A novel mutation leading to a premature stop codon in *inlA* of *Listeria monocytogenes* isolated from neonatal listeriosis

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SUMMARY

The study objective was to investigate whether the strain of *L. monocytogenes* serotype 1/2c isolated from neonatal listeriosis carries a premature stop codon (PMSC) mutation in the *inlA* gene. The strain was characterized by serotyping, macrorestriction analysis after digestion with the restriction enzyme *Asc*I, and sequencing of the *inlA* gene. The tested strain of serotype 1/2c and pulsotype 1 possesses a new type of point mutation leading to a PMSC in the *inlA* gene and production of truncated internalin A. The case of early onset form of neonatal listeriosis caused by serotype 1/2c with a PMSC mutation in the *inlA* gene confirmed the transplacental transmission potential of this strain.

KEY WORDS: Truncated internalin A, Sequencing, Newborn, Trans-placental barrier, Listeria.

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L. monocytogenes causes serious invasive disease in a susceptible human population. Listeriosis manifests itself as sepsis, meningitis or, in the case of pregnant women, can be the cause of miscarriage or premature birth (Vázquez-Boland *et al.,* 2001). Listeriosis is 18 times more common during pregnancy than in the non-pregnant population (Lamont *et al.,* 2011). Interaction of internalin A with E-cadherin, a transmembrane adhesion protein required for adherens junction formation in epithelial cells, is exploited to cross the intestinal and also placental barrier (Bierne *et al.,* 2007). Strains of *L. monocytogenes* producing a truncated form of internalin A show attenuated invasion of Caco-2 cells in tissue cultures and exhibit low virulence in mammalian hosts (Olier *et al.,* 2003; Jacquet *et al.,* 2004; Nightingale *et al*., 2005; Nightingale *et al.,* 2008).

To date, 18 naturally occurring mutations leading to a PMSC which result in the expression of truncated internalin A have been identified in the *inlA* gene (Van Stelten *et al.,* 2010). The occurrence of *L. monocytogenes* strains with mutations encoding the production of truncated internalin A has been reported in different countries, such as the USA (Nightingale *et al*., 2005; Orsi *et al.,* 2007; Shen *et al.,* 2013), Canada (Kovacevic *et al*., 2013), France (Olier *et al.,* 2003; Jacquet *et al.,* 2004; Rousseaux *et al.,* 2004), Portugal (Felício *et al.,* 2007) and Japan (Handa-Miya *et al.,* 2007). According to Van Stelten *et al.* (2010) the most common is PMSC mutation type 3 which together with types 1 and 4 represent more than 90% of the PMSC mutations in the USA.

In particular, strains of serotype 1/2c, which rarely cause listeriosis in humans, have been associated with the frequent occurrence of truncated internalin A (Olier *et al.,* 2003; Jacquet *et al*., 2004; Kovacevic *et al.,* 2013) and

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cases of asymptomatic human carriage (Olier *et al.,* 2003). The aim of the present study was to check for PMSC in the *inlA* gene in a strain of serotype 1/2c from neonatal listeriosis.

The tested strain was obtained in 2011 within the activities of the Czech National Reference Laboratory (NRL) for listeria. The strain was isolated from a newborn with the early onset neonatal form of listeriosis (stomach content) and stored in a BHI medium with 20% glycerol at -75°C. The strain was cultivated on blood agar (Bio-Rad, USA) aerobically at 37°C for 24 hours before the typing. Serotyping was performed by the slide agglutination method using commercially available antisera (Denka Seiken, Japan) and subsequently confirmed by a multiplex PCR method (Borucki and Call, 2003; Doumith *et al.,* 2004). Macrorestriction analysis using endonuclease *Asc*I (New England BioLabs, USA) was performed according to the EU RL protocol (Anses, Paris, France). Amplification of the complete *inlA* gene was carried out using three pairs of primers:

1) S_F1: GATATCACTAAACGGCTCC, S_R1: TAGTTTTGTTAGACCCGACA (1060 bp);
2) S_F2: TAAATCGGCTAGAACTATCCA, S_R2: GTCAATAAATTCCCAGCTTC (1063 bp);
3) S_F3: CTATACCTTTAGCCAACCTG, S_R3: TTCATTTTGTGTCACTGCATC (1300 bp).

Sequencing of both strands using the aforementioned primers was performed in a sequencing facility of Eurofins MWG Operon (Ebersberg, Germany). The presence of PMSC in a sequence was evaluated by *in silico* translation. The GenBank accession number for the *inlA* gene sequence of *L. monocytogenes* L3102 strain is KJ129607.

Many authors consider internalin A as a molecular marker for evaluating the potentially attenuated virulence of *L. monocytogenes* (Jacquet *et al.,* 2004; Nightingale *et al.,* 2005; Van Stelten and Nightingale, 2008; Chen *et al.,* 2011). Many studies have shown that *L. monocytogenes* strains isolated from foods more often carry mutations leading to the release of truncated internalin A than strains isolated from humans (Jacquet *et al.,* 2004; Nightingale *et al.,* 2005; Van Stelten and Nightingale, 2008; Van Stelten *et al.,* 2010). In the USA, mutations in the *inlA* gene leading to PMSCs were identified in 45% of isolates originating from ready-to-eat foods and only in 5.1% of strains isolated from

TABLE 1 - *Position of PMSCs identified in inlA gene of L. monocytogenes strains*

PMSC mutation type	*Nucleotide position of mutation*	*Lenght of truncated internalin A*	*The strain number*	*References*
1	1818 (T→A)	605	FSL F2-563	Nightingale *et al*., 2005
2	1966 (C→T)	655	FSL R2-074	Nightingale *et al*, 2005
3	2100 (C→G)	699	FSL F2-516	Nightingale *et al*., 2005
4	12 (deletion A)	8	F7-061	Felício *et al*., 2007
5	565 (C→T)	188	FSL R2-080	Van Stelten and Nightingale, 2008
6	1474 (C→T)	491	H1	Olier *et al.*, 2003
7	1684 (C→T)	561	FSL T1-061	Van Stelten and Nightingale, 2008
8	1380 (G→A)	459	NV8	Rousseaux *et al*., 2004
9	1540 (deletion G)	518	NV7	Rousseaux *et al*., 2004
10	1961 (insertion T)	676	NV4	Rousseaux *et al*., 2004
11	2054 (G→A)	684	NV5	Rousseaux *et al*., 2004
12	1637 (deletion A)	576	LO28	Jonquières *et al*., 1998
13	1579 (A→T)	526	36-25-1	Handa-Miya *et al*., 2007
14	1615 (C→T)	538	LM57179	Ragon *et al*., 2008
15	229 (C→T)	76	NRRL_B-57040	Van Stelten *et al*., 2010
16	508 (G→T)	169	NRRL_B-33873	Van Stelten *et al*., 2010
17	758 (T→A)	252	NRRL_B-57066	Van Stelten *et al*., 2010
18	1165 (deletion T)	403	NRRL_B-33591	Van Stelten *et al*., 2010
Novel	976 (G→T)	325	L3102	This study

clinical cases of listeriosis (Van Stelten *et al.,* 2010). The likely cause of this finding can be the predominant occurrence of serotypes 1/2a and 1/2c in foods because these mutations prevail among lineage II strains (Nightingale *et al.,* 2008; Van Stelten *et al.,* 2010).

In the Czech Republic, only one case of human listeriosis was noted to be caused by serotype 1/2c, which at the same time confirmed a possible transplacental transmission of a *L. monocytogenes* strain carrying a mutation in the *inlA* gene leading to PMSC. In the strain of serotype 1/2c, pulsotype 1 isolated from a newborn with early onset infection, the sequencing analysis revealed a nonsense mutation at position 976 (GAA→TAA) of the *inlA* gene where a change of glutamic acid codon to a stop codon occurs. A comparison with the hitherto known PMSC mutation types (Van Stelten *et al.,* 2010) showed that this type of point mutation in the *inlA* gene is new (Table 1).

Strains of *L. monocytogenes* carrying *inlA* PMSCs can be up to 10 000 times less virulent than strains containing a functional internalin A (Chen *et al.,* 2011). However, strains of *L. monocytogenes* carrying mutations in *inlA* leading to the synthesis of truncated internalin A can be implicated in human listeriosis (Jacquet *et al.,* 2004; Van Stelten *et al.,* 2010). Holch *et al. (*2013) demonstrated that persistent strains of *L. monocytogenes* isolated from food processing plants, carrying mutations leading to PMSC in the *inlA* gene, were able to cross the placental barrier after oral exposure of pregnant mice and guinea pigs. Crossing of the placental barrier most probably occurs by a mechanism that is independent on the interaction between E-cadherin and internalin A not only in animals (Holch *et al.,* 2013) but also in humans. In our study we detected a point mutation in *inlA* leading to a premature stop codon in the strain of *L. monocytogenes* serotype 1/2c, isolated from a case of early onset neonatal listeriosis.

Neither mice or guinea pigs represent an ideal animal model for *L. monocytogenes* pathogenesis study because listerial internalin A does not bind E-cadherin in mice and internalin B does not activate Met receptor in guinea pigs (D'Orazio, 2014). Conjugated action of both *inlA* and *inlB* mediates crossing of the placental barrier (Bonazzi *et al.,* 2009). When assessing virulence of *L. monocytogenes* it is necessary to consider that there are also a number of other factors responsible for the pathogenicity of these bacteria.

It was interesting to find that the only case of neonatal listeriosis caused by serotype 1/2c in the Czech Republic was associated with the strain carrying a point mutation in the *inlA* gene leading to the production of truncated internalin A. This result shows that strains of *L. monocytogenes* producing truncated internalin A may still pose a potential risk to pregnant women and neonates.

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