

SHORT REPORT

More than one variant of *Listeria monocytogenes* isolated from each of two human cases of invasive listeriosis

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SUMMARY

Two variants of *Listeria monocytogenes* were isolated from blood cultures from each of two patients with listeriosis. Each variant displayed a two-band difference in DNA profile from the other by pulsed-field gel electrophoresis. Although this difference in profile is insufficient to distinguish clearly between the variants, the possibility of co-infection with different strains of *L. monocytogenes* needs to be considered. We suggest that more than one colony should be selected for molecular typing to aid interpretation during investigation of the sources and routes of *Listeria* infection.

Listeria monocytogenes is a foodborne Gram-positive bacterium that can cause severe sepsis particularly meningitis, encephalitis and spontaneous abortions. The first report of a foodborne outbreak of listeriosis [1] led to a number of epidemiological investigations into the reservoirs and transmission of *L. monocytogenes* and the description of a variety of molecular methods for the typing of strains [2]. Pulsed-field gel electrophoresis (PFGE) of DNA digests generated with rare-cutting restriction endonucleases has become the 'gold standard' for the typing of *L. monocytogenes*, and many other bacterial species owing to its reproducibility and discriminatory power [2]. There are a number of reports on the diversity of *L. monocytogenes* strains isolated from humans, food and the food environment. Boerlin *et al.* [3] found that a limited number of clonally similar and possibly related PFGE subtypes emerged slowly over a 5-year outbreak in Switzerland and concluded that

the evolution of these variants was the consequence of a single gene insertion event in the genome of the index strain over time.

We describe here the recovery of PFGE variants within the same blood samples from each of two patients with listeriosis. Blood samples (10 ml) were taken from the two patients (A and B) and inoculated into BacT/ALERT FA bottles (Organon Teknika Corp., Durham, NC, USA). The bottles were sent by regular post without cooling and arrived the next day at our laboratory, where they were kept refrigerated until analysis within 1 week; 0.1 ml from each sample was spread on 5% bovine blood agar plates and incubated at 37 °C for 24 h. Forty-six colonies of *L. monocytogenes* were isolated from patient A and 50 colonies from patient B. All isolates were frozen in brain heart infusion broth (Merck, Darmstadt, Germany) containing 20% glycerol, at –70 °C, for later characterization.

Each isolate was characterized by restriction enzyme analysis with *AseI*, followed by PFGE according to the PulseNet standardized protocol [4] with some modifications. Pharmacia Gene Navigator

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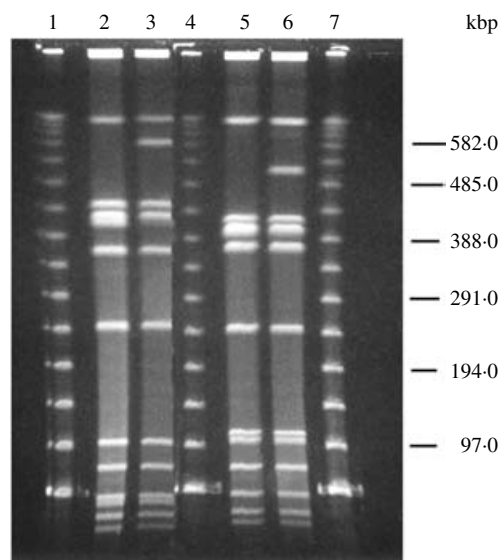


Fig. Pulsed-field gel electrophoresis patterns from *AscI* digests of DNA of *Listeria monocytogenes* isolates. Lanes 1, 4, 7, lambda ladder; lane 2, SLU2916 (patient A, variant 1); lane 3, SLU2918 (patient A, variant 2); lane 5, SLU3267 (patient B, variant 1); lane 6, SLU3268 (patient B, variant 2). Note: The thick band slightly above the 388 kbp marker band of isolate SLU3267 (lane 5) was separated into two bands when the gel was run for another 24 h.

(Pharmacia, Uppsala, Sweden) was used with a run time of 24 h, an initial pulse time of 4 s and final pulse time of 40 s; a lambda ladder PFG marker no. 340 S (New England Bio-Labs Inc., Beverly, MA, USA) was used as molecular size markers. Isolates showing variation in profile were examined further by restriction with *ApaI* and *SmaI* and with different migration protocols. DNA was prepared three times from isolates representing each variant and analysed by PFGE to ensure repeatability. In addition, the BacT/ALERT FA bottle from patient A was kept for 6 years in the refrigerator (+6 to +8 °C) and a further 16 colonies were isolated after this time and characterized by restriction with *AscI*. One representative isolate of each of the PFGE variants was confirmed as *L. monocytogenes* by their catalase and haemolysin production, Gram reaction and fermentation of rhamnose and xylose. The isolates were serotyped with *Listeria* O and H antisera (Mast Diagnostics, Bootle, UK), according to the manufacturer's instructions.

L. monocytogenes was recovered in pure culture from each sample. Two PFGE variants, differing by two bands in *AscI* digests, were isolated (43 isolates of variant 1 and three of variant 2) from patient A.

Patient B also exhibited two variants that differed in two bands (37 isolates of variant 1 and 13 of variant 2) (Fig.). All isolates were confirmed as *L. monocytogenes* serovar 1/2a. The two-band difference in both pairs of variants was also found with *ApaI* restriction. However, all isolates from patient A were identical in *SmaI* digests but this enzyme confirmed the presence of two variants in patient B. Of the 16 isolates recovered after 6 years' refrigeration, 14 were identified as variant 1 and the remainder, variant 2.

Boerlin *et al.* [3] analysed five isolates of *L. monocytogenes* from different body sites of a single patient, sampled the same day, and all gave the same PFGE *ApaI* and *SmaI* patterns thus confirming the validity of macrorestriction analysis for short-term epidemiological tracing of this species. In the long term Swiss epidemic outbreak PFGE profiles were found to vary over time which was probably to be expected particularly as a result of rearrangements involving insertion, deletion, or transposition of genes [3]. The present study, however, shows that more than one variant of *L. monocytogenes* can be recovered from a single blood sample. This finding may be interpreted as either genetic instability of a single strain population or infection with mixed populations of the organism; the latter scenario is feasible if they were acquired through ingestion of food contaminated with multiple strains.

Baxter *et al.* [5] found that multiple isolates of *L. monocytogenes* from a single site of a sheep had identical enzyme electrophoretic types as did isolates from different tissues within an individual sheep. Wiedmann *et al.* [6], however, found that RAPD patterns for brain isolates from a goat were different (the degree of difference is unknown) from the pattern for the cerebrospinal fluid isolates from the same animal. In contradiction of their own results, they discounted co-infection with two different *L. monocytogenes* strains as being unlikely as in such a case both strains would be expected to be present in the brain. However, two different strains of *L. monocytogenes* in a human [7] and an animal case of listeriosis [8] have been described. In each of those cases, the strains were clearly unrelated by PFGE and thought to have been ingested through food and feed respectively.

Although a considerable degree of strain diversity in PFGE profiles exists in *L. monocytogenes* populations [2] the number of band differences required to discriminate between different strains is contentious. Strains differing by several bands are clearly distinct

from each other but some strains from temporally and geographically distinct sources may exhibit few differences in DNA profile and by the Tenover criteria could be considered epidemiologically related [9]. However, the latter criteria have not been validated for typing *L. monocytogenes* and studies addressing the variation of populations in single specimens are thus necessary. The evidence for dual infection in the two patients presented here is admittedly circumstantial but the study does highlight the fact that an approach of typing a single isolate from a clinical site of patient may pose problems for interpretation of DNA profiles when investigating sources and routes of *Listeria* infection. The examination of multiple isolates from blood cultures and other patient sites and from mother–baby pairs is therefore recommended.

DECLARATION OF INTEREST

None.

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