

## A molecular *Listeria monocytogenes* database to centralize and share typing data

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*Listeria monocytogenes* (Lm) is an ubiquitous bacterium that causes a severe illness. The listeriosis is contracted through contaminated food consumption with a case fatality rate of 20-30%. The incidence is higher for risk groups having a weak immunity and lower for healthy people. The infection depends on the dose and the genetic group of the strain ingested. Lm needs to be extensively surveyed in food production. This is the reason why a molecular surveillance is applied. For 4 years, Ifip and Anses have been working together in the frame of the Armada joint technological unit, a national collaborative project. It enabled the two partners to harmonize their typing method according to standard operating procedures for PFGE. The need for exchanging typing data recently resulted in the creation of a joint national molecular typing database. Its objective is to share epidemiological and genetic data related to strains owned by both Anses and Ifip. Ultimately, this database will be shared between four other French technical institutes and Anses units involved in the national surveillance of Lm. This database contains 1200 strains all typed by PFGE, sharing 256 combined Apal-Ascl PFGE pulsotypes. This key tool may enable an improved surveillance of strains circulating throughout the pig and pork sector.

### Une base de données de typage partagée pour la surveillance de *Listeria monocytogenes*

*Listeria monocytogenes* (Lm) est une bactérie ubiquitaire responsable d'une infection rare mais grave : la listériose. Transmise par la consommation d'aliments contaminés, la listériose s'avère mortelle dans 20 à 30 % des cas. Elle touche principalement les personnes faibles immunitairement et plus rarement des personnes en bonne santé. L'infection dépend de la dose et de la virulence du groupe génétique de la souche ingérée. De ce fait, la surveillance génétique des souches isolées de la chaîne alimentaire et de l'environnement de production est essentielle. Dans le cadre de l'Unité mixte technologique (UMT) Armada, l'Ifip et l'Anses ont travaillé depuis 4 ans à l'harmonisation de leurs protocoles de typage PFGE. Le besoin d'échanger leurs données de typage a abouti à la création d'une base de données nationale de typage partagée. L'objectif de cette base est de mettre en commun les données épidémiologiques et génétiques des souches détenues par les deux instituts. A terme, elle sera partagée entre quatre instituts techniques français ainsi que les laboratoires de l'Anses impliqués dans la surveillance de Lm. Elle contient actuellement 1200 souches typées par PFGE, partageant 256 profils combinés Apal/Ascl. Cet outil permettra une surveillance accrue des souches circulant dans la filière porcine.

**Keywords:** *Listeria monocytogenes*, molecular database, surveillance, pig and pork sector

**Mots clés :** *Listeria monocytogenes*, base de données de typage, surveillance, filière porcine

## Introduction

*Listeria monocytogenes* (*Lm*) is an ubiquitous saprophytic bacterium known to cause the foodborne disease listeriosis in humans. Listeriosis is a rare (5.7 cases per million population in 2014; InVS) but potentially deadly infection. It predominantly affects people with weakened immune systems (older adults, immunocompromised people, or pregnant women) although in rarer cases it can also affect otherwise healthy people. Infectiveness depends on dose ingested and the virulence of the ingested strain.

Food contamination can occur via animal or plant raw materials but the main route is via the production-site environment in which *Lm* strains manage to survive, persist and replicate.

The pork industry has been hit hard by a series of *Lm*-related food poisoning outbreaks over the past few decades (Tourdjman *et al.*, 2014). It has been demonstrated that the production-site contamination and the persistence of certain strains in the processing-plant environment are often the root cause of this public health problem. To help safeguard consumer health, EU regulations (EC No 2073/2005) set microbiological safety as an absence of *Lm* in 25 g food samples for all ready-to-eat food commodities, except those for which the food business operator has demonstrated that bacterial growth is kept below 100 CFU/g throughout the shelf-life up until the use-by date. Under EC No 2073/2005, food business operators are expected to implement drastic microbiological testing and food safety control plans and, in some cases, food recalls from the marketplace.

In this context, molecular typing methods can help (i) identify the sources of contamination at each stage in the food chain and (ii) define the genetic lineage of the strains isolated. For *Lm* surveillance in France, as in surveillance networks worldwide, pulsed-field gel electrophoresis (PFGE) is still the gold standard molecular typing method for clinical and food strain isolates (Tourdjman *et al.* 2014).

The “*Listeria*” team at the SEL (*Salmonella*–*E.coli*–*Listeria*) Unit of the Anses Food Safety Laboratory (FSL) in Maisons-Alfort is one of several support units for European Union Reference Laboratory (EU-RL) and National Reference Laboratory (NRL) activities on *Lm*. Ifip—the French pig and pork industry agency—has been handling *Lm* surveillance on pig farms through to processed products for over 15 years now.

Under the Joint Research Unit (UMT) framework “UMT–Armada”, the Ifip and the Anses-run SEL Unit have set up a multi-sector molecular database (DB) containing both in-depth epidemiological information and typing data (PFGE, MLST, serotypes) on strains isolated from processing-plant environments, raw food materials and finished-product foods in France over the last decade.

This DB is designed to serve as a platform for exchanging and pooling data from different food industry sectors. Building out from this shared DB, we have developed a pork sector specific subbase to help support *Lm* surveillance across the pork industry and gain deeper insight into the genetic diversity of *Lm* strain isolates.

## Materials and Methods

### Background to isolation of strains

Anses-SEL receives non-human *Lm* strains (i) voluntarily sent in by departmental reference laboratories and food-feed industry analysis laboratories, (ii) forwarded on under surveillance programmes and self-monitoring programmes implemented by the DGAL (French Directorate General for Food), (iii) isolated for food recall and outbreak warning investigations, in its role as NRL, and (iv) submitted under research projects. The Ifip collects the *Lm* strains isolated via self-monitoring by food-operators (dry-cured, cold-prepared and processed meat products) or submitted under research projects in its role as pig and pork industry agency.

### Characterization of strains

When either Anses or Ifip lead public health surveillance work, the *Lm* strains received are put through exactly the same characterization process: the strains are first serotyped and classed into serovars IIa (1/2a, 3a), IIb (1/2b, 3b, 7), IIc (1/2c, 3c), IVb (4b, 4ab, 4d, 4e) or IVa (4a, 4c) (Doumith *et al.*, 2004, Kerouanton *et al.* 2010), then sub-typed by PFGE (to get the molecular profile fingerprint). Under the UMT–Armada framework, the Ifip and Anses harmonized their PFGE typing and profile interpretation methods to align with standardized protocols (Michelon *et al.* 2015). For each strain, two profiles were generated—one with the *ApaI* enzyme, the other with the *AscI* enzyme—to enable optimal discrimination between isolates.

### The individual databases available

All the epidemiological information and typing data on strains collected by both Ifip and Anses gets independently integrated into their respective parent DBs.

#### The Anses database

The Anses DB counts 3500 non-human isolates, of which 474 were isolated from pork-sector sources. All the isolates have been PFGE-typed and their profiles are centralized in the NRL DB, all managed on the BioNumerics v7.5 software platform (Applied Maths, Belgium). The EU-RL is heavily engaged in the development of the EFSA’s European molecular typing DB (Félix *et al.* 2014). This



strains were submitted from and where they were geographically isolated from, as described previously in Félix *et al.* (2014; 2015). The UMT–Armada DB is networked to the EFSA DB on BioNumerics v7.5 via a user connection (EFSA 2014). The first layer of European-level data curation is then retranscribed down to the national-level layer via a synchronization system cascading the UMT–Armada DB with its user DBs.

There are currently four food–farming industry technical institutes (Ifip, Aerial, Actalia—La-Roche-sur-Foron, and ADRIA Développement) and two Anses laboratories (Anses–SEL and Anses–Ploufragan/HQPAP, the farmed pig and poultry food health and hygiene unit) that can use the UMT–Armada DB.

The UMT–Armada DB pulls together 1602 combined (PFGE-matched) molecular profiles generated with twin *Ascl/ApaI* restriction enzymes, of which 1136 profiles have been validated through the curation process and 319 are in the curation process pipeline. These profiles are regrouped into 538 distinct combined pulsotypes differing by at least one band, as recommended under international standards

(Barrett *et al.* 2006). MLST typing has been completed for 167 strains in 167 different combined pulsotypes and made available to users in two of the DB fields (‘sequence type’ and ‘clonal complex’). The strains are sub-mapped by food-product source: meat (524 strains, including 241 isolates from pork-based products), dairy (189 strains) fish (179 strains), ready-made/multi-category foods (213 strains), plants/vegetables (59 strains), non-food animal and environmental samples (45 strains).

Inside the UMT–Armada DB, we have developed a French pork sector-specific subset that counts around 560 strains isolated from pig farming, slaughter/butchery and processing-plant environments, and finished-product foods. This subset pulls together the combined molecular profiles of strains from three laboratories—Ifip, Anses–SEL and Anses–Ploufragan/HQPAP (Boscher *et al.* 2012; Denis *et al.* 2011).

### Database governance

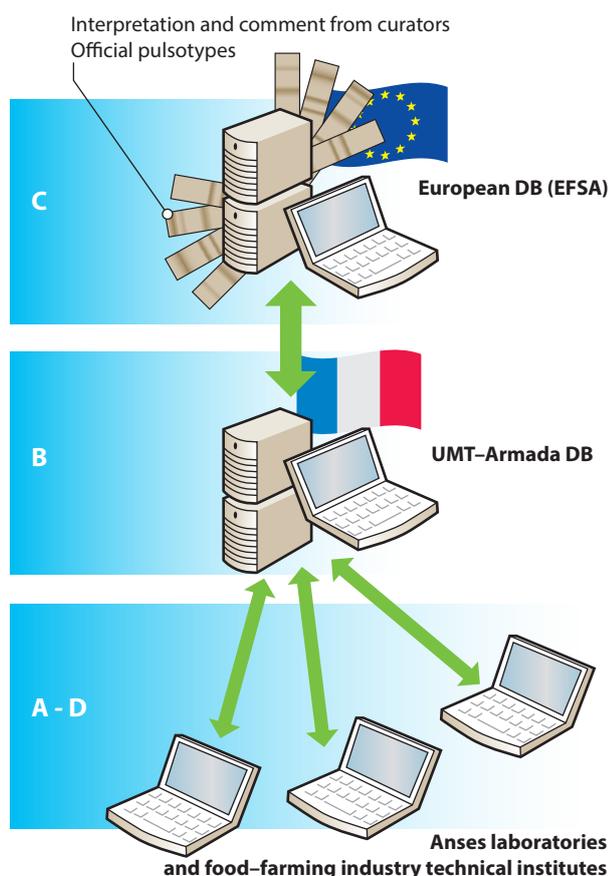
- The UMT–Armada DB has a steering committee, composed of a representative from each lead UMT partner-stakeholder, i.e. Anses, Ifip and Actalia—La-Roche-sur-Foron. Its role is to mandate orientations and issue guidance to users on information exchange and networking, and specifically:
  - to guarantee the robustness of the DB content
  - to oversee new functionality additions to the DB, such as the provision of multi-locus sequence typing (MLST) or whole genome sequence typing data.

### Charter

Molecular profiles are submitted on a purely voluntary basis, and only after first securing agreement between the food operator business from where the strain(s) came and the laboratory that typed the strain. Each user-laboratory must register both PFGE-*ApaI* and PFGE-*Ascl* profiles simultaneously. Both serovar and all requisite strain(s)-related epidemiological data must be completed before the profiles get submitted.

Anses then examines the PFGE profiles submitted and issues a curation-process report stating date of profile submission, date of quality review by the curator, and decision to accept or reject the profile. When a profile gets accepted, it is integrated into the DB and assigned a code number. The user is also updated on the frequency of this isolate pulsotype in the DB. When a profile gets rejected, the curator issues guidance recommendations along with the reason(s) for the decision to reject. The curation-process report is sent out 7 days after the user submitted the profiles.

All data entered, validated and integrated by the curator into the UMT–Armada *Lm* DB is definitively an integral



**Figure 2: Information flows and interfaces — (A) molecular profile submissions, (B) networked data sharing between UMT–Armada DB users, (C) European-level data curation and (D) curation-process feedback to users**

part of the DB and thus, by extension, also becomes part of the EFSA DB.

### User-side possibilities

Users can consult all the typing data and epidemiological information on strains registered into UMT–Armada *Lm* DB even if they do not submit their own PFGE profiles. Users can match their own profiles against existing in-DB profile entries and/or screen in-DB strains for PFGE profiles matching to specific epidemiological criteria (serovar, isolate host-sector, etc.).

Users can also definitively submit any PFGE profiles they want to compare. Registering profiles is key to building up and expanding the UMT–Armada *Lm* DB.

Users are thus given unlimited access to the UMT–Armada *Lm* DB for registering and automatically consulting profiles.

### Data ownership and confidentiality issues

Users register data in the UMT–Armada *Lm* DB on the understanding that there is no transfer of data ownership involved. Users thus retain ownership of their data and are free to publish their own PFGE profiles. Anses may not release the UMT–Armada *Lm* DB to another DB system or any third party without prior unanimous joint agreement from the steering committee members.

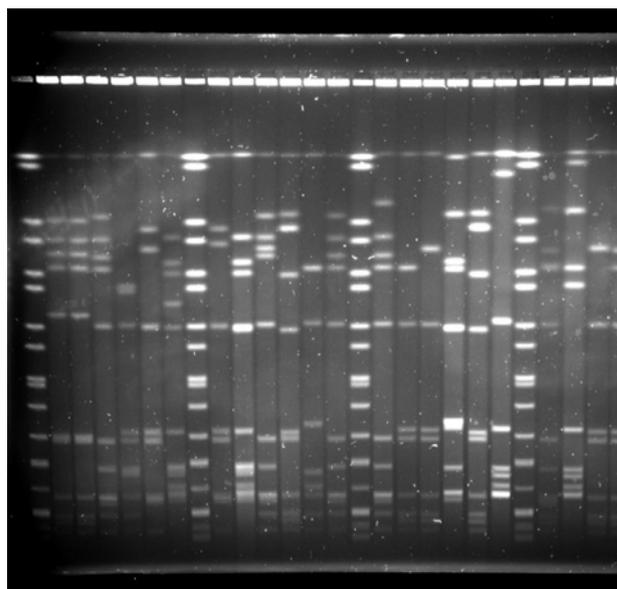
Anses is responsible for securing and maintaining data integrity and confidentiality on all information recorded in the UMT–Armada *Lm* DB, which it does via user access rights management to control information access privileges.

The following item, in particular, are considered sensitive information: laboratory ID, initial strain number, region/department, city and postcode—country of origin is the only mandatory field. All this information is effectively stored in-DB but is only freely consultable by DB users.

The French Directorate General for Food (DGAL) may file to consult this information but only if mandated by law as part of an official government inquiry, in which case it may need access to all the in-DB data and would file a request directly with Anses via the Food Safety Laboratory in Maisons-Alfort. Users who own the relevant data will be notified in advance before any information is handed over.

### Examples of use-case scenarios

The shared DB can be profitably used by a food business operator for *Lm* surveillance in its slaughter/butchery or processing-plant environments. This would give the food business operator a vision on the diversity of the strains in circulation in its plant-facility environments



PFGE-*Ascl* restriction enzyme profiles of *Listeria monocytogenes*

and connect this diversity to that of strains isolated from raw food materials and finished-product foods to better forward/backward trace the sources of meat-cut/finished-food contamination. A food business operator can use the multi-sector DB to visualize the frequency of its strain PFGE profiles across its own activity sector or even in other food industry sectors. If the DB comes up with information for strains presenting the same combined PFGE-*Apal* and PFGE-*Ascl* profiles, then the food business operator can use this information to evaluate the harmfulness of its strains.

The *Lm* DB can also be used to resolve spot contamination incidents— as long as the food business operator is running day-to-day *Lm* surveillance in its facilities, then it will be far quicker to connect the detection of an inline-contaminated food to a contamination source (equipment, raw food material) by cross-matching the typing data in the DB. This would give the food business operator a headstart to swiftly contain and counter any further contamination and, in some cases, to backward trace to the supplier(s) of the contaminated raw material.

The food business operator can also use this same approach to evaluate the effectiveness and efficiency of its facility and equipment cleaning–disinfection procedures against its circulating or persistent strains.

### Conclusion

This shared DB is a key tool for public health surveillance efforts and for bigger and better knowledge on *Lm*.

Its design brief is to help food business operators manage *Lm* risk in food-sector production chains, particularly the pork sector, by enabling deeper understanding of (1) how

strains are vectored between links of the food production chain and (2) how strains gain a foothold in food business operator environments, and by better characterization of the *Lm* strains in circulation.

It can only fully leverage this role if every link of the food production chain (farms, slaughterers/butchers, processors, distribution) takes it aboard and participates heavily. Looking beyond the UMT-Armada framework, which is scheduled to wrap up in late 2016, Anses and the food-farming industry technical institutes will continue to popu-

late and grow this DB. It will thus continue to support the national surveillance network on *Lm* strains circulating up and down the pig and pork industry.

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