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Development of a European molecular typing database for food, environmental and veterinary *Listeria monocytogenes* strains

Benjamin Félix[1*] (benjamin.felix@ANSES.fr), Corinne Danan[1], Pia Makela[2], Ivo Van Walle[3], Renaud Lailler[1], Thomas Texier[4], Bertrand Lombard[1],
Anne Brisabois[1], Sophie Roussel[1]

1. University Paris-Est, ANSES, Laboratory for Food Safety, European Union Reference Laboratory for *Listeria monocytogenes*, Maisons-Alfort, France.
2. EFSA, Unit of the Biological Monitoring Unit, EFSA, European Food Safety Authority, Via Carlo Magno 1A43126 Parma, Italy.
3. ECDC, European Centre for Disease Prevention and Control, Food- and Waterborne Diseases and Zoonoses Programme, Tomtebodavägen 11 A, SE-17183 Stockholm, Sweden
4. University Paris-Est, ANSES, Information Technology Services, Maisons-Alfort, France

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Abstract

The European Union Reference Laboratory (EURL) for *Listeria monocytogenes* (*Lm*) is collaborating with a network of 35 European National Reference Laboratories (NRLs). These NRLs are responsible for typing *Lm* strains of food, feed and animals. The consolidation of NRL capacity for standardised typing resulted in the recent creation of a centralized molecular database. Data (typing results and epidemiologic information) are provided by each NRL and shared within the network. This database, together with databases on human strains, is to become part of the European surveillance system in order to improve the traceability of *Lm* strains circulating throughout Europe.

Introduction

In several European countries, after a long decline of human cases, the incidence of listeriosis has increased over the last decade (EFSA-ECDC, 2010; EFSA-ECDC, 2012; Goulet *et al,* 2008). Listeriosis, a consequence of the ingestion of *Listeria monocytogenes* (*Lm*), was reported in 26 European Union (EU) Member States (MSs) in 2012 (EFSA-ECDC, 2012).

Molecular typing of food-borne bacteria is an essential tool for various surveillance purposes, such as (1) monitoring the spread of clones and strains, (2) providing an essential tool for epidemiological investigations and early detection of scattered national or international outbreaks, and (3) predicting epidemic potential. For *Lm* typing, pulsed-field gel electrophoresis (PFGE) remains currently the "Gold Standard" method. Finding a PFGE profile of a strain isolated in food that matches a human strain profile does not necessary imply that this food is the source of the contamination. It could simply reflect circulation of this particular strain. Nevertheless detecting a human strain profile in food should improve the rapidity and precision of outbreak detection. PFGE profiles combined with epidemiological data must be considered together to conclude on case attribution. The goal is to collect as much scientific evidence as possible, to help decision makers to withdraw or to recall a product from the market.

In recent years, interest in developing a European surveillance network for listeriosis has led to enhanced surveillance activities in several countries and has generally heightened awareness of the public health importance of *Lm*. The surveillance network PulseNet Europe ceased to be active in 2006 by lack of funding (Swaminathan *et al,* 2006). However, efficient networks have been set up since and they cooperate closely to improve information exchange and molecular testing.

The ANSES Maisons-Alfort Laboratory for Food Safety has been designated European Union Reference Laboratory (EURL) for *Lm* (**http://www.ANSESpro.fr/eurl-listeria/**) by the Directorate-General for Heath and Consumers (DG Sanco) of the European Commission. It coordinates a network of 35 National Reference Laboratories (NRLs) in 29 Member States (MSs) and Norway. The majority of these NRLs are responsible for typing *Lm* strains of food, environmental and veterinary origin isolated nationally. Out of 35 NRLs, 6 NRLs are in charge of typing of clinical strains.

The European Food Safety Authority (EFSA) collects information from the Member States on food and animals as well as food-borne outbreaks on an annual basis. For this purpose, EFSA has created a Task Force on Zoonoses Data Collection with participants from all Member States, EEA countries, Switzerland, DG SANCO, and ECDC. For this data collection, EFSA runs a web-based reporting application as well as a more automated Data Collection Framework. The European Center for Disease Prevention and Control (ECDC) coordinates a network of national public health surveillance institutes and national public health laboratory (NPHLs), in particular responsible for typing *Lm* strains isolated from national clinical cases (ECDC). ECDC has also developed The European Surveillance System (TESSy) (van Walle, 2013) molecular surveillance database in the objective to be used to timely share molecular epidemiologic information and PFGE data from strains isolated from human cases and quickly recognize outbreaks at the European scale. At the European level, other than TESSy, there was no molecular database for centralizing and sharing molecular data obtained from food strains. For this reason, the EURL has recently set up a database for *Lm* that includes typing results as well as epidemiological information related to strains isolated from food, environmental or animal samples. This database, known as the "EURL *Lm* Database" (EURL *Lm* DB), is shared within the NRL network. Its objective is to compile data sets as complete as possible on *Lm* typing and epidemiological data to document the European food chain. This article describes in a first part the

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EURL typing activities which have led to the setting up of the EURL *Lm* DB, in a second part the different steps involved in developing the EURL *Lm* DB and lastly addresses an example of the use of this database.

Consolidation of NRL capabilities for standardized *Lm* typing

The EURL has developed NRLs standardized serotyping and PFGE methods for *Lm* that have been shared with all collaborating NRLs (Félix *et al,* 2012b). The EURL PFGE method was recently compared with the PulseNet USA method. The results were basically indistinguishable for both methods (Félix, personal communication). The EURL has developed a PFGE profile interpretation standard operating procedure (SOP) (Félix *et al,* 2012a) which reduces operator-dependent subjectivity. The EURL has also organized regular typing training sessions, annual workshops, and three typing proficiency testing (PT) trials (Félix *et al,* 2012b; Félix *et al,* 2013) for the NRLs over the past six years. The recent PT trial organized by the EURL and the ECDC focused on PFGE analysis and profiles interpretation according to the PFGE profile interpretation SOP (Félix *et al,* 2012a). Out of 28 participating laboratories, 16 including 10 NRLs and 6 NPHLs were considered as competent for PFGE typing of *Lm*. This PT trial provided a valuable opportunity to facilitate and to stimulate exchanges of reproducible PFGE profiles between human and food reference laboratories (Félix *et al,* 2013). All these activities (1) stimulate NRLs for typing (2) reinforce and consolidate NRL typing capabilities (3) harmonize *Lm* typing methods throughout Europe.

Development of the EURL *Lm* DB

Technical support

The EURL *Lm* DB is hosted by the EURL. A network management platform dedicated to molecular *Lm* typing data exchange was implemented (**https://moleculartyping-db.ANSES.fr/EUListNet**). Web networking is managed by a web server (BioNumerics (BN) Server Web Edition, version 6.1, Applied Maths, Sint-Martens-Latem, Belgium). The network is based on machine-to-machine communication over the Internet, allowing NRL databases to be shared with the EURL *Lm* DB. The EURL *Lm* DB was developed using modified PulseNet USA communication scripts and protocols with the agreement of the U.S. Center for Disease Control and Prevention (CDC). Modifications include use of the latest Web Edition of the BN Server, facilitating data exchange through Internet-based protocols. The various functionalities included in the database are described in **Figure 1**.

Organization

The EURL *Lm* DB steering committee (SCOM) comprise an equal number of representatives from eight participating NRLs and from the ECDC, EFSA and the EURL (administrator and curator of the EURL *Lm* DB).

The registration number of each strain is randomly generated by the BN server at submission (unique identification (UID) code of 33 alphabetic characters) and used as the central database identifier. The strain identification is given by two other information fields. The first field gives the identity of the submitting laboratory as XXYY. The code is composed of two characters (XX) given the national identity of the submitting NRL (e.g. IT is the ISO 3166-1-alpha-2 code used for Italy), the next two characters (YY) represent the submitting laboratory's national number (e.g. 01 would be the code for the first Italian laboratory subscribing to the EURL *Lm* DB project). The second information field is the initial strain number given by the submitting laboratory. Only the random anonymous key would be available to users in order to keep data owner identity anonymous.

Pulsotype nomenclature is defined according to the PulseNet USA pulsotype format (Gerner-Smidt *et al,* 2006) : pulsotypes are labeled with the "EU" tag, e.g. for a AscI profile: GX6A16.0001. EU, GX6 meaning *Lm*, A16 meaning the restriction enzyme AscI, 0001 the pulsotype number and EU the European tag. Each pulsotype is associated to information on its frequency within the whole database.

Epidemiological classification

The epidemiological data are recorded in accordance with a detailed epidemiological classification (**Figure 2**) which consists of several consecutive fields associated with predefined pick lists in the software. The epidemiologic classification structure was based on the dataset required in the EFSA epidemiological reporting system (EFSA, 2012). However, to simplify reporting, epidemiological information in the EURL *Lm* DB is restricted to food classification typically used in *Lm* risk assessments.

Data management

The EURL is the curator and administrator of the EURL *Lm* DB. The administrator manages the participant connections (logins and passwords), communication scripts and is responsible for EURL *Lm* DB maintenance (**Figure 1**).

The curator is in charge of validating each new profile submitted. The curator can directly modify the gel image processing parameters, and the profile. Each profile is analysed and identified according to the EURL PFGE profile interpretation method using an identification group-based SOP. The technical skills of the curator for PFGE gel interpretation are regularly updated in an internal assessment process that establishes the test for curator qualification. After curation, the curator designates profiles as either "confirmed" or "unsatisfactory".

Any change made by the curator is traced in the EURL *Lm* DB and visible to the user. These changes are automatically implemented in the local NRL database. The update process is automatic and can be activated by the NRL at any time. Script functionality allows the NRL to generate a report on its own database listing all the changes made by the curator on a given profile.

Participants

Criteria for joining the EURL *Lm* DB include (1) the successful participation in the most recent EURL PFGE and PFGE profile interpretation PT trials and (2) the availability of PFGE analysis software (BioNumerics version 6.6 or higher) equipped with a specific BN server communication script.

Participants must comply with the memorandum of understanding (MoU) that regulates EURL *Lm* DB use. The first item of the MoU stipulates that, by submitting data, all participants accept disclosure of the data to the other participating NRLs and the EURL. It also specifies that NRLs take part in the database on a voluntary basis and that they are responsible for the content of the data submitted. The NRLs retain ownership of their data and are free to publish their own PFGE profiles even if they have been submitted to the EURL *Lm* DB. The MoU also establishes that disclosure of EURL *Lm* DB data by the NRLs or the EURL requires a joint written

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agreement between partners. EURL *Lm* DB citations are only permitted if they do not disclose any specific data other than those owned by the disclosing party. The MoU also establishes the information routes in case of EURL *Lm* DB solicitation by DG SANCO, MSs or external bodies. Finally, the MoU specifies that NRLs complying with the MoU can consult the EURL *Lm* DB without restriction.

The NRLs are trained to the use of the EURL *Lm* DB through individual training sessions, scheduled at the EURL, on site, by phone, or by video. Moreover, individual technical follow-up and technical assistance by the EURL are available for each NRL.

Data consultation and submission (Figures 1 and 2)

Submission of data to the central database

These submitted data included PFGE profiles, serotype (molecular or conventional serotype) data and epidemiological information (see Figure 2). For PFGE data, participants have to submit *ApaI* and *AscI* profiles simultaneously.

Consultation of data of the central database

Users can consult the EURL *Lm* DB in two ways: (1) by matching their own profile against existing entries in the central database or (2) by consulting the database according to queries from epidemiological and typing data. In both cases, profiles obtained following database consultation may be downloaded from the EURL *Lm* DB to the user's local database. These downloaded profiles are available as profiles of the local database for statistical analysis and comparison until the active session of the consultation software is closed.

Benefits of the EURL *Lm* DB use by the NRLs

The NRLs can use the EURL *Lm* DB to interpret a PFGE profile in their own database. All PFGE profiles available in the EUR *Lm* DB can be compared with local NRL profiles. The EURL *Lm* DB can thus be considered as a routine assessment tool for NRLs. The use of EURL *Lm* DB facilitates the harmonisation of the PFGE profiles in the local NRL database.

For a given PFGE profile, the NRL can access following information available in the EURL *Lm* DB: (1) serotype, (2) food matrix, (3) sampling date and (4) profile frequency in the EURL *Lm* DB. The use of the EURL *Lm* DB thus enables NRLs to collect information useful for epidemiological investigation in case of an outbreak.

The database also could accept data resulting from alternative typing techniques such as multi-locus sequence typing (MLST), multi-locus variable number tandem repeats analysis (MLVA), and whole genome sequencing data. Data generated using these methods can be compared with PFGE and serotyping data to determine their congruity and the structure of profile groups.

Example of use of the EURL *Lm* DB

The EFSA set up a monitoring program (baseline survey) on the prevalence of *Lm* in selected categories of ready-to-eat (RTE) foods (Decision 2010/678/EU) in 2010-2011 in EU Member States (MSs). This survey should allow the comparison of *Lm* contamination in ready-to-eat food in the Community and Member States and the verification of the Community food safety criteria for *Lm*. The ECDC and the EURL *Lm*, in collaboration with EFSA and with approval of the European Commission and the EU MSs, have launched a joint project to compare the PFGE profiles of food isolates from the coordinated European monitoring programme with strains from human listeriosis cases isolated during the same period. At the ECDC, the European *Listeria* Typing Exercise (ELiTE) project focuses on PFGE typing of human *Listeria* strains that have been isolated and stored by NPHLs in 2010-2011. The EURL *Lm* coordinates PFGE typing of the strains collected by NRLs and isolated from certain RTE food categories sold at retail stage in EU MSs in 2010-201. NRLs, trained by the EURL and having demonstrated their competence through participation in inter-laboratory proficiency trials organised by the EURL, type these strains isolated on the national level using serotyping and PFGE methods. Likewise, the EURL types strains from other countries. Human and food typing data are compared to allow a better estimation of the importance of certain foods as sources of human listeriosis.

As part of the European baseline survey, nine NRLs currently use the EURL *Lm* DB to submit and share the PFGE profiles of strains isolated in certain RTE foods at the national level. The EURL also contributes the typing and epidemiological data on strains sent by NRLs of 16 other countries to the EURL *Lm* DB. The close collaboration between the ECDC and the EURL will foster the exchange and comparison of typing data, as part of the ECDC's ELiTE project. The SOP and PFGE profile nomenclature used for the ELiTE project and the TESSy pilot study are currently being discussed by the ECDC, the EURL for *Lm* and EFSA, taking into account those used for the EURL *Lm* DB.

Conclusion - Prospects

The use of the EURL *Lm* DB should encourage individual countries to strengthen national surveillance of *Lm* infections, by facilitating the implementation and wide use of the typing national databases in each country. The functionalities of EURL *Lm* DB associated with curation work make it easier to harmonise PFGE profiles and the epidemiological data circulating within the NRL/EURL database network.

It is expected that the EURL *Lm* DB will be used together with databases on human strains and by microbiologists and epidemiologists. Used in combination with collaborative epidemiological investigations, the EURL *Lm* DB should improve the surveillance of *Lm* in the food chain by (1) enhancing the detection of European, eventually transboundary, contamination clusters, (2) optimising the detection of emerging *Lm* strains potentially pathogenic for consumers, (3) facilitating communication between NRLs, the EURL, EFSA and the ECDC and (4) suggesting links with potential sources of contamination.

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References

ECDC http://ecdc.europa.eu/en/activities/surveillance/strategies_principles/Pages/case_definitions.aspx

EFSA-ECDC (2010) The Community Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. *EFSA Journal* 2010 **8(1):1496**: 137-165

EFSA-ECDC (2012) The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010. *EFSA Journal* 2012;**10(3):2597**: 133-160

EFSA. (2012) Manual for reporting of food-borne outbreaks in accordance with Directive 2003/99/EC from the year 2011.

Félix B, Brisabois A, Dao TT, Lombard B, Asséré A, Roussel S (2012a) The use of Pulsed Field Gel Electrophoresis in *Listeria monocytogenes* sub-typing : harmonization at the European Union level. In *Gel Electrophoresis : Principles and Basics*, INTECH (ed), Vol. 1, Dr. Sameh Magdeldin edn, 14, pp 241-254. Niigata University, Japan

Félix B, Dao TT, Grout J, Lombard B, Assere A, Brisabois A, Roussel S (2012b) Pulsed-Field Gel Electrophoresis, Conventional, and Molecular Serotyping of *Listeria monocytogenes* from Food Proficiency Testing Trials Toward an Harmonization of Subtyping at European Level. *Foodborne Pathog Dis* **9**: 719-726

Félix B, Vingadassalon N, Dao TT, Asséré A, Lombard B, Brisabois A, Roussel S (2013) PFGE Proficiency Testing Trials: toward an harmonization of typing of *Listeria monocytogenes* food and clinical strains at European Level. *Foodborne Pathog Dis* In press

Gerner-Smidt P, Hise K, Kincaid J, Hunter S, Rolando S, Hyytia-Trees E, Ribot EM, Swaminathan B (2006) PulseNet USA: a five-year update. *Foodborne Pathog Dis* **3**: 9-19

Goulet V, Hedberg C, Le Monnier A, de Valk H (2008) Increasing incidence of listeriosis in France and other European countries. *Emerg Infect Dis* **14**: 734-740

Graves LM, Swaminathan B (2001) PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. *Int J Food Microbiol* **65**: 55-62

Swaminathan B, Gerner-Smidt P, Ng LK, Lukinmaa S, Kam KM, Rolando S, Gutierrez EP, Binsztein N (2006) Building PulseNet International: an interconnected system of laboratory networks to facilitate timely public health recognition and response to foodborne disease outbreaks and emerging foodborne diseases. *Foodborne Pathog Dis* **3**: 36-50

van Walle I (2013) ECDC starts pilot phase for collection of molecular typing data. *Euro Surveill* **18 (3)**

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Figure 1. Schematic representation of the EURL *Lm* DB data processing for administrator side (red), database automatic consultation (clear blue) and data submission (deep blue). Black circles symbolize when processes start and circles with dot when processes end up. The black circle marked with A shows the way for requesting the database based on molecular profiles, the black circle marked with B shows the way for requesting the database based on epidemiological information including serotyping data.

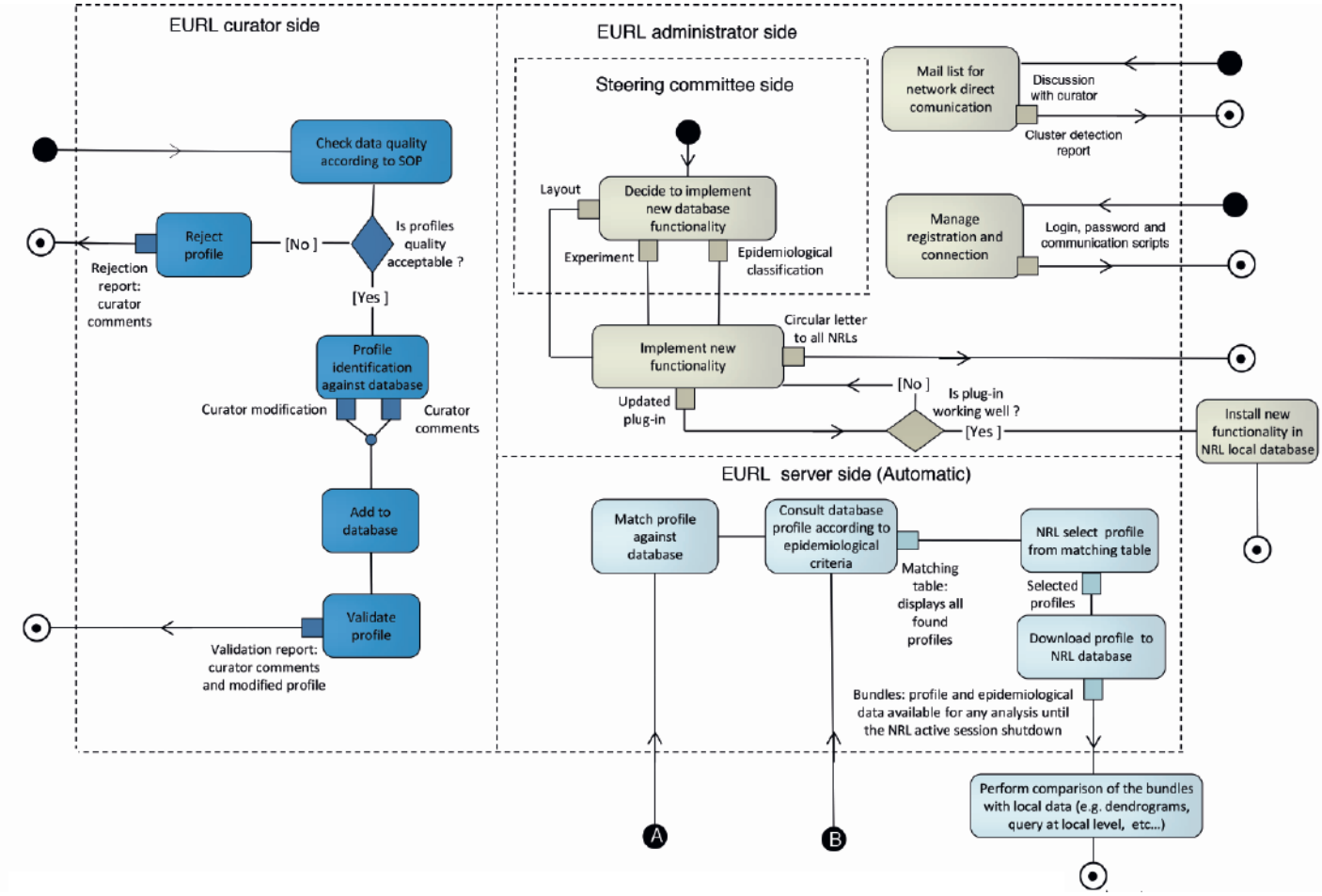

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Figure 2. Description of the samples submitted to the EURL *Lm* DB, according to seven main information blocks (A, B, C, D, E, F, G, H, I). Block A describes the sample (Food, Feed, Animal or Environment) and is further subdivided into subcategories describing where and how the sample was taken. Block B describes the food matrix, with first eight large categories of food products (Meat and meat products, Fish products, Elaborated food products combining several food categories or other food products, etc.) and further, specific information describing in detail the product and its processing. Block C describes the type of food sources which compose the product. Block D describes the level in the food chain where the sample was taken (from Farm to Retail or Borders). Block E describes the context of sampling, block H the date and block G the geographic information. Block F is composed of molecular or conventional serotyping data. Bloc I is an open field for the reporting of additional information.