Ongoing outbreak of invasive listeriosis due to serotype 1/2a *Listeria monocytogenes*, Ancona province, Italy, January 2015 to February 2016

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In the first seven weeks of 2016, five serotype 1/2a *Listeria monocytogenes* isolates were collected from patients with invasive listeriosis in Ancona province in Italy. These strains and six 1/2a isolates identified in 2015 in the same area were typed by ERIC-PCR and PFGE. A clonal relationship, documented between the two sets of isolates, suggested a listeriosis outbreak in Ancona that started most probably in 2015. Investigation into the source of infection is still ongoing.

In the first seven weeks of 2016, six cases of invasive listeriosis were recorded in Ancona province, Italy. Five strains of *Listeria monocytogenes* serotype 1/2a were isolated and typed by enterobacterial repetitive intergenic consensus (ERIC)-PCR and PFGE, indicating clonality. In addition, seven serotype 1/2a *L. monocytogenes* strains from cases of invasive listeriosis recorded in the same area in 2015 were also typed and showed relatedness. Here we provide details of the ongoing outbreak.

**Outbreak description**

From 4 January to 15 February 2016, six *L. monocytogenes* strains (3 from blood, 3 from cerebrospinal fluid (CSF)) were isolated from six patients diagnosed with invasive listeriosis at the Clinical Microbiology Laboratory of Ancona Regional Hospital (eastern Italy) of Area Vasta 2 (AV2) which encompasses Ancona, Fabriano, Senigallia, and Jesi. Patients had been admitted to four different departments: emergency room (ER) (n=2), oncology (n=2), infectious diseases (n=1), and intensive care unit (ICU) (n=1). Four of the six patients were women and the most common risk factors/underlying conditions were: age (n=5; >71 years), cancer (n=2), and diabetes (n=1). Clinical manifestations included sepsicaemia (n=3), meningitis (n=2) and meningoencephalitis (n=1). In addition to the cases detected in 2016, eight *L. monocytogenes* strains (5 from blood and 3 from CSF) had been isolated in AV2 (from 7 cases) and nearby Ascoli Piceno (from 1 case) in 2015 (Figure 1); clinical samples came from six hospital departments: ER (n=1), general medicine (n=3), nephrology (n=1), vascular surgery (n=1), infectious diseases (n=1), and ICU (n=1). Five patients were men and the mean patient age was 73.6 years (range: 55–84; median: 75); a 77 year-old man died.

The 2015 and 2016 isolates were identified as *L. monocytogenes* by Gram staining and the Vitek MS system (bioMérieux Italia SpA, Firenze, Italy). Susceptibility to ampicillin, meropenem, erythromycin, and sulfaphmethoxazole-trimethoprim was tested by the E-Test (Liofilchem, Teramo, Italy) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [1]. All strains were susceptible to all the antibiotics tested.

**Molecular typing**

In order to identify relatedness, the 2015 and 2016 *L. monocytogenes* isolates were sent to our laboratory (Unit of Microbiology, Department of Biomedical Sciences and Public Health, Polytechnic University of Marche, Ancona) for molecular typing. Multiplex PCR serotyping [2] assigned five 2016 isolates and seven 2015 isolates to serotype 1/2a; the remaining isolates were serotype 4b (2016) and serotype 1/2c (2015).

Genetic relatedness was explored by ERIC-PCR [3] and by PFGE after ApaI digestion of total DNA [4]. ERIC pattern similarity was determined on the basis of the Dice similarity coefficient; the matrix thus generated was subjected to clustering using TREECON software (Bioinformatics and Evolutionary Genomics, Gent, Belgium). The 1/2a 2015 and 2016 isolates shared a
five-band pattern ranging from 1,500 to 900 bp (Figure 2).

Moreover, four of five serotype 1/2a 2016 isolates (76622, 80466, 80864, 81753) displayed identical ERIC PCR profiles; the remaining isolate (73844) differed by one band (>90% similarity index). The profile of the serotype 4b strain (77660) was completely different (<50% similarity index). All 1/2a 2015 isolates showed a high degree of similarity (>85%) with respect to the 1/2a 2016 isolates. Notably, the profile of the single serotype 1/2c isolate (09707) was closely related to that of the 1/2a isolates.

PFGE analysis confirmed ERIC PCR results, except for two 1/2a isolates, i.e. strain 56053 (Ascoli Piceno) and strain 02470 (the first 2015 isolate) (data not shown). The DNA of serotype 1/2c strain 09707 was not digested by Apal.

**Background**

*L. monocytogenes* is widely distributed in the environment and is frequently isolated from a variety of sources, including soil, vegetation, food of animal origin such as meat and dairy products, silage, fecal material, sewage, and water [5]. Listeriosis is most often transmitted through food and primarily affects older adults, pregnant women, newborns, and adults with weakened immune systems [5]. Serotyping is a universally accepted typing method for *L. monocytogenes*, with more than 14 serotypes being recognised according to variation in somatic (O) and flagellar (H) antigens [6]. Multiplex PCR serotyping is a practical alternative to slide agglutination serotyping, since it differentiates among the five major serogroups, each of which...
includes multiple serotypes: serogroup IVb (serotypes 4b, 4d and 4e), serogroup Ila (serotypes 1/2a and 3a), serogroup Iib (serotypes 1/2b, 3b and 7), serogroup IIC (serotypes 1/2c and 3c), and serogroup IVa (serotypes 4a and 4c). By use of suitably designed primer pairs, the four major serotypes 1/2a, 1/2b, 1/2c, and 4b produce four distinct PCR profiles [2]. PFGE is considered as the gold standard molecular typing approach for L. monocytogenes, owing to its high reproducibility and discrimination ability [4].ERIC PCR is a relatively simple, cost-effective, and discriminatory typing method based on ERIC sequences, 124 to 127 base-long elements consisting of highly conserved central inverted repeats found in the extragenic regions of the bacterial genome [3].

Discussion and conclusion

The incidence of listeriosis has been rising since the early 2000s in several European countries, mainly in immunocompromised patients older than 65 years [7-9]. In particular, a statistically significant increase was reported in Austria, Denmark, Hungary, Italy, France, Spain, and Sweden from 2005 to 2009 [10]. In the past 30 years, outbreaks of listeriosis have been mostly linked to serotype 1/2a and 4b clones [8]. A shift to serotype 1/2a has been observed in Europe and North America in the last decade [8]. In Italy, surveillance of invasive listeriosis has found an increase in serotype 1/2a isolates over the same period, mainly in the central and northern regions (about 80% of cases) [10-14].

Listeriosis is an infection of great concern to public health due to its clinical severity and high case fatality rate, despite its low incidence compared with other foodborne diseases such as salmonellosis or campylobacteriosis. The present data suggest an ongoing outbreak of listeriosis due to serotype 1/2a L. monocytogenes in AV2 that most probably started in 2015, since the strain was already present in the area in 2015. As in other European countries, most cases were associated with an underlying condition and involved elderly people [8,9]. Local authorities are working with the Italian national public health institute (the Istituto Superiore di Sanità, Rome) and the regional Istituto Zooprofilattico Umbria and Marche to identify the sources of food contamination. A recent press release [15] points out that there are findings which suggest contamination of a pork product as a possible vehicle of infection for at least one human case. At present, however, no clear link can be established between the contaminated pork product and the infections. Investigation into the source of infection in AV2 is still in progress.

Conflict of interest

None declared.

Authors’ contributions

E. Marini, G. Magi and B. Facinelli designed and developed the experimental design. E. Manso and C. Vincenzi collected bacterial strains and epidemiological data; E. Marini and G. Magi performed experiments; B. Facinelli, E. Marini and G. Magi performed data analysis and wrote the manuscript. All authors reviewed and approved the study.

References


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