

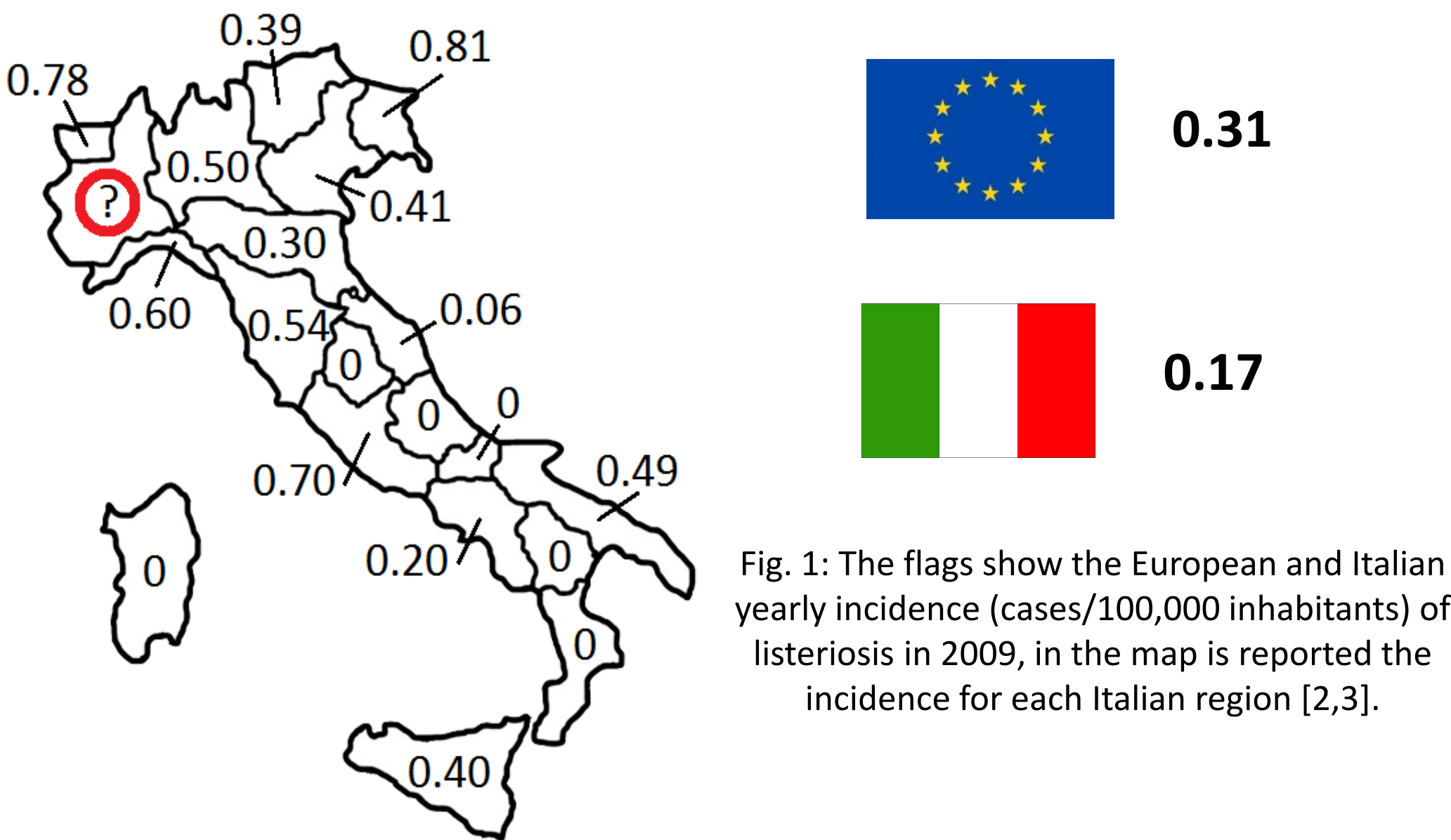
# Human Listeriosis in Piedmont, Northern Italy (2010-2013): an insight on surveillance efficacy and strain diffusion.

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### Objectives

Listeriosis is a notifiable foodborne disease, with high hospitalization (up to 90%) and fatality rates (20-50%) [1]. Still, due to unspecific symptoms and long incubation time, the epidemiology of this disease is often unclear. In 2011, the incidence of listeriosis was 0.31 cases/100.000 inhabitants in Europe, and 0.17 cases/100.000 in Italy, with some Italian regions regularly reporting no cases and others with an incidence of 0.88 cases/100.000 [2, 3]. Our aim was to assess whether the number of reported listeriosis cases in Piedmont is accurate and to type the isolates of human food and animal origin retrieved in the same area through genomic subtyping.



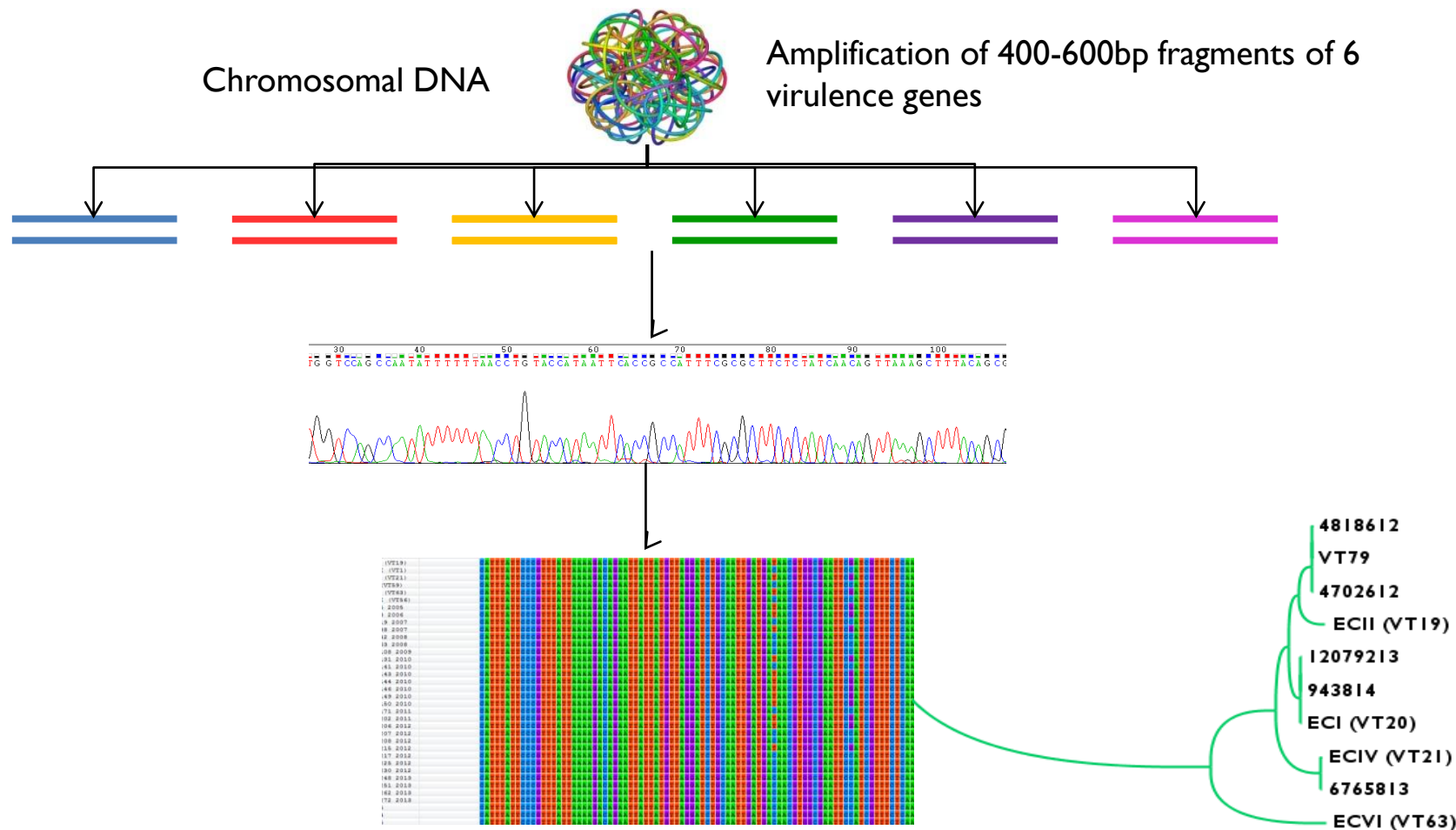
### Materials and Methods

#### Retrospective database analysis

Notification rate was assessed by comparing the regional notification database with Hospital Dismissal Records (HDRs) listing listeriosis as diagnosis, for the period 2010-2014. The two databases were merged using R software [4], using date of birth and city of residence as identifiers for the same clinical case. Multiple hospitalizations of the same individual in a time span shorter than three months were considered as single listeriosis cases.

#### Strain collection and typing

Hospitals were asked to send *Listeria monocytogenes* clinical isolates for characterization with Multi-Virulence-Locus-Sequence-Typing (MVLST). MVLST results were compared with reference database and a neighbor-joining tree based on the number of nucleotide differences in the gene fragments analyzed was constructed using MEGA version 6 [5].



### Results

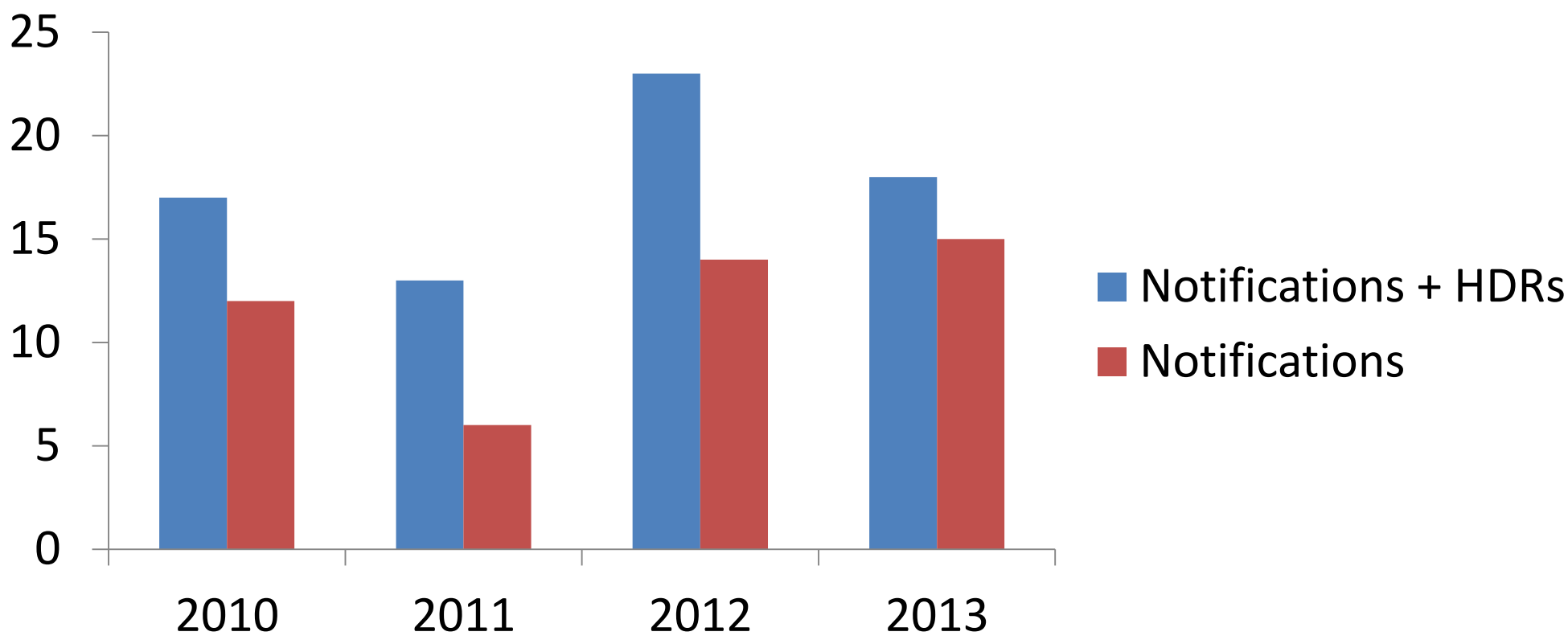
#### Retrospective database analysis

Overall, 62% of total listeriosis cases were observed in patients above 65 years of age, 2% of cases were pregnancy related. Our analysis showed that on average each year 35% of listeriosis cases are not notified, with a total of 28 cases not notified over the time span considered (Fig. 2). When only the notification data were considered, the observed yearly incidence in Piedmont ranged from 0.13-0.34 cases/100.000 inhabitants. This rate was adjusted to 0.29-0.53 cases/100.000 inhabitants when HDRs data were considered (Tab. 1).

#### Strain collection and typing

To date, 19 clinical strains of *L. monocytogenes* have been collected. MVLST (Fig. 3) revealed that 42% of the strains (n=8) had a genetic profile (Virulence Type - VT) matching that of an Epidemic Clone (EC), previously defined as strains or group of strains responsible for at least two listeriosis outbreaks not related in space and time [6]. Four cases belonged to VT79 (n=2) and VT80 (n=2), VTs previously identified as outbreak clones (OCs) during the 2012 ricotta salata outbreak [7]. Two isolates have been identified as VT11, often found worldwide in food and food processing environment [7]. One isolate belonged to VT113, previously observed in several cheese production plants in Piedmont [7]. Finally, one isolate did not match any previously observed VT and therefore was arbitrarily assigned a new one (VT121) (Fig. 3).

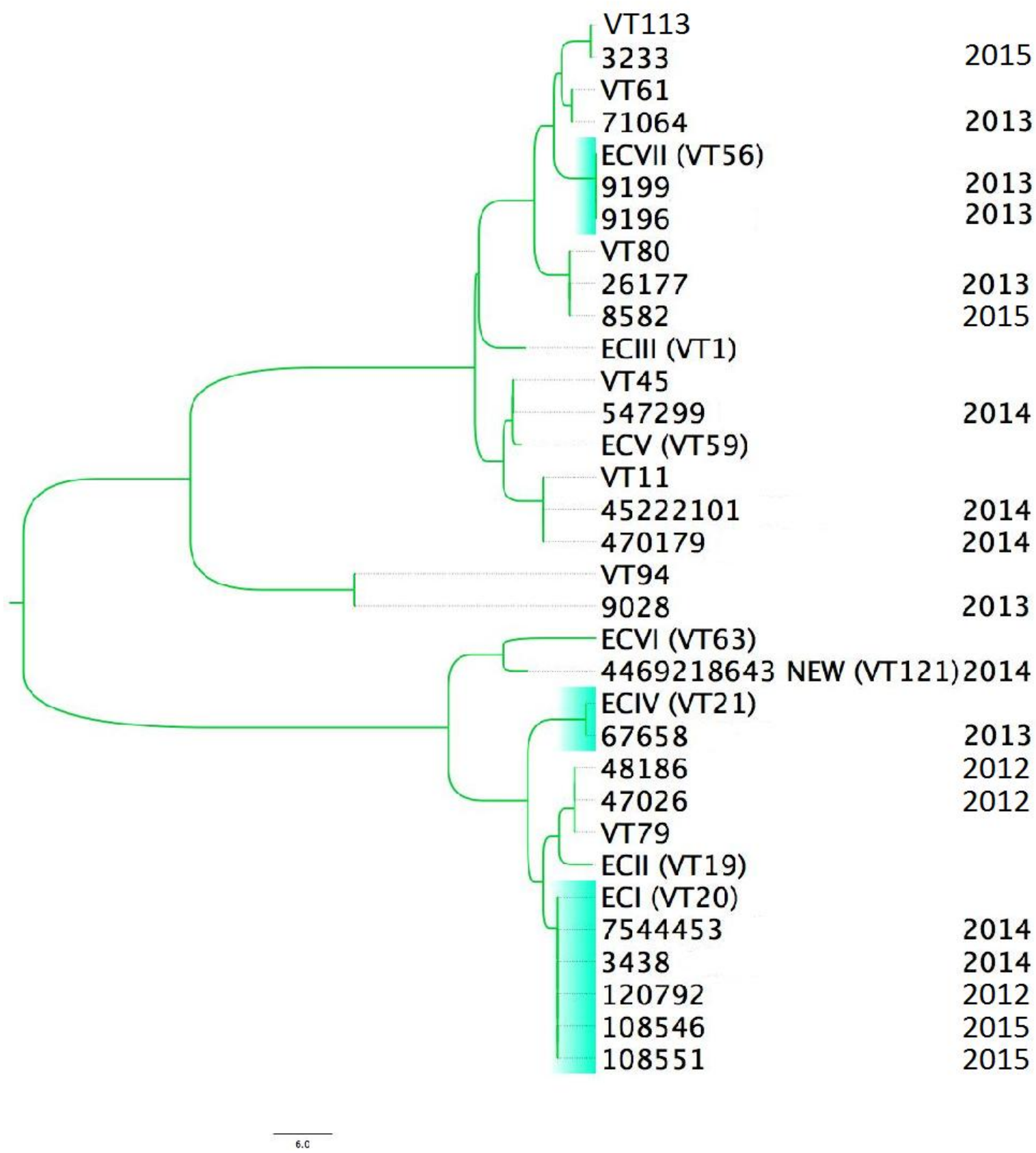
Fig. 2: Yearly count of listeriosis cases notified in Piedmont (red) and sum of notification and Hospital Dismissal Records (HDRs) (blue).



Tab. 1: Yearly and average incidence (cases/100,000 inhabitants) of listeriosis in Piedmont, calculated on notification data (a) and adjusted when taking in account data from the Hospital Dismissal Records (b).

Year	Incidence reported through notifications <sup>a</sup>	Incidence adjusted with HDRs <sup>b</sup>	% of cases not notified
2010	0,27	0,38	29,41
2011	0,13	0,29	53,85
2012	0,32	0,53	39,13
2013	0,34	0,41	16,67
Average	0,27	0,40	34,76

Fig. 3: Unrooted neighbor-joining tree for MVLST data obtained for the 19 *Listeria monocytogenes* isolates collected in this study, and year of isolation. Green highlighting indicates strains classified as epidemic clones.



### Conclusions

Currently there is no systematic clinical *Listeria* strain collection in Piedmont and thus, the collection of even relatively few human isolates is important to start implementing listeriosis surveillance strategies. Overall, the percentage of pregnancy related cases is lower compared to other countries (reporting 10-20%) [2], probably because causes of miscarriage are seldom investigated. Moreover, 35% of human listeriosis cases diagnosed in Piedmont every year are not notified, highlighting the need to raise awareness of notification procedures among medical staff. Stressing the importance of collecting and typing clinical strains, starting at regional level, might be an efficient way to reach this goal.

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### Further information

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