

Original Article

Listeria infection in Chinese pregnant women and neonates from Shandong

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Abstract: Objective: This study aims to investigate the characters of pregnant women and neonates with listeria monocytogenes infections (*L. monocytogenes*) in Shandong of China. Method: Pregnant women with premature delivery or prenatal fever were recruited in 2013. Bacterial culture was performed for the umbilical cord blood and placental swab after delivery. The strains isolated were then analyzed for the virulence factors and classified based on the serotype and MLST typing. Results: Four cases (0.34‰) of the neonates were identified with listeriosis. Prenatal fever was observed in mothers of all infected neonates. The pathological examination of placenta showed non-specific inflammatory manifestations and two serotypes including 1/2b and 4b were detected. Six kinds of virulence factors including hly, inlA, actA, plcB, prfA and iap were all identified in the infected neonates. Conclusion: The results suggested that mother-to-infant was an important transmission mode for listeria. Antibiotic treatment, bacterial culture and placenta pathological examination were highly recommended in the diagnosis and treatment of listeriosis.

Keywords: Listeria, neonates, serotype, MLST

Introduction

Listeria monocytogenes (*L. monocytogenes*) can cause the listeriosis through contaminated food. It was reported that pregnant women accounted for 27% of listeriosis cases [1] and listeriosis in pregnant women can lead to premature delivery, miscarriage, stillbirth and other serious health problems for the neonates. The incidence of premature delivery and miscarriage caused by listeriosis was about 2.4‰ and 5.5‰ respectively. The incidence of listeriosis was about 0.52‰ in neonates and the incubation period of pregnancy-associated cases was 17-67 days [2]. However, the incidence of listeriosis in pregnant women and neonates in China is unknown.

It was reported that the mortality rate of neonatal listeriosis was as high as 30% even immediate and effective treatment was given [3]. Early antibiotic treatment was considered to reduce the mortality significantly. The association of the severity of neonatal listeriosis with mothers' treatment has also been reported. There

are two main clinical manifestations: sepsis for early stage and meningitis for late stage. However, both manifestations are non-specific.

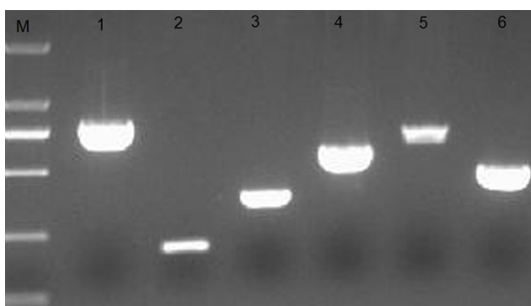
Sixteen serotypes of *L. monocytogenes* such as 1/2a, 3a have been identified by characterizing surface antigens such as O antigens (teichoic acids) and H antigens (proteins), in which the serovars 1/2a, 1/2b and 4b account for up to 90% of the listeriosis, other serotypes are rarely reported [4-6]. In this study, serum typing, multilocus sequence typing (MLST) and virulence factors analysis were performed for better understanding the pathogenicity and transmissions of listeria.

Materials and methods

Pregnant women with premature delivery or prenatal fever in 2013 were collected in the study. Medical history such as gestational age, pregnancy complication, infection and corresponding treatment was well recorded. Neonatal venous blood was immediately collected for bacterial culture in case of C-reactive

Table 1. Primers used in the MLST test

Genes	Primer Sequence (5'-3')
abcZ	GTTTTCCAGTCACGACGTTCTATCGCTGCTGCCACTTTTATCCA TTGTGAGCGGATAACAATTTCTCAAGGTCGCCGTTTAGAG
bglA	GTTTTCCAGTCACGACGTTGTAGCCGACTTTTATGGGGTGGAG TTGTGAGCGGATAACAATTTCCGATTAAATACGGTGCGGTCATA
cat	GTTTTCCAGTCACGACGTTGTAATTGGCGCATTTTGATAGAGA TTGTGAGCGGATAACAATTTGAGATTGACGATTCTGCTTTTG
dapE	GTTTTCCAGTCACGACGTTGTACGACTAATGGGCATGAAGAACAAG TTGTGAGCGGATAACAATTTTCATCGAACTATGGGCATTTTACC
dat	GTTTTCCAGTCACGACGTTGTAGAAAGAGAAGATGCCACAGTTGA TTGTGAGCGGATAACAATTTCTGCGTCCATAATACACCATCTTT
ldh	GTTTTCCAGTCACGACGTTGTAGTATGATTGACATAGATAAAGA TTGTGAGCGGATAACAATTTCTATAAATGTCGTTTCATACCAT
lhkA	GTTTTCCAGTCACGACGTTGTAAGAATGCCAACGACGAAACC TTGTGAGCGGATAACAATTTCTGGGAAACATCAGCAATAAAC

**Figure 1.** Electrophoretogram of the six genes tested in the MLST. 1. Hemolysin; 2. Internalin A; 3. Internalin B; 4. Actin; 5. Phospholipase C; 6. Modulin; M: Marker.

proteins increased and *L. monocytogenes* existed in the umbilical cord blood test.

Listeria was isolated from the placental secretions and neonatal blood. Serotypes were identified by characterizing O antigens and H antigens.

PCR amplification for seven housekeeping genes was performed to determine the MLST types with specific primers (Table 1). The reaction solution (50 µl) consisted of: 5.0 µl of the 10×pfu buffer, 200 µM of each dNTP, 50 pmol each of the two primers, 1.5U of Taq DNA Polymerase, 0.5U of pfu DNA polymerase and 100 ng of NDA template. The reaction conditions were as follows: pre-denaturation at 94°C for 5 minutes, followed by 30 amplification cycles of 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds, with a final

extension step of 72°C for 5 min. The PCR products were then purified and sequenced, followed by alignment to sequences in the database of Pasteur institute.

Results

Four cases of the fetus (0.34‰ in 11795) were identified with listeriosis by bacteria cultures. Meconium stain of amniotic fluid and fetal distress were all observed in the 4 cases. The clinical manifestations including asphyxia, pallor, poor reaction, dyspnea, low muscular tension were

respectively observed. Increased leukocyte or neutrophils, monocytes and C-reactive proteins were found in the blood examination in all cases. The cerebrospinal fluid cultures (CSF) showed meningitis changes with increased cell and proteins but decreased sugar and chlorides. Patchy shadows were all observed in the X-ray examination, suggesting inflammatory lesion. Meropenem was immediately given to neonates within 20 minutes after delivery. Penicillin or amoxicillin and clavulanate potassium was then added after identification of listeriosis in the blood culture. Intravenous antibiotics therapy was respectively continued for a total of 12 and 26 days. Four cases fully recovered.

Type 1/2b for 3 cases and type 4b for one case were identified in the serum typing test. The results of MLST revealed the cases belonged to MLST87, MLST3 and MLST 1 respectively. Six kinds of virulence factors including *hly*, *inlA*, *actA*, *plcB*, *prfA*, *iap* were all identified in the infected neonates (Figure 1; Table 2).

Non-specific inflammation changes such as membranitis and acute inflammation of the chorionic plate were observed in the pathological examination of placenta in the cases (Figure 2).

Discussion

L. monocytogenes is strongly pathogenic to human beings by expressing the hemolytic exotoxin. It has been isolated from soil, water and other environmental sources. At least 42 mam-

Table 2. Etiologic results of the strains isolated from 4 cases

Case	Samples	Sero-type	virulence factor						MLST
			actA	prfA	plcB	hly	inlA	iap	
1	Placenta (M)	1/2b	+	+	+	+	+	+	87
	Placenta (S)	1/2b	+	+	+	+	+	+	87
	Blood (S)	1/2b	+	+	+	+	+	+	87
2	Placenta (M)	1/2b	+	+	+	+	+	+	3
	Placenta (S)	1/2b	+	+	+	+	+	+	3
	Blood (M)	1/2b	+	+	+	+	+	+	3
3	Placenta (M)	4b	+	+	+	+	+	+	1
	Placenta (S)	4b	+	+	+	+	+	+	1
	Blood (S)	4b	+	+	+	+	+	+	1
4	Placenta (M)	1/2b	+	+	+	+	+	+	3
	Placenta (S)	1/2b	+	+	+	+	+	+	3
	Blood (S)	1/2b	+	+	+	+	+	+	3

M: mother; S: son; MLST: multilocus sequence typing.

malian species as well as 22 species of birds have been identified to be carriers. Numerous bacteria were discharged outside even into raw milk, thus entering the human food chain. It was reported that 1% to 5% of the healthy people and 10% to 20% of the slaughterhouse staff were the main source of infections as their asymptomatic carrier [7, 8].

In the present study, 0.34% of neonates suffered from listeriosis, which was similar to the incidence of Euramerican countries. Early onset listeriosis occurring within two days after delivery was often considered to be vertically transmitted by circulation cross the placenta. We found 4 neonatal patients with listeria infection immediately after delivery in this study. In addition, significant non-specific inflammatory manifestations were only observed in the infected neonates, which indicated that the pathological examination of placenta may be helpful in the diagnosis of listeriosis.

Smith et al. has suggested the negative correlation between gestational age and severity of the listeriosis [9]. We also found the phenomenon in this study. Relationship between the severity of neonatal listeriosis and mothers' treatment has also been reported [10]. In our study, pregnant mothers with corresponding symptoms did not receive antibiotic treatment until a high fever appeared, resulting in serious neonatal listeria infections, so listeriosis should

be highly suspected when mother was found of prenatal fever, polluted amniotic fluid with bad smell, respiratory tract infection and diarrhea.

It was reported that early surveillance and effective treatment play important roles in improving the patient's survival rate and prognosis [11, 12]. In this study, three mothers were found of eating undercooked crab and sushi. Pregnant women should be advised to avoid cold and long-term storage food which may be contaminated by *L. Monocytogenes*. Although various therapies could be selected for patients

with listeria infection, effective antibiotic treatment is still the cornerstone that is recommended in such cases before bacterial culture and susceptibility test. In our study, meropenem, penicillin or amoxicillin and clavulanate potassium were immediately given, leading to full recovery for 4 cases. Pregnancy should be early terminated if abnormalities of the amniotic fluid and fetal heart were observed. Moreover, intrauterine and prophylactic antibiotics were also recommended.

Studies have revealed effects of the strain types on the severity and prognosis of the disease [13]. In this study, there cases were identified as 1/2b type and one was 4b type, which was not consistent with results from the western countries that indicated 4b as the most common type [14]. However, similar results were also observed in other study [15]. Regional difference may contribute to this discrepancy.

As the major virulence factor, Hemolysin LLO encoded by *hly* gene was suggested to closely associate with the pathogenicity of listeria [16]. *Listeria monocytogenes* with the *hly*, *inlA*, *actA*, *plcB* and *prfA* genes can pass through blood brain barrier and was considered to be more pathogenic with the highly invasive abilities [17]. In this study, 4 infected infants showed serious diseases, which may be due to existence of the 6 virulence factors. Previous studies have also reported that bacterial adaptive ability, environmental pH value and patient

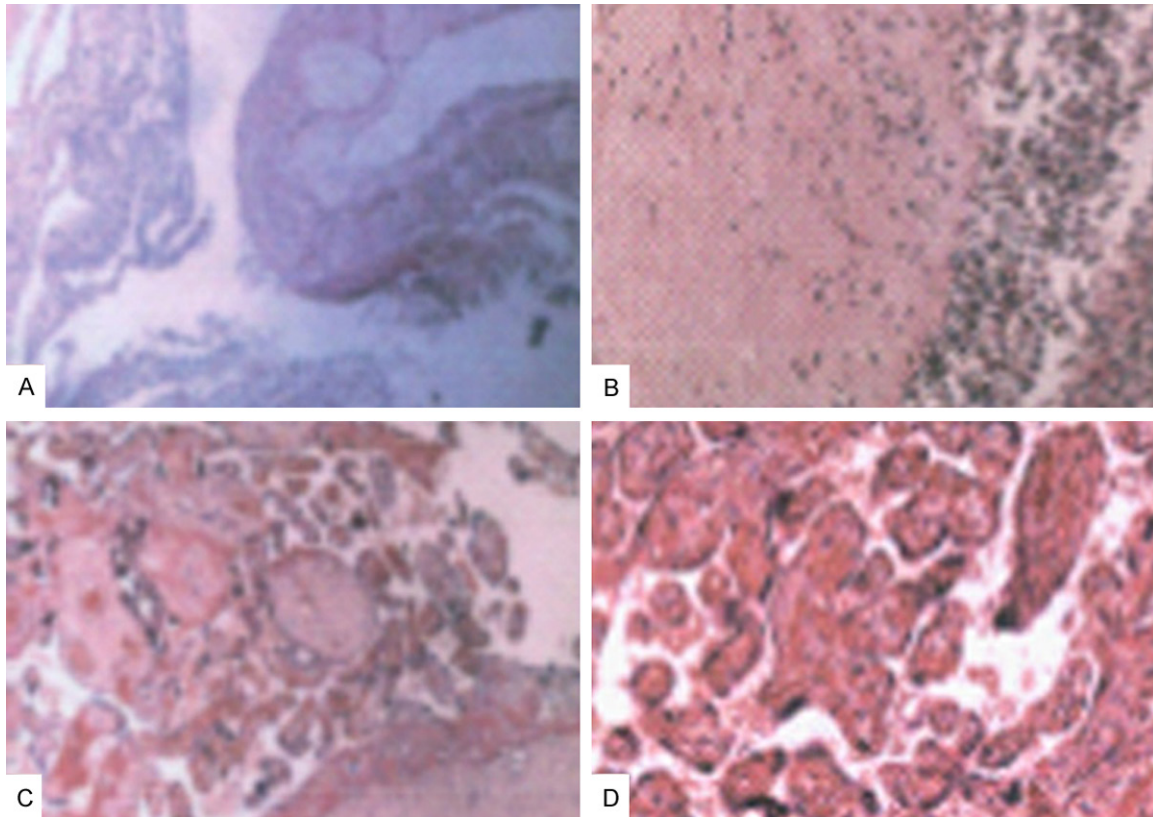


Figure 2. Pathological results of the placentas from 4 cases. A: Mature placenta of case 1. Acute membranitis, acute inflammation of the chorionic plate, locally congestive villus, narrow intervillous space were observed. B: Partially mature placenta of case 2. Severe acute membranitis, acute inflammation of the chorionic plate, inflammatory exudates in the intervillous space, infarcted villus, acute umbilical vasculitis and perivasculitis can be observed. C: Mature placenta of case 3. Congestive villus, severe acute membranitis, severe acute inflammation of chorionic plate, acute umbilical vasculitis and perivasculitis were found. D: Immature placenta of case 4. Acute inflammation of epichorion, acute inflammation of chorionic plate, acute umbilical vasculitis and perivasculitis can be observed.

immunity were associated with pathogenicity of listeria. However, further studies are still required to identify specific genes [18].

In this study four cases with listeria septicemia were fully recovered although positive virulence factors were found, which may be due to immediate antibiotic treatment and termination of pregnancy. So early monitoring, empirical antibiotic treatment, bacterial culture and placenta pathological examination were thus highly recommended.

Disclosure of conflict of interest

None.

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