J PREV MED HYG 2020; 61: E167-E172 OPEN ACCESS

ORIGINAL ARTICLE

Laboratory-based surveillance of invasive listeriosis in Northern Italy over a fourteen-year period: epidemiological and clinical results

M. GORI[1], G. CICERI[1], S. BIANCHI[1], D. CEREDA[2], S. SENATORE[2], M. GRAMEGNA[2], A. AMENDOLA[1, 3], M. PONTELLO[3], E. TANZI[1, 3]
[1] Department of Biomedical Sciences for Health, University of Milan, Milan, Italy; [2] DG Welfare, UO Prevenzione, Milan, Italy; [3] Coordinated Research Center "EpiSoMI", University of Milan, Italy

Keywords

Listeria monocytogenes • Listeriosis • Surveillance

Summary

Introduction. *Invasive listeriosis is a rare foodborne disease with a significant impact on public health worldwide, because of the severity of its clinical manifestations and high fatality rate. In this study, we provide a snapshot of epidemiology of listeriosis in Lombardy Region, Northern Italy, reviewing enhanced surveillance data collected over fourteen years, after the implementation of a voluntary laboratory-based surveillance system for the referral of clinical isolates of Listeria monocytogenes to a regional reference laboratory, since 2005.*
Methods. *Invasive listeriosis cases data from 2005 to 2018 were extracted from the regional laboratory-based surveillance system database and compared with the regional mandatory notification disease system data.*
Results. *Over the fourteen-year period under study, 533 Listeria monocytogenes isolates were detected by the laboratory surveillance system, 55 of which from pregnancy-related cases. The median age of non-pregnancy-associated patients was 71 years, with 64.6% of cases observed in the elderly. Cases with underlying medical risk conditions accounted for 92.1%, and the fatality rate was 26.2%. By integrating data from the mandatory notification system and the laboratory-based surveillance system, a total of 935 cases were recorded. The collection of data through the laboratory surveillance system allowed to increase the surveillance sensitivity by 18%.*
Conclusions. *Our results documented the growing epidemiological relevance of listeriosis through the analysis of two information sources, the regional mandatory notification system and the regional laboratory-based surveillance system. The data we obtained were consistent with the literature, except for pregnancy-related cases, which are often underdiagnosed. This study highlighted the importance of laboratory-based surveillance system, which led to a significant increase in the sensitivity of the mandatory notification system.*

Introduction

Listeriosis is a foodborne illness caused by the bacterium *Listeria monocytogenes*, with a relatively low incidence of disease among the 28 European Union Member States (0.47 per 100,000 population) [1]. Nevertheless, it is of major public health concern, because of the severity of its clinical manifestations (hospitalization rate > 90%) and the high fatality rate (20-30%) [2-4], mainly among the pregnant women, neonates, elderly and immunocompromised patients [5].
In Italy, since 1990, invasive listeriosis is subjected to mandatory notification, and the notification rate in 2018 was 0.29 per 100,000 population [1]. The majority of notified listeriosis cases (55%) is reported by Lombardy Region (Northern Italy), the most populous region in Italy, with 17% of the population [6], and a notification rate of 0.70 per 100,000 population [7]. Investigation on the transmission route is complex because of the long incubation period of the invasive infection (up to 70 days), foods with a long shelf-life and the probable high number of asymptomatic infections in people exposed to the same infection vehicle [4, 8, 9]. Moreover, the contemporary exposition of several people to the same food vector may result in a succession of apparently unrelated cases [10]. Therefore, it is essential that conventional epidemiology be supported by the molecular investigation, which allow to distinguish between outbreak-related and sporadic cases, and link cases to particular food and animal sources [11]. Since 2005, Lombardy Region holds an enhanced surveillance system that involves a voluntary laboratory-based network [12]. Hospital laboratories participating in the surveillance network voluntarily send clinical isolates to the Regional Reference Laboratory (RRL), which collects clinical and epidemiological data and carries out molecular subtyping. All the *Listeria monocytogenes* isolates are sent to the ECDC Operational Contact Point for *Listeria monocytogenes*, at the National Institute of Health (Istituto Superiore di Sanità, ISS), for further typing and characterization. The present study was undertaken to describe the epidemiological and clinical results of the laboratory-based surveillance system in Lombardy Region from the beginning of the surveillance (2005) until December 2018. The aims were to make a comparison with mandatory notification system data over the same period and to assess how much laboratory-

based network has increased the sensitivity of the surveillance system in Lombardy Region.

Methods

The laboratory-based surveillance program in Lombardy Region is performed with the delivery of *Listeria monocytogenes* isolates from hospitalized clinical cases to the RRL, which routinely carries out pulsed-field gel electrophoresis (PFGE) typing and whole-genome sequencing (WGS). The isolates are sent along with a standardized report form, agreed with ISS, used to collect: i) demographic data (e.g. age, gender, province of residence); ii) clinical data (e.g. symptoms, clinical form of disease, existence of underlying conditions, outcome); and iii) microbiological data. All data of the *Listeria monocytogenes* isolates from human cases occurred in Lombardy Region between 2005 and 2018 and collected by the RRL were included in our study. In case there were two or more isolates from the same patient only the first one was included in the analysis. For the purposes of the present work, an invasive listeriosis case was defined as an isolate of *Listeria monocytogenes* from a normally sterile site. A pregnancy-related listeriosis case was defined an isolate of *Listeria monocytogenes* from clinical sample of pregnant woman or foetus, stillborn, new-born aged < 28 days. Confirmed cases with isolation of *Listeria monocytogenes* from both mother and infant were considered as single cases. In order to make a comparison with regional mandatory notification system data, all available information regarding listeriosis cases were extracted from the regional web-based database MAINF (Sistema di sorveglianza notifiche di malattie infettive), and all listeriosis cases from MAINF and RRL were cross-matched according to whether they were present or absent in each data source. The detection of the same individuals in the two sources was determined by name, sex, age and date of hospitalization or symptom onset, and a joint database was generated, in order to include all reported cases of both systems. Observed cases were defined as the sum of cases reported only in MAINF, those recorded only by RRL and those present in both surveillance systems.
The statistical analyses were performed by chi-square test, using OpenEpi software (version 3.01).

Results

A total of 533 *Listeria monocytogenes* isolates were collected by the RRL between 2005 and 2018, 55 of which from pregnancy-related infection cases (10.3%) (Tab. I). The median age of non-pregnancy-associated patients was 71 years, with 64.6% of cases observed in people older than 65 years. The prevalent clinical manifestations were sepsis (62.1%) and central nervous system infections (28.9%). Atypical and rare forms of the infection were also detected: two cases of endocarditis, two cases of endophthalmitis and a case of periprosthetic joint infection. The number of patients whose outcome was known was 267, and the fatality rate was 26.2%. Cases with underlying medical risk conditions accounted for 92.1%, including cancer (32.2%), renal failure (10.1%), hemopathies (7.8%) and diabetes (7.3%). Fourteen pregnancy-related infection cases (31.8%) resulted in foetal death, miscarriage or stillbirth.
In the same period, MAINF recorded a total of 790 notified cases (Tab. I). Sixty-eight were pregnancy-related cases (8.6%). The median age of non-pregnancy related patients was 72 years, with 67.4% of cases observed in elderly patients (≥ 65 years). Sepsis was the most common clinical manifestation (49.6%), followed by meningitis and other central nervous system infections (28.7%). Cases with known outcome were 454, and the fatality rate was equal to 24.7%. Five hundred and ninety-six notified cases (90.3%) had at least one underlying risk condition for developing listeriosis: among others, cancer (30.1%), renal failure (8.3%), diabetes (8.2%) and hemopathies (3.6%). Seventeen pregnancy-related infection cases (33.3%) resulted in foetal death, miscarriage or stillbirth.
No significant differences were found between the variables reported in Table I, based on the chi-square test.
The distribution by year of listeriosis cases from the two data sources MAINF and RRL and total observed cases by year are presented in Table II. Overall, the two sources observed a total of 935 cases over a 14-year period, with an average of 66.8 cases per year. The incidence of the observed listeriosis cases in Lombardy is included in the range 0.29 per 100,000 population (2005) - 0.97 per 100,000 population (2011), with an average of 0.68 per 100,000 population. Considering the cases reported by the two sources and the cases observed through the cross-matched data, the increasing tendency is evident, as well as the presence of two peaks, one in 2009-2011 and one in 2017 (Fig. 1).
The distribution of listeriosis cases in the twelve provinces of Lombardy is shown in Table III and Figure 2. A third of the observed cases (311 out of 935) were recorded in the province of Milan, which with over 3,000,000 inhabitants corresponds to a third of the Lombardy population. The distribution of cases is not homogeneous, the incidence varies in a wide range, from 0.21 per 100,000 inhabitants to 1.00 per 100,000 inhabitants (Fig. 2).
The collection of data through the RRL system, which identified 145 cases not notified to the MAINF system, allowed to increase the surveillance sensitivity by 18% (Tab. II). The increase in sensitivity is particularly evident in 2007 (+ 35.3%) when there were 46 cases, 34 of which reported by the official notification system and 23 by the laboratory surveillance system, with an overlap of only 11 cases. As shown in Table III, the laboratory surveillance system resulted in an increase in sensitivity not uniformly distributed in the twelve provinces and, above all, concerned two of them, Brescia and Lodi (+ 40.0% and + 47.1%, respectively).

Tab. I. RRL and MAINF databases: main features of non-pregnancy and pregnancy-related listeriosis cases occurred in Lombardy Region, 2005-2018. All variables were calculated for patients with known information.

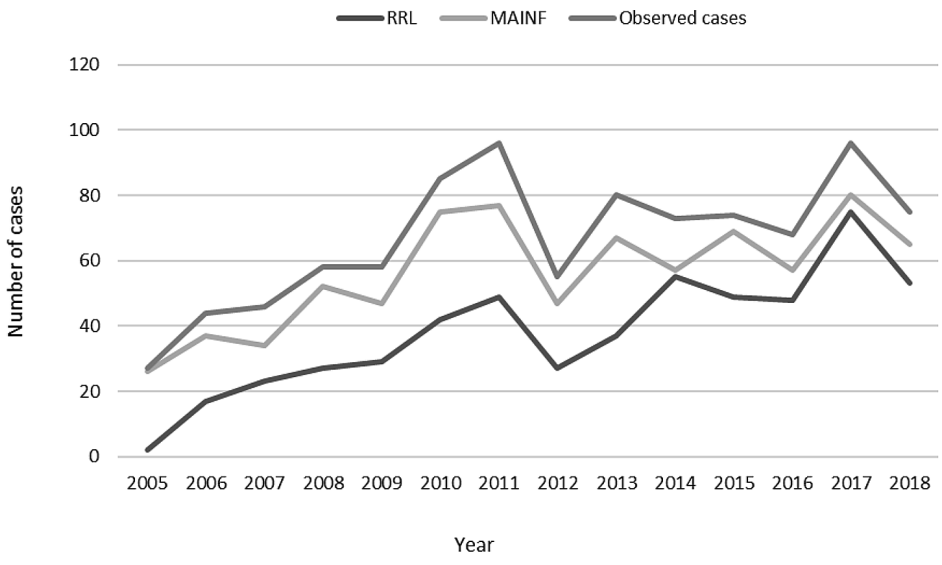
Characteristics	RRL (%)		MAINF (%)	
Number of cases	533		790	
Pregnancy-related cases	55	(10.3)	68	(8.6)
– fatality rate*	14	(31.8)	17	(33.3)
Non-pregnancy related cases	478	(89.7)	722	(91.4)
– median of age (years)	71		72	
– patients ≥ 65 years	309	(64.6)	487	(67.5)
– m:f	1.19		1.23	
– underlying condition	399	(92.1)	596	(90.3)
– fatality rate	70	(26.2)	112	(24.7)

*: no maternal fatality was recorded.

Tab. II. Listeriosis cases from two data sources (MAINF and RRL) and total observed cases by years of diagnosis.

Year	MAINF	RRL	MAINF only (A)	RRL only (B)	Both sources (C)	Observed cases (A+B+C)	B/A+C	Incidence per 100,000 population
2005	26	2	25	1	1	27	3.9%	0.29
2006	37	17	27	7	10	44	18.9%	0.46
2007	34	23	23	12	11	46	35.3%	0.48
2008	52	27	31	6	21	58	11.5%	0.60
2009	47	29	29	11	18	58	23.4%	0.60
2010	75	42	43	10	32	85	13.3%	0.87
2011	77	49	47	19	30	96	24.7%	0.97
2012	47	27	28	8	19	55	17.0%	0.57
2013	67	37	43	13	24	80	19.4%	0.82
2014	57	55	18	16	39	73	28.1%	0.72
2015	69	49	25	5	44	74	7.3%	0.74
2016	57	48	20	11	37	68	19.3%	0.68
2017	80	75	21	16	59	96	20.0%	0.96
2018	65	53	22	10	43	75	15.4%	0.75
Total	**790**	**533**	**402**	**145**	**388**	**935**	**18.4%**	**0.68**

Fig. 1. Listeriosis cases from two data sources (MAINF and RRL) and total observed cases by years of diagnosis.



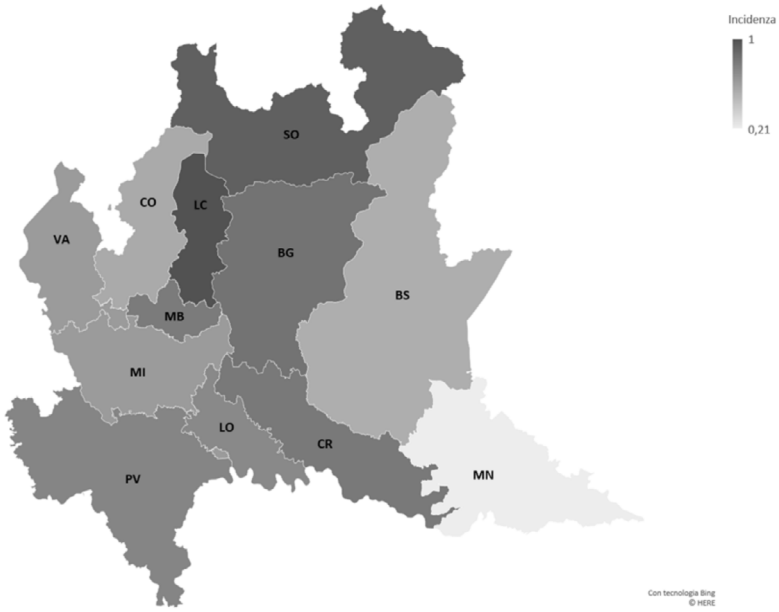
Discussion and conclusions

Although listeriosis has been included in the mandatory notification-based surveillance system in Italy since 1990, accurate data on epidemiology of this severe foodborne disease are still very limited. This study showed the results of the laboratory-based surveillance of listeriosis in Lombardy Region over the period 2005-2018, in comparison with mandatory notification system data, in order to learn more about the health impact of *Listeria monocytogenes* infections. The epidemiological scenario, without significant differences between the

Tab. III. Listeriosis cases from two data sources (MAINF and RRL) and total observed cases by Lombardy's provinces.

Province	MAINF	RRL	MAINF only (A)	RRL only (B)	Both sources (C)	Observed cases (A+B+C)	B/A+C	Incidence per 100,000 population
BG	113	77	48	12	65	125	10.6%	0.83
BS	70	66	32	28	38	98	40.0%	0.53
CO	37	24	23	10	14	47	27.0%	0.55
CR	38	8	31	1	7	39	2.6%	0.80
LC	44	15	30	1	14	45	2.3%	1.00
LO	17	19	6	8	11	25	47.1%	0.72
MN	11	2	9	0	2	11	0.0%	0.21
MI	249	197	114	62	135	311	24.9%	0.63
MB	67	59	21	13	46	80	19.4%	0.79
PV	47	30	24	7	23	54	14.9%	0.74
SO	24	0	24	0	0	24	0.0%	0.93
VA	73	36	40	3	33	76	4.1%	0.63
Tot	**790**	**533**	**402**	**145**	**388**	**935**	**18.4%**	**0.68**

Fig. 2. Geographical distribution of the mean annual incidence (cases per 100,000 population) of observed listeriosis cases in Lombardy Region during the period 2005-2018.



two sources, is consistent with the literature, except for the proportion of pregnancy-related cases, lower than reported in literature (20-43%) [13]. It is not clear if this lower frequency is real or due to underdiagnosis or underreporting of pregnancy related cases. Underdiagnosis could be more likely, considering that earlier stages miscarriage may be seldom investigated. On the other hand, the use of diagnostic tool for the detection of the pathogens in blood culture and CSF culture in non-pregnancy-related cases, mostly sepsis and meningitis in immunocompromised patients, are routinely performed [13].

As expected, the most affected age group was the elderly people [4] and, in most cases, one or more underlying immunosuppressive conditions were present. In the elderly, the most common clinical manifestation was sepsis. The fatality rate for non-pregnancy-associated cases (26.2%) was higher than that reported by the last ECDC report (15.6%), while for pregnancy-related cases the fatal outcome was observed in the 25% of cases [1]. This difference could be explained by a higher propensity to diagnose and report severe cases, with a frequently fatal outcome.

In Lombardy, the mean annual incidence of observed cases (0.68 per 100,000 population), obtained by integrating data from the two surveillance systems, was not homogeneously distributed neither in the twelve provinces (0.21 per 100,000 population - 1.0 per 100,000 population) nor in the considered years (0.29 per 100,000 population - 0.97 per 100,000 population). In particular, although no epidemic events were officially notified, a higher concentration of the number of cases in spotted areas and in certain periods was observed. More in detail, peaks in the years 2009-2011 in the provinces of Bergamo and Milan and in 2017 in the provinces of Brescia and Milan were identified. The laboratory-based surveillance allowed to identify a major listeriosis outbreak, linked to soft cheese, occurred in 2009-2011,

which went undetected by local health authorities [14]. Concerning the second peak occurred in 2017, the epidemiological investigation did not point out any link between cases, even though an outbreak was suspected.

In Europe, outbreak-related cases are apparently not frequent: in the report published in 2019 by the ECDC [1], only 158 out of 2561 cases reported in Europe (6.2%) belong to an outbreak (14 outbreaks in total). This rare outbreak identification could only be apparent and could represent the consequence of a series of difficulties deriving from the peculiar characteristics of *Listeria monocytogenes* infections. Indeed, the epidemiological investigation is often intricate because of many concurrent factors, such as the contamination of foods with long shelf lives, the long incubation period and the low attack rate of exposed people that may allow a listeriosis outbreak to occur as a succession of apparently unrelated cases [4, 10].

Laboratory-based surveillance system, introduced in Lombardy Region fifteen years ago, not only has supported the recognition of outbreaks, but has also led to a significant increase in the sensitivity of official surveillance (overall + 18% in the study period). However, it is surprising that this increase in sensitivity is included in a very wide range (+ 3.8 in 2005, year of start of RRL and, only two years later, in 2007, + 35.4%) and fluctuating over the fourteen years observed. This oscillation could be explained by hypothesizing a different sensitivity of the laboratory surveillance, which acts on a voluntary basis and which is conditioned by the difficulties in sending the isolates from the provinces distant from the RRL [12]. This difficulty does not concern MAINF, since notifications are mandatory, and the surveillance system is web-based.

Overall, our results document a not inconsiderable incidence of listeriosis, equal to 0.68 per 100,000 population if we consider all the "observed cases", 0.60 per 100,000 population and 0.40 per 100,000 population if we calculate the incidence from MAINF system and laboratory-based system, respectively. The increasing tendency in incidence, observed by both independent data sources, is probably real, as it is not possible to hypothesize – at least for the MAINF system – significant changes in the sensitivity of the surveillance over the considered period. It is also probable that the data of our Region, even referring only to the officially notified cases, have strongly contributed to the increase observed at national level, and highlighted in the latest European report, which document a significant increase in listeriosis incidence for nine European countries, including Italy, in the period 2014-2018 [1].

The explanation of this increase could be related to the progressive increase of population at risk, both for age and for other underlying conditions (e.g. diabetes, cancer), without excluding the role of the eating habits of groups characterized by situations of social hardship or ethnic groups, as already reported in the United Kingdom [15, 16].

In conclusion, having documented the growing epidemiological relevance of listeriosis through the analysis of two information sources, in order to consolidate surveillance, reduce underestimation and promote a more effective ability to suspect and recognize epidemic events, we believe that notification system should be integrated with laboratory-based system. Laboratory-based surveillance system must not be a voluntary-based channel alongside the official notification system, but the results of molecular analysis should systematically and as soon as possible be merged into the same database, in order to identify clusters of possibly related listeriosis cases and outbreaks undetected by the traditional surveillance systems. In this direction, in Italy, listeriosis surveillance has been improved since the end of 2019, with the creation of the Integrated Rapid Infectious Disease Analysis (IRIDA) Advanced Research Infrastructure for Experimentation in GenomicS (ARIES) platform [17]. IRIDA-ARIES is an open-source software set up and maintained at the ISS, which collects genomic and epidemiological data from the regional reference laboratories, in order to get real time data for timely detection of clusters of listeriosis and other foodborne diseases.

Acknowledgements

The authors would like to thank Marco Mentasti, at Directorate General of Welfare of Lombardy Region, for his help in retrieving the epidemiological data from the regional database MAINF, and Monica Gianfranceschi, Antonietta Gattuso and Alfonsina Fiore at the ECDC Operational Contact Point for *Listeria monocytogenes* (Istituto Superiore di Sanità, Rome), for their kind support and precious collaboration.

Funding sources: this work was supported by funds from Lombardy Region.

Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

MG, GC and SB managed the databases, analysed and interpreted the data. MG, GC, MP and ET wrote the paper. MP and ET conceived the work and led the study group. AA contributed to the conception and design of the study and critically revised the manuscript. MG, SS and DC coordinated surveillance and control activities in Lombardy Region. All authors have read and approved the final version of the manuscript.

References

[1] EFSA (European Food Safety Authority). Scientific report on the European Union One Health 2018 Zoonoses Report. EFSA Journal 2019;17:5926. https://doi.org/10. 2903/j.efsa.2019.5926

[2] Hedberg C. Listeria in Europe: the need for a European surveillance network is growing. Euro Surveill 2006;11:pii=628. https://doi.org/10.2807/esm.11.06.00628-en

[3] Todd ECD, Notermans S. Surveillance of listeriosis and its causative pathogen, Listeria monocytogenes. Food Control 2011;22:1484-90. https://doi.org/10.1016/j.foodcont.2010.07.021

[4] Hernandez-Milian A, Payeras-Cifre A. What is new in listeriosis? Biomed Res Int 2014;2014:358051. https://doi.org/10.1155/2014/358051

[5] Allerberger F, Wagner M. Listeriosis: a resurgent foodborne infection. Clin Microbiol Infect 2010;16:16-23. https://doi.org/10.1111/j.1469-0691.2009.03109.x

[6] Pontello M, Guaita A, Sala G, Cipolla M, Gattuso A, Sonnessa M, Gianfranceschi MV. Listeria monocytogenes serotypes in human infections (Italy, 2000-2010). Ann Ist Super Sanita 2012;48:146-50. https://doi.org/10.4415/ANN_12_02_07

[7] MAINF. Surveillance, notification, control of infective diseases: revision and reorganization of preventive interventions in Lombardy Region. Regione Lombardia. Deliberation n. VII/18853, 30/09/2004.

[8] Swaminathan B, Gerner-Smidt P. The epidemiology of human listeriosis. Microbes Infect 2007;9:1236-43. https://doi.org/10.1016/j.micinf.2007.05.011

[9] Silk BJ, Date KA, Jackson KA, Pouillot R, Holt KG, Graves LM, Ong KL, Hurd S, Meyer R, Marcus R, Shiferaw B, Norton DM, Medus C, Zansky SM, Cronquist AB, Henao OL, Jones TF, Vugia DJ, Farley MM, Mahon BE. Invasive listeriosis in the foodborne diseases active surveillance network (FoodNet), 2004-2009: further targeted prevention needed for higher-risk groups. Clin Infect Dis 2012;54(Suppl 5):S396-404. https://doi.org/10.1093/cid/cis268

[10] Kathariou S. Foodborne outbreaks of listeriosis and epidemic-associated lineages of Listeria monocytogenes. In: Torrence ME, Isaacson RE, editors. Microbial food safety in animal agriculture. Ames, Iowa: Iowa State University Press 2003, pp. 243-56.

[11] Sauders BD, Fortes ED, Morse DL, Dumas N, Kiehlbauch JA, Schukken Y, Hibbs JR, Wiedmann M. Molecular subtyping to detect human listeriosis clusters. Emerg Infect Dis 2003;9:67280. https://doi.org/10.3201/eid0906.020702

[12] Zolin A, Amato E, D'Auria M, Gori M, Huedo P, Bossi A, Pontello M. Estimating the real incidence of invasive listeriosis through an integrated surveillance model in use in Lombardy (Italy, 2006-2014). Epidemiol Infect 2017;145:2072-80. https://doi.org/10.1017/S0950268817000711

[13] Filipello V, Amato E, Gori M, Huedo P, Ciceri G, Lomonaco S, Pontello M. Epidemiology and molecular typing of pregnancy-associated listeriosis cases in Lombardy, Italy, over a 10-year period (2005-2014). Infect Dis Obstet Gynecol 2017;2017:6479121. https://doi.org/10.1155/2017/6479121

[14] Amato E, Filipello V, Gori M, Lomonaco S, Losio MN, Parisi A, Huedo P, Knabel SJ, Pontello M. Identification of a major Listeria monocytogenes outbreak clone linked to soft cheese in Northern Italy - 2009-2011. BMC Infect Dis 2017;17:342. https://doi.org/10.1186/s12879-017-2441-6

[15] Mook P, Grant KA, Little CL, Kafatos G, Gillespie IA. Emergence of pregnancy-related listeriosis amongst ethnic minorities in England and Wales. Euro Surveill 2010;15:17-23. https://doi.org/10.2807/ese.15.27.19610-en

[16] Gillespie IA, Mook P, Little CL, Grant KA, McLauchlin J. Human listeriosis in England, 2001-2007: association with neighbourhood deprivation. Euro Surveillance 2010;15:7-16. https://doi.org/10.2807/ese.15.27.19609-en

[17] The Integrated Rapid Infectious Disease Analysis (IRIDA) Advanced Research Infrastructure for Experimentation in GenomicS (ARIES) Platform. Available at: https://irida.iss.it (accessed on: 22/12/2019).

Received on January 17, 2020. Accepted on February 27, 2020.

Correspondence: Maria Gori, Department of Biomedical Sciences for Health, University of Milan, via Carlo Pascal 36, 20133 Milan, Italy. Tel. +39 02 40222471 - + 39 02 50315124 - E-mail: mgorimaria@gmail.com

How to cite this article: Gori M, Ciceri G, Bianchi S, Cereda D, Senatore S, Gramegna M, Amendola A, Pontello M, Tanzi E. Laboratory-based surveillance of invasive listeriosis in Northern Italy over a fourteen-year period: epidemiological and clinical results. J Prev Med Hyg 2020;61:E167-E172. https://doi.org/10.15167/2421-4248/jpmh2020.61.2.1473