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A novel virulent core genome multilocus sequence type CT 11424 of *Listeria monocytogenes* isolate causing stillbirth in Bangladesh

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Abstract

Background *Listeria monocytogenes* is a foodborne pathogen that can lead to severe pregnancy outcomes. This study reports the clinical and genomic characteristics of a *Listeria*-mediated stillbirth identified in January 2022 through the Child Health and Mortality Prevention Surveillance (CHAMPS) project in Bangladesh. The *Lm*-BD-CHAMPS-01 isolate was recovered from the blood and cerebrospinal fluid (CSF) of a male stillborn. Maternal history, clinical, and demographic data were collected by the CHAMPS surveillance platform. An expert panel evaluated all reports to determine the role of *L. monocytogenes* infection in the causal chain of stillbirth. Genomic characterization included multilocus sequence typing (MLST), core genome MLST (cgMLST), serotyping, and the presence or absence of virulence genes. Genetic divergence and phylogenetic analyses were conducted to determine the relationship with other reported isolates globally.

Results The isolate *Lm*-BD-CHAMPS-01 was identified as a novel cgMLST CT11424. It belonged to ST 308, Serotype 4b, Clonal Complex 1, and Phylogenetic Lineage 1. Key *L. monocytogenes* virulence genes facilitating the crossing of the placental barrier, including full-length *inlA*, LIPI-1, and LIPI-3, were detected. The isolate was closely related to clinical *L. monocytogenes* isolates, as determined by GrapeTree based on cgMLST. SNP-based phylogenetic analysis found *Lm*-BD-CHAMPS-01 to be the most distant from other CC1 isolates in the database. Possible sources of infection included the consumption of contaminated raw vegetables or exposure to pigeons.

Conclusions This is the first genome sequence of clinical *L. monocytogenes* from Bangladesh, which also caused stillbirth. Rural healthcare professionals should be aware of *L. monocytogenes* infection risks during pregnancy. Pregnant women should be counseled on the dangers of exposure to animals or birds and consumption of potentially contaminated raw food to prevent adverse pregnancy outcomes due to *L. monocytogenes* infection.

Keywords *Listeria monocytogenes*, Stillbirth, Bangladesh, Pregnancy, CHAMPS, Mother-to-child transmission, Animals

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Introduction

Listeria monocytogenes infection in humans and ruminants leads to septicemia, gastroenteritis, and central nervous system infections [1]. *L. monocytogenes* multiplies intracellularly and is capable of cell-to-cell transmission, avoiding the extracellular space [2]. The pathogen is vertically transmitted from pregnant women to fetuses by crossing the placenta [3]. Nearly, 27% of all infections with *L. monocytogenes* occur in pregnant women and infection during pregnancy can lead to 20% of fetal loss [1]. Listeriosis during pregnancy is often asymptomatic or presents with mild or non-specific symptoms. Symptoms may exert as fever, diarrhoea, respiratory tract symptoms or preterm rupture of membrane [4]. Therefore, many *L. monocytogenes* infections during pregnancy remain undiagnosed, especially in low- and middle-income countries like Bangladesh.

L. monocytogenes is commonly found in the environment [5, 6]. This pathogen has been reported in the feces of sheep/goats, cattle [7], dogs, rats [8], chickens, pigeons [9, 10], and crows [11], as well as in cloacal swabs of chickens and gulls [12]. These animals are potential sources of contamination for both raw and processed food consumed by humans. In developed countries, outbreaks of *L. monocytogenes* infection have been linked to various food sources, including meat, fish, mixed dishes, vegetables, juices, and dairy products [5]. *L. monocytogenes* contamination in market and restaurant produce, such as cabbage, corn, carrots, lettuce, cucumbers, parsley, and salad mixes, is well reported [13–16]. *L. monocytogenes* enters food processing facilities through contaminated raw ingredients, making it a significant food safety concern. Certain strains can persist for extended periods in processing plants, acting as constant sources of cross-contamination due to their ability to adhere to abiotic surfaces. Eradicating *L. monocytogenes*, even with the most stringent safety protocols, can be challenging [17].

The key virulence related genes of *L. monocytogenes* are *hly*,*actA*,*plcA*,*plcB* and *mpl* gene found in *Listeria* pathogenicity island-1 [18]. A crucial virulence factors is internalins including *inlA* and *inlB* [19].

L. monocytogenes is classified into four distinct evolutionary lineages denoted I, II, III, and IV, with most isolates belonging to I and II [20]. Serovar 1/2b and 4b of lineage I are responsible for 95% of listeriosis cases in humans, with 4b being the predominant serovar among clinical isolates and outbreaks [19]. Major clonal complexes (CC) CC1, CC2, CC4, and CC6 of lineage I are associated with clinical listeriosis [21]. Few reports on genomic investigation of *L. monocytogenes* are available from South Asia. *L. monocytogenes* sequences reported from India (animal and human aborted material) from 2006 to 2014, belongs to phylogenetic lineage I and clonal complex CC1. As of July 2023, there are few publications available regarding the detection of *L. monocytogenes* from Bangladesh [22–24]. *L. monocytogenes* has been detected in goat/sheep abattoir environments, sick goats, frozen shrimp, and locally manufactured cosmetics. The only reported clinical listeriosis case was from a female cancer patient in Bangladesh [25]. All of these studies used culture-based detection except one, which used molecular detection by PCR system.

We isolated *L. monocytogenes* from postmortem specimens of a stillbirth collected through Child Health and Mortality Prevention Surveillance (CHAMPS) using microbial culture and detected the bacteria using TaqMan Array Card based Real-time PCR system [26]. Till now whole genome sequence of *L. monocytogenes* is unavailable from Bangladesh. Availability of genomic data will aid public health authorities, to be aware regarding pathogenicity of locally circulating clinical *L. monocytogenes* strains specially during pregnancy and guide future epidemiological studies. Here, we report on an *L. monocytogenes* mediated stillbirth as well as the only clinical *L. monocytogenes* isolate reported from Bangladesh, using next-generation sequencing to understand the molecular characteristics and virulence factors of the pathogen.

Methods

Study settings

The CHAMPS is a multi-country project collecting data from six countries of sub-Saharan Africa and South Asia (only Bangladesh) as of 2022. In Bangladesh, the sites are the Rajbari (Baliakandi) and Faridpur districts. These districts are about 130 km away from capital of Bangladesh, the Dhaka city. The project has implemented a minimally invasive tissue sampling (MITS) technique for collecting postmortem specimens from stillbirths and under-5 deaths in low-and-middle income settings. Collected specimens from stillbirths are blood, CSF, rectal swab, lung tissue for microbiological investigations. Clinical, demographic and laboratory diagnosis information of the deceased along with maternal information are reviewed by a panel of experts termed DeCoDe (Determination of Cause of Death) panel to identify the cause of death. More information regarding the CHAMPS protocols and methods can be found at [27–29].

Specimen collection

The body was received for postmortem sampling within 1 h of delivery of the stillbirth. After receiving the body, a skilled technician collected anthromorphic measurement and performed gross inspection. Gross inspection included recording fresh or grade of maceration. The body was disinfected using iodine solution at the area of the body to be punctured. A spinal puncture needle

was used to collect CSF from the cisterna magna. Using another spinal puncture needle, blood was collected from midway between the sternal notch and acromioclavicular joint. Collected whole blood and CSF was distributed for both microbiological culture and molecular detection of infectious agents.

Identification and antibiotic susceptibility test of *L. monocytogenes* isolate

Microbial culture was performed from postmortem blood and cerebrospinal fluid (CSF) specimens. The bacterial isolate on blood agar were identified using the Vitek-2 system by Biomerieux (France). The antimicrobial sensitivity was determined following manufacturer's instruction for Vitek-2 P628 AST card applying CLSI criteria for Enterococci [30]. The isolate was named *Lm*-BD-CHAMPS-01. Additionally, Blood and CSF was investigated using TaqMan Array Card based Real-time PCR (TAC) system for detection of *L. monocytogenes* DNA [31].

Whole genome sequencing

Lm-BD-CHAMPS-01 DNA was extracted from a pure culture using the DNeasy Blood and Tissue Kits with lysozyme pretreatment (Qiagen, Germany; cat. no 51604). DNA library for whole genome sequencing was prepared from 1 ng of DNA with Nextera XT DNA Library Preparation Kit (Illumina Inc, San Diego, CA, USA; cat. no 20060059). The sequencing procedure was carried out on the MiSeq platform (IlluminaInc, USA), employing the v3 reagent cartridge using 522 cycles with standards 251-bp paired-end reads for sequencing (Illumina Inc, San Diego, CA, United States; cat no MS-102-3003). All experiments were performed following the manufacturer's guidelines.

Genome assembly and characterization

The quality of the sequenced raw reads was assessed with FastQC v.0.11.9, followed by trimming of the low-quality reads using Trimmomatic v.0.39 [32, 33]. *De novo* genome assembly was performed with SPAdes v.3.15.2 [34]. Reordering contigs of the draft genome was done using ABACAS v.1.3.2 [35]. The quality of the draft genome assembly was evaluated with QUAST v.5.2.0, excluding contigs < 200 bp and annotated using the prokka v.1.14.6 [36, 37]. Both K-mer and ribosomal multilocus sequence typing (rMLST) was used to identify the species of the draft assembly using KmerFinder v.3.2 from Center for Genome Epidemiology and rMLST from PubMLST, respectively [38, 39]. The Bacterial Isolate Genome Sequence Database (BIGSdb) for the *L. monocytogenes* was used to deduce the seven-gene multilocus sequence typing (ST), the core genome multilocus sequence typing (cgMLST) and CC. The BIGSdb server was also used to screen for the presence of virulence genes, antimicrobial resistance genes, and disinfectants resistance genes [40]. The detection of phage and plasmid was performed using Phaster and Platon v.1.7, respectively [41, 42]. Syntenic relationship was visualized using SimpleSynteny v.1.4 [43].

Minimum spanning and SNP-based phylogenetic analysis

A Minimum Spanning Tree (MST) was constructed using the GrapeTree plugin of PubMLST with default parameters and a manually prepared metadata table (Supplementary Table 1) [44]. *L. monocytogenes* isolates with a unique cgMLST profile ID ($n = 695$) in the BIGSdb were chosen for the MST. A phylogenetic tree was built with 19 isolates from evolutionary lineage I of human source, one isolate from food source and *L. innocua* as an outgroup. BIGSdb was used to retrieve the isolates information and the selection criteria ensured only one isolate per clonal complex per year from each country and contained the raw reads information (Supplementary Table 2) [40]. Raw reads of the selected isolates were retrieved from National Center for Biotechnology Information, which were used as inputs to get the preserved SNP consensus output file using the CFSAN pipeline v.2.2.1. Default configure file of CFSAN was updated by adding out_group option to FilterRegions_ExtraParams parameter for excluding outgroup from SNP filtering [45]. IQtreeweb server was used to build a maximum likelihood tree from the preserved SNP consensus file using best auto detected substitution model (TVMe + ASC) with default parameters and visualized using interactive tree of life (iTOL) [46, 47]. This whole genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JBFRXX000000000.

Results

Clinical narrative

CHAMPS project has been operating in Rajbari (Baliakandi) and Faridpur district of Bangladesh since September 2017. As of December 2023, a total of 848 MITS have been conducted from stillbirths and postmortem under-5 deaths to determine the cause of death. On 25 January 2022, *L. monocytogenes* was isolated from one case ($n = 1$ of 848) during the study period. The 22-year-old pregnant woman from Baliakandi at 32-week gestational age was admitted to Bangabandhu Sheikh Mujib Medical College& Hospital (BSMMCH). The household reported maintaining a flock of five pigeons and a single hen. During pregnancy period, the mother reportedly consumed cow milk but boiled. Maternal complaints during pregnancy were severe headaches, blurring of vision, pre-eclampsia and anemia (Table 1). From the mother's recall, she received a total of seven antenatal care visits during this pregnancy. Maternal complaints during

Table 1 Clinical features available from the pregnant women and the stillborn infected with *Lm*-BD-CHAMPS-01

Maternal features	Parameters
Clinical symptoms	
Fever before delivery	Yes
Anemia	Yes
Headache	Yes
Dizziness	Yes
Pre-eclampsia	Yes
Malnutrition	Yes
Burning sensation during micturition	Yes
Laboratory diagnosis (on admission after delivery)	
Hemoglobin (g/dL)	9.8
Random Blood Sugar (mmol/L)	4.9
Thyroid-stimulating hormone (µl U/ml)	3.1
HBsAg	Negative
Urine Routine Examination	Normal
Maternal Medications during pregnancy	
Medications	**Indication**
Iron	Anemia
Misoprostol	Medical induction of labour
Paracetamol	Fever
Stillborn features	
Maceration Level	Level 1 - Maceration
Anthropomorphic measurements during MITS	
Weight (g)	1820
Height or Length (cm)	45
MUAC (cm)*	7.5
Head Circumference (cm)	29
Right Leg Length (cm)	6
Right Foot Length (cm)	6.5

*MUAC = Mid upper arm circumfluence

Table 2 Antibiotic susceptibility of *L. monocytogenes* isolate *Lm*-BD-CHAMPS-01 determined using Vitek-2 system applying CLSI criteria for Enterococci

Antibiotics name	Minimum Inhibitory Concentration (µg/ml)	Antibiotic sensitivity interpretation	
		Blood	CSF
Levofloxacin	2	S	S
Moxifloxacin	1	S	S
Erythromycin	≤ 0.12	S	S
Clindamycin	≥ 1	R	R
Linezolid	≤ 2	S	S
Vancomycin	1	S	S
Tetracycline	≤ 0.25	S	S
Tigecycline	0.12	S	S
Chloramphenicol	4	S	S
Trimethoprim/sulfamethoxazole	≤ 10	S	S

admission for delivery were intrauterine fetal demise confirmed by ultrasonography, fever, burning sensation during micturition and lower abdominal pain for seven hours. The post-admission examination failed to detect fetal movement and heartbeat. After admission, a maternal complete urine routine examination provided normal findings. After a few hours of hospital admission, a male stillborn baby with breech presentation was delivered by normal vaginal delivery. Following CHAMPS protocol, the family was approached for consent to conduct MITS. The body was macerated without any other significant physical observations all over the body. Body weight during MITS was recorded at 1820 g. CHAMPS laboratory platform isolated *L. monocytogenes* from both blood and CSF culture. Resistance was observed against clindamycin among tested antibiotics (Table 2). *L. monocytogenes* DNA was detected from whole blood specimen using TAC (Cycle threshold 26.2). The DeCoDe panel determined the underlying cause of this antepartum stillbirth as intrauterine infection by *L. monocytogenes*. The panel also suggested maternal infection was the main condition affecting the fetus. To understand the virulence gene landscape and phylogeny of the *Lm*-CHAMPS-BD-01, we used next-generation sequencing to analyze the draft genome sequence. The antibiotic resistance pattern was same for both blood and CSF derived isolate (Table 2). As *L. monocytogenes* was detected from blood sample using both microbial culture and TAC; we selected this isolate for whole genome sequencing.

Lm-BD-CHAMPS-01 has a novel cgMLST type of *L. monocytogenes*

The *Lm*-BD-CHAMPS-01 genome had 13 contigs, a genome size of 2,932,029 bp, and a 37% GC content. The genome coverage was 127x, with an N50 of 1,491,565 bp and an L50 of 1 bp. Prokka annotation found 2873 CDS, 5 rRNA, 1 repeat region, 56 tRNA, and 1 tmRNA (Fig. 1). In comparison, reordered annotated genome using F2365 as reference strain found 2872 CDS, 2 rRNA, 1 repeat region, 41 tRNA, and 1tmRNA (Supplementary Fig. 1). MLST and K-mer dependent analysis confirmed the sequence as *L. monocytogenes*. Using the BIGSdb tool, the isolate was identified as ST 308, CC1, lineage I, sublineage SL150, and PCR serogroup 4b. cgMLST analysis declared the isolates as a novel type, and a new cgMLST profile identification number, CT 11424, was provided.

Virulence and stress-associated genes

Lm-BD-CHAMPS-01 was found to contain 65 virulence genes (Supplementary Table 3). Genes *lap*, *dltA*, *fbpA*, *inlJ*, *lapB*, *actA* and *inlF* were found which are known to aid bacterial adhesion. Invasion-related genes *inlA*, *inlB*, *inlE*, *vip*, *ant*, *iap*, *lgt*, and *lepA* without any premature stop codon (full length) were also identified.

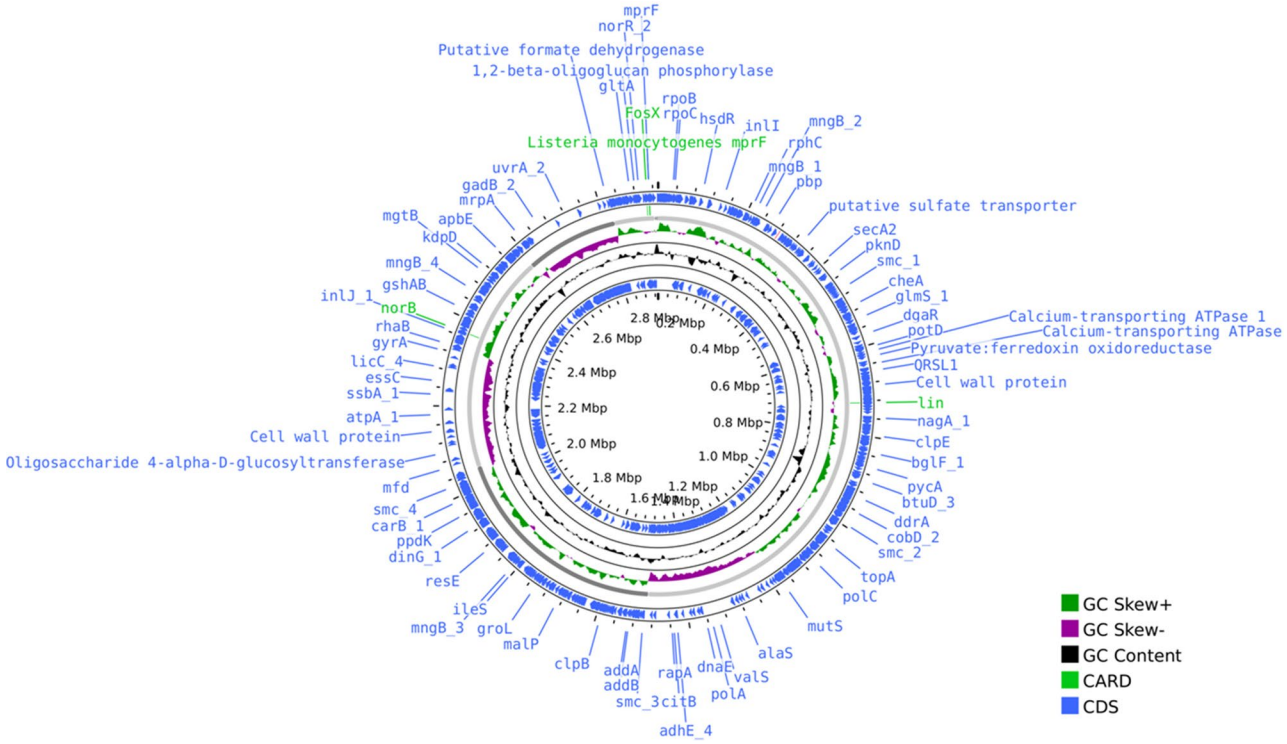

Fig. 1 Genomic features of *Lm*-BD-CHAMPS-01. The Circular presentation of annotated genetic features of genome of *Lm*-BD-CHAMPS-01 was generated using Proksee

The isolate harbored several genes to survive inside intracellular environment including *llo* (Listeriolysin O), *plcA*, *plcB* (enhance phagosomal membrane disruption), *prsA2*, *lsp*, *svpA*. For intracellular growth *hpt*, *IpA1* and *oppA* genes (survival inside macrophage) were detected. *L. monocytogenes* 4b serotype-specific gene cassette *gltA-gltB* was present. These genes were found arranged inside *Listeria* pathogenicity island (LIPI-1) 1 (*hly*,*mpl*,*actA*,*plcB*,*plcA*,*prfA*) and LIPI-3 (*llsA*, *llsG*, *llsH*, *llsX*, *llsB*, *llsY*, *llsD*, *llsP*) [48]. Gene synteny of these two pathogenicity islands was similar when compared with the F2635 reference strain (Supplementary Fig. 2). *Listeria* genomic island locus, LGI-2_*LM*OSA2310 and LGI-2_*LM*OSA2320 were found *Lm*-BD-CHAMPS-01. The presence or absence of genes was illustrated using the isolates included in the phylogenetic analysis (Fig. 2). The analysis showed that *Lm*-BD-CHAMPS-01 has the same virulence and stress adaptation profile as the all CC1 isolates, except one *L. monocytogenes* from Chile. It also contains multidrug resistance transporters *mdrM*. The isolate contains one intact phage (PHAGE_Lister_vB_*Lm*oS_293; GenBank accession number NC_02892). The intact phage is 42.4 kb long and has 64 total proteins. From this draft genome of *Lm*-BD-CHAMPS-01, we were unable to find any plasmid.

We compared allelic profile of the gene from *Listeria* Pathogenicity Island I and II with those from lineage I and clonal complex I. In *Lm*-BD-CHAMPS-01 had same allelic profile compared with other isolates except for *actA*, *llsB* and *llsD*. For these three genes allelic profile of *Lm*-BD-CHAMPS-01 was 325, 38 and 11 respectively (Supplementary Table 4). These allelic profiles are unique to *Lm*-BD-CHAMPS-01.

Genetic relatedness and phylogenetic analysis of *L. monocytogenes* isolate

We calculated the MST, to visualize the genomic relatedness of *Lm*-BD-CHAMPS-01 in comparison to cgMLSTs of *L. monocytogenes* from different sources (Fig. 3). Among 1748 core genes, 1746 were detected from the *Lm*-BD-CHAMPS-01; only two hypothetical genes were missing. In MST, the isolate was differed with clinical origin cgMLST CT 5635 with 484 alleles. *L. monocytogenes* CT 5635 was located in the center of the cluster. Most genetically adjacent of *L. monocytogenes* CT5635, was from a food source (CT 2830, 64 allelic difference) and animal feed (CT6145, 23 allelic difference). The other connected clinical isolate was CT2872 with 29 allelic differences (Fig. 3).

To investigate the evolutionary origin of *Lm*-BD-CHAMPS-01 phylogenetic analysis was performed with selected sequences of lineage I from global isolates (Fig. 4). The log-likelihood of the tree was – 360.7421. The *Lm*-BD-CHAMPS-01 diverged from *L. monocytogenes*

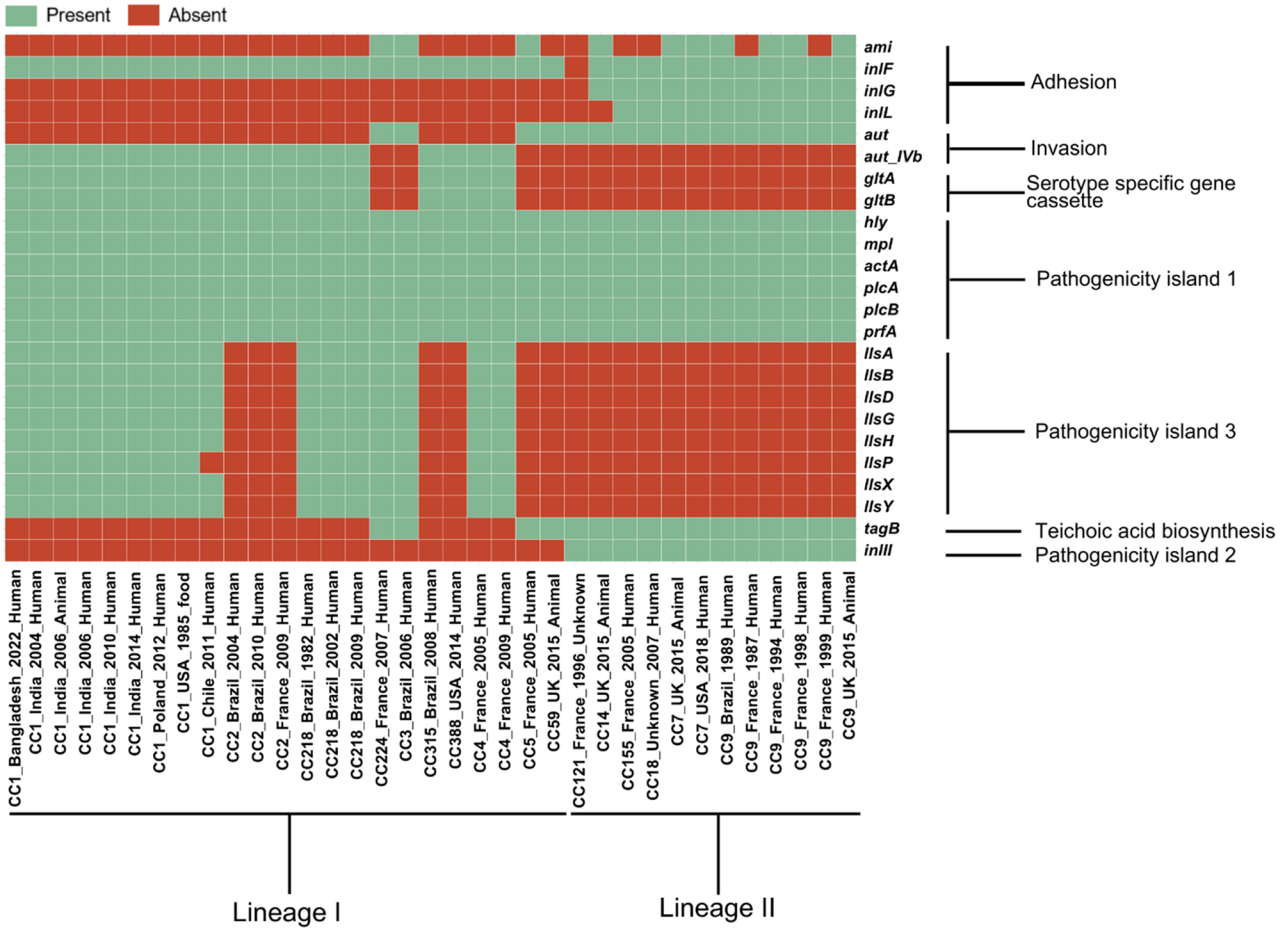

Fig. 2 Presence and absence of virulence genes in *Lm*-BD-CHAMPS-01.*Lm*-BD-CHAMPS-01 was compared with clonal complexes from lineage I and II (x-axis). The y-axis represents the function of virulence genes. The data for the presence/absence of virulence genes was generated using BIGSdb server

reported from the food source of the USA in 1985. Indian isolates reported from 2004 to 2014 all clustered together and were adjacent to *Lm*-BD-CHAMPS-01. Among all the CC1 isolates, *Lm*-BD-CHAMPS-01 is placed in a distant position indicating that it has acquired more changes (most distant on the tree scale at 0.63) compared to nearest clades which includes Indian sequences (Supplementary Table 5).

Discussion

Here we report, genomic analysis of a *L. monocytogenes* strain causing stillbirth in Bangladesh. Phylogenetic analysis based on cgMLST identified the *L. monocytogenes* as a novel cgMLST CT 11424. The clinical narrative aligns with the genomic landscape of the isolate showing all major virulence genes required for crossing the placental barrier through inter-cellular movement.

The *Lm*-BD-CHAMPS-01 isolate is a virulent strain to cause stillbirth which is indicated by the presence of listeriolysin Oexpressing gene *llo* and other genes required for intracellular survival and intercellular movement to cross the placental barrier [49]. The presence of full-length *lap* (adhesion protein) mediates adhesion of with host cell and gene *inlA* facilitates its ability to cross the intestinal as well as placental barrier [50, 51]. In mice models, it has been shown that the *inlF* gene enhances the early stage of infection by modulating host inflammatory responses [52]. Recently, it has been reported that the *actA* gene enhances the shedding of *L. monocytogenes* by several magnitudes which leads to better transmission from host to environment [53]. It also expresses multidrug resistance transporter *mdrM* which triggers host immunity leading to activation of the placental immune system against the fetus.

Although virulent, the *Lm*-BD-CHAMPS-01 remains sensitive to a wide range of antibiotics. Resistance was only observed for clindamycin against which is commonly reported for *L. monocytogenes* [54]. However, the mechanism of clindamycin resistance in this pathogen is still debated. *L. monocytogenes* possesses natural resistance against cephalosporin antibiotics, hence was untested [55]. The antibiotic sensitivity pattern and health care-seeking approach (7 visits) of the pregnant women discussed here showed that the antenatal

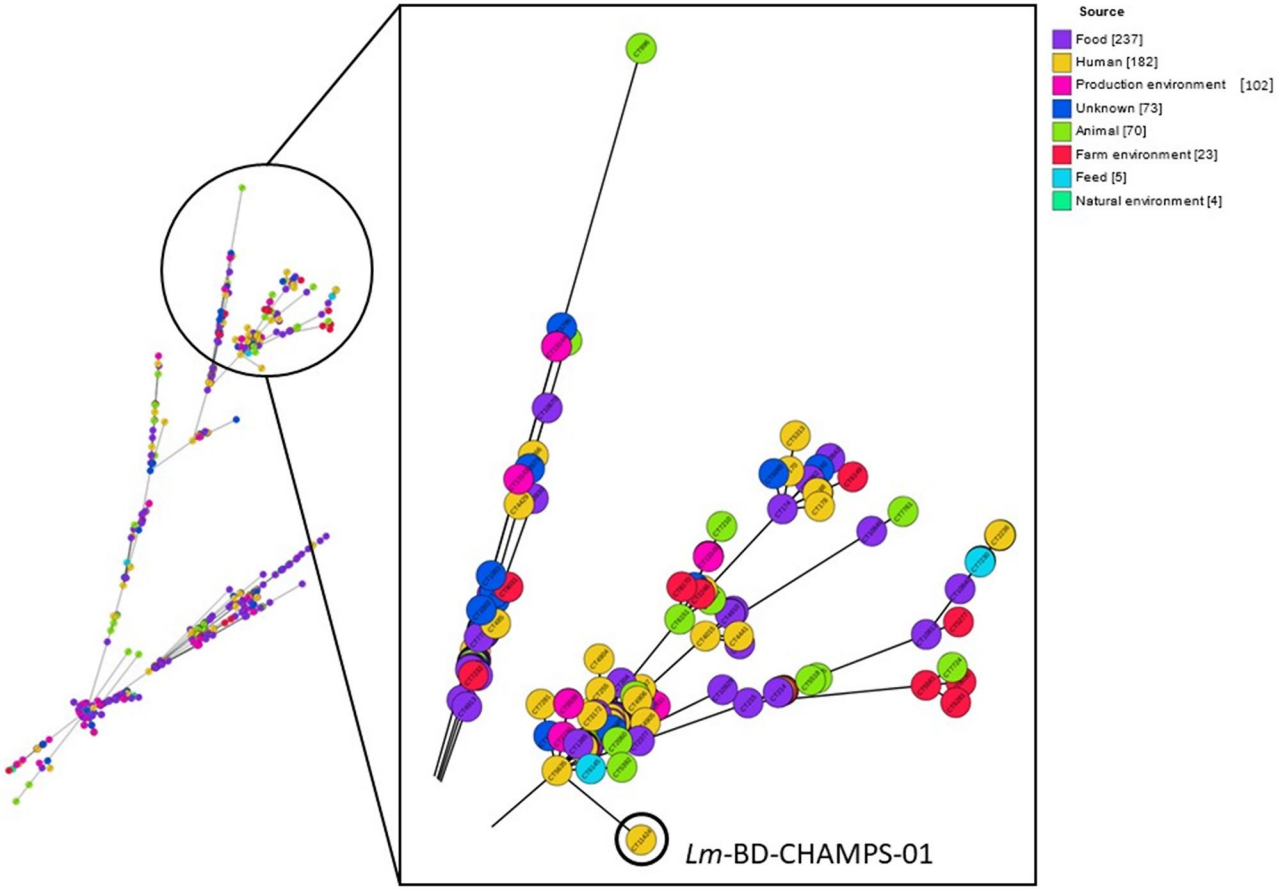

Fig. 3 Minimum Spanning tree demonstrating the divergence of *Lm*-BD-CHAMPS-01 with other *L. monocytogenes* sequences. The MST was built with 695 *Lm*cgMLST sequences available in the BIGSdb-LM database. *Lm*-BD-CHAMPS-01 resides within the circle in the left figure. Zooming in on the circle shows the location of *Lm*-BD-CHAMPS-01 (CT11424) with respect to the nearest isolate. *Lm*-BD-CHAMPS-01 is 484 alleles different from the closest isolate (CT5635), which is a clinical isolate

care system was unable to identify the infection. Timely diagnosis could have prevented this *L. monocytogenes* mediated stillbirth using antibiotics such as erythromycin which is considered safe during pregnancy [56]. This indicates the importance of availability of microbiological diagnosis during antenatal care. Any plasmids remain undetected however a prophage was found in *Lm*-BD-CHAMPS-01. The prophage PHAGE_Lister_vB_*Lmo*S_293 has been reported recently from South Africa from both pathogenic *L. monocytogenes* and less-pathogenic *L. innocua* [57, 58]. However, the exact role of this prophage in *L. monocytogenes* virulence or evolution is yet to be discovered.

Globally, reporting of *L. monocytogenes* outbreaks are uncommon, reports of at least two cases from same place are considered as outbreaks [59]. A major reason for unreported listeriosis is the lack of severe symptoms in non-pregnant individuals. As a result, laboratory diagnosis is limited especially in low- and middle-income countries. Postmortem investigation of neonatal deaths across CHAMPS sites have reported 6 *L. monocytogenes* mediated neonatal deaths including 4 cases within first week of birth [60]. During pregnancy, *L. monocytogenes* invades the placenta where inflammatory and immune-regulatory T-cell response is modulated to promote maternal tolerance to the semi-allogeneic fetus. This leads to increased host susceptibility to *L. monocytogenes* infection. However, *L. monocytogenes* infection triggers innate immune response which ultimately triggers host immune response in the placenta leading to fetal damage or demise [61].

The ST308 was reported previously from Chinese fresh aquatic products (2011–2016) and confiscated foods from non-European passengers (2012–2013) at a Spanish airport [62, 63]. *Lm*-BD-CHAMPS-01 also belongs to serovar 4b which is one of the predominant serovar reported to cause listeriosis in humans and ruminants. Strain of serover 4b is more adapted to mammalian host than other strains [64, 65]. Strains from lineage I and serovar 4b have shown the lowest diversity among the other lineages; and lowest levels of recombination among the lineages indicating genomic stability [55]. This is also

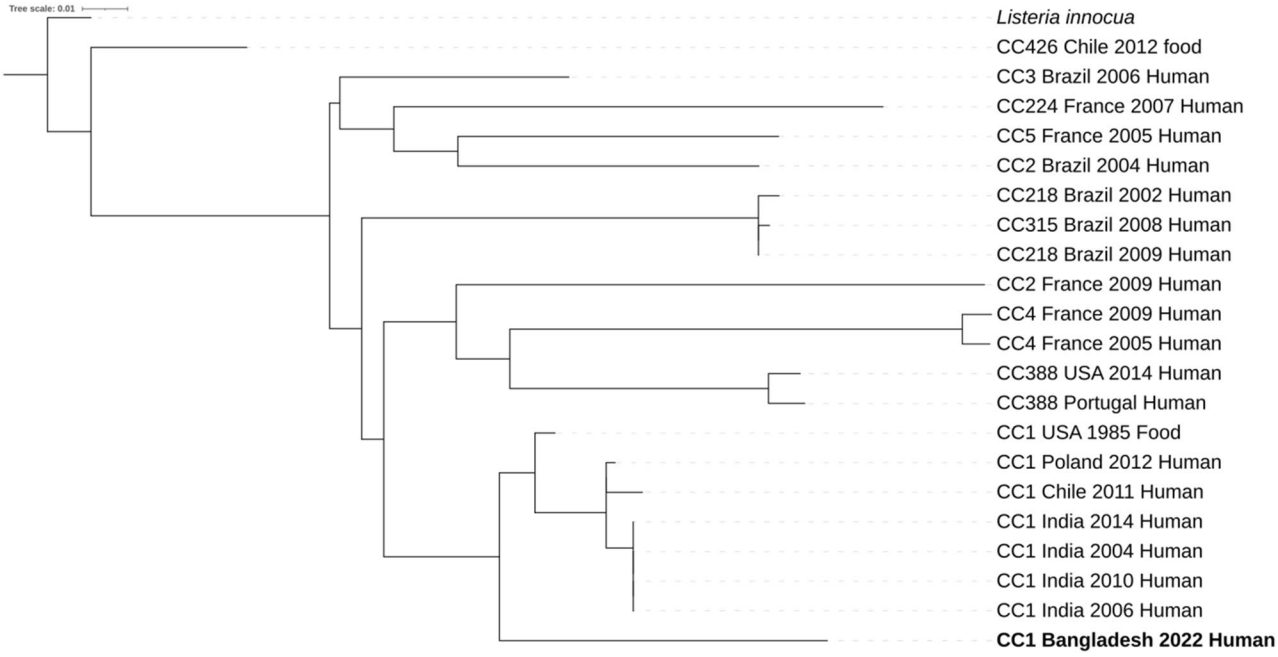

Fig. 4 SNP based Phylogenetic analysis of *Lm*-BD-CHAMPS-01. SNP based maximum likelihood tree indicating *Lm*-BD-CHAMPS-01 (CC1 Bangladesh 2022 Human) is the most diverged among all the CC1. *Listeria innocua* was used as an outgroup. The tree was visualized in iTOL

evident from the detection of similar isolates from 2004 to 2014 in India, which shares a large border with Bangladesh. *L. monocytogenes* serotype 4b has been reported from an outbreak of urban poultry flocks in US [66]. The same serotype has been also reported from fecal samples of hen in Germany [67]. Although out of scope of this analysis, the presence of domestic pigeon and hen in the household could be a potential source of *L. monocytogenes* infecting the pregnant women or through her food.

Allelic distance using core genome MST analysis indicates *Lm*-BD-CHAMPS-01 has gathered more mutations from other analyzed *L. monocytogenes* isolates. Analysis of SNPs also showed unique allelic profile for important virulence genes including *actA*. The phenotypic change due to this allelic profile is beyond the scope of this analysis. Additionally, analysis showed the isolate is phylogenetically distant from neighboring strain reported from India. A limitation of evolutionary analysis is lack of whole genome sequences from nearby geographic location and lack of sequences from recent time. It can be anticipated that if more sequence data from Bangladesh were available, Bangladeshi isolates might form their subclade. However, the low evolutionary rate of *L. monocytogenes* serovar 4b strains, it can be assumed that the isolate *Lm*-BD-CHAMPS-01 has been also circulating in the population for a long time.

In summary, the *Lm*-BD-CHAMPS-01 isolate is a genetically stable and virulent strain likely circulating at least in the community of our study area. Circulation of such virulent strains indicates the necessity to consider *L. monocytogenes* infection as a notifiable disease for diagnostic facilities. Backyard poultry along with free living birds could be investigated as a potential carrier of *L. monocytogenes* in Bangladeshi households. However, consumption of raw food contaminated with the pathogen cannot be ruled out. Overall, dietary guidelines and exposure to animals adapted for local food habit and local culture should be developed considering potential *L. monocytogenes* infection during pregnancy.

Conclusions

This study presents the identification and characterization of a virulent *Listeria monocytogenes* strain, *Lm*-BD-CHAMPS-01, causing stillbirth in Bangladesh. Our findings highlight the presence of key virulence genes that enable the pathogen to cross the placental barrier, posing significant risks during pregnancy. The genomic stability and evolutionary insights suggest this strain's prolonged history within the region. Importantly, the sensitivity of *Lm*-BD-CHAMPS-01 to several tested antibiotics underscores the potential for prevention and treatment through timely diagnosis and quality antenatal care. The findings also suggest that backyard poultry, such as hens, and free-living birds, such as pigeons, could be potential reservoirs, necessitating further investigation. The development of locally adapted dietary and animal exposure guidelines to prevent *L. monocytogenes* infections during pregnancy will be useful. This report contributes valuable insights to the field of public health microbiology as well as maternal and neonatal health.

The findings suggest advocacy for enhanced surveillance and preventive measures against *Listeria* infections.

Abbreviations

BD	Bangladesh
CC	Clonal Complex
cgMLST	core genome Multilocus Sequence Typing
CHAMPS	Child Health and Mortality Prevention Surveillance
CLSI	Clinical and Laboratory Standards Institute
CSF	Cerebrospinal Fluid
DeCoDe	Determination of Cause of Death
LIPI	Listeria Pathogenicity Island
L. monocytogenes	Listeria monocytogenes
MITS	Minimally Invasive Tissue Sampling
MLST	Multilocus Sequence Typing
MST	Minimum Spanning Tree
TAC	TaqMan Array Card

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-024-03650-5.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

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Author contributions

M.A. drafted the manuscript and guided the overall analysis. M.S.I., M.I.J., Z.I. and A.S.D. generated the whole genome sequencing data, conducted the bioinformatics analysis and generated the figures/tables. A.R., K.M.I., and M.Z.H. were responsible for collecting and analyzing the clinical data and generated the tables. A.I.C. gathered the demographic information. D.A. isolated the bacteria. S.E.A. and E.S.G. provided overall supervision for the CHAMPS project, secured funding, reviewed the manuscript. M.R. supervised the laboratory work and reviewed the manuscript.

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Data availability

This whole genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JBFRXX000000000. The codes used in the study is available in the Supplementary material 1.

Declarations

Ethics approval and consent to participate

The CHAMPS study was conducted following the approval of the research review committee (icddr, b protocol number PR-16082) and the ethical review committee of icddr, b. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from the legal guardians or family members of all stillbirths or deceased children included in the study. The consent process ensured that participants were fully informed about the purpose, procedures, risks, and benefits of the study, and their participation was entirely voluntary. Written consent forms were signed by the legal guardians or family members before any study-related procedures were initiated. No animal was included in this investigation.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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