ciprofloxacin, ertapenem, gentamicin, imipenem, levofloxacin, moxifloxacin, tigecycline, and trimethoprim-sulfamethoxazole.

Comparative genomics analysis clearly classified our genome collection into four distinct evolutionary lineages with most isolates grouped into lineages I and II (Figure 1), which is in line with the previous studies.[12,13,15] Eight and five isolates detected in this study were grouping into lineages I and II, respectively. It is obviously seen that the isolates were clustered together based on STs and serotypes. Not surprisingly, identical isolates (Lmo9017 and Lmo2001) were observed from different sampling sites in the same patient, which is consistent with genotype and phenotypic results (Table S1 and Figure 1). Interestingly, we found that clinical isolate Lmo5272 detected in this study exhibited highly identity with food isolate S12003 (SAMN09388362) also identified in Hangzhou, Zhejiang province. It is worthy to note that Lmo5272 was isolated in 2018, whereas S12003 was collected in 2007. Moreover, Lmo2050 also exhibited highly identity with isolate SHL007 from Shanghai in 2011. In addition, we found high similarity isolates between cases. Lmo13 and Lmo5414 are identical, but two isolates were not clinically linked as two cases occurred in different patients with 2 years apart with no evident epidemiological association.

Discussion

In the present work, we detected and characterized 13 *L. monocytogenes* strains recovered from pregnancy-associated listeriosis in China and conducted a comparative genomics and phylogenetic analysis against publicly available data from diverse sources and locations from China. Our

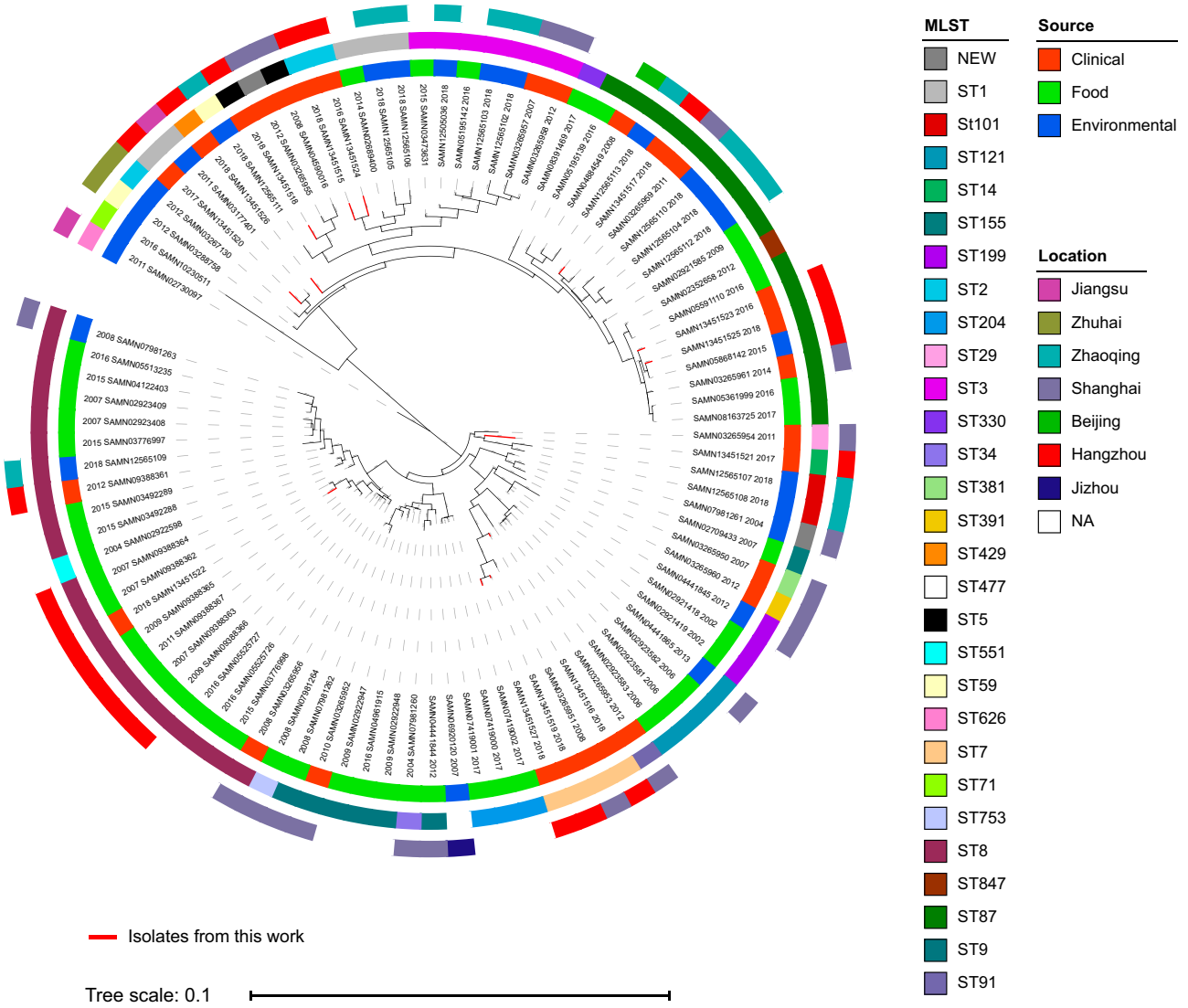

Figure 1 Core-genome-based phylogenic tree of 95 representative *L. monocytogenes* from China, including 13 isolates from this study and 82 strains downloaded from NCBI genome database. The location of the isolates is labelled in the outer ring. The source of the strains is colored in the middle ring. STs of the isolates were presented in the inner ring. Isolates identified in this study were colored in red. NA, details regarding the region of the strains are not available. The bar shows 0.1 nucleotide substitution per position.

study showed that *L. monocytogenes* from China displayed a divergent population structure with extensive diversification, suggesting a genetic diversity of *L. monocytogenes*.

Previous studies revealed a marked difference in the prevalence of STs between clinical and food isolates. The most predominant STs in contaminated foods were ST1, ST5, ST8, and ST9.[13,21-26] An investigation on the prevalence of *L. monocytogenes* in retail foods showed that ST9 (23.3%), ST155 (16.4%), and ST8 (12.3%) were the main dominant types in Zhejiang province.[27] Their observations combined the results from this work imply the epidemiological difference between clinical and food isolates from Zhejiang province, although prevalent serotypes 1/2a, 1/2b, and 4b were also identified in that study. Of note, ST87 clone was the predominant ST in clinical *L. monocytogenes* isolates and closely related to pregnancy-associated infections in China.[22,23] Interestingly, ST87 was rarely described in food, environmental or clinical isolates in Western countries.[28] Additionally, to the best of our knowledge, this study detected the ST429 infection case for the first time.

This is the first pregnancy-associated listeriosis surveillance in China. Detection of genetic-related clone with no evident epidemiological association highlighted that the application of a WGS-based analysis as a powerful surveillance tool.[3] It also demonstrated that some *L. monocytogenes* isolates, such as Lmo5272 and S12003

(Figure 1) probably exist in this area for a long period, suggesting a more active surveillance system is warranted.

This study is limited by the relatively small number of pregnancy-associated listeriosis cases identified; only 13 *L. monocytogenes* isolates were detected during the 3-year period. Notwithstanding the limitations of this work, our findings indicate that pregnancy-associated listeriosis rate is low in Zhejiang province. Future investigations involving more human and non-human isolates with a "one health" strategy are needed for prediction of the listeriosis risk associated with a typical prevalent clone in China, such as ST87.

Data Sharing Statement

The Whole Genome Shotgun BioProject for the 13 *L. monocytogenes* isolates has been deposited at DDBJ/EMBL/GenBank under BioProject accession no. PRJNA592908.

Ethics and Consent Statement

Informed consent was obtained from all patients according to the ethical protocol approved by the Ethics Committee of First Affiliated Hospital of Zhejiang University (no. 2016-349).

Consent for Publication

Not applicable.

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Disclosure

The authors report no conflicts of interest in this work.

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