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What role do NRLs and NRCs play in disease surveillance?

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The core functions of NRLs and NRCs

In their technical report for 2010 (ECDC, 2010) "Core functions of microbiology reference laboratories for communicable diseases", the authors provide a clear and concise definition for five types of activity, the "core-functions", for National Reference Laboratories and Centres in Europe:

- function 1: Reference diagnostics;
- function 2: Reference material resources;
- function 3: Scientific advice;
- function 4: Collaboration and research;
- function 5: Monitoring, alert and response.

The document is the result of discussions between representatives of National Microbiology Focal Points (NMFPs) in the different countries of the EU. It is designed to encourage cooperation between "experts" and reference laboratories and to serve as a core document for future discussions concerning the European system of reference laboratories. "National Reference Laboratory (NRL)" and "National Reference Centre (NRC)" are both commonly-used terms. However, usage is often country-specific and different interpretations exist. To avoid confusion and to ensure that the report establishes a common reference point, we shall here use the term "microbiology reference laboratory" in this context (see ECDC, 2010).

Here we will discuss *Core Function 5*, which we see as an essential aspect of the work of microbiology reference laboratories: monitoring, alert and response. These are the activities on which hinge the interactions between a microbiology reference laboratory and the body in charge of epidemiological disease surveillance at national level (or regional level depending on the degree of decentralisation of this responsibility in each country).

The goals of Function 5 can be summarised as follows for a given pathogen:

1 – to measure at specific intervals (yearly, half yearly, monthly, etc.) spatio-temporal changes in the presence and number of identifications of the pathogen and its key characteristics (resistance to antibiotics, antivirals and antiparasitics, new serotypes, etc.);

2 – to alert the public health authorities of any unusual or unexpected event concerning this pathogen: appearance of any new resistance to antibiotics, emergence of a new serotype, shift in serotype, new virulence factor, unusual cluster of cases, etc.;

3 – in the event of an outbreak or a real epidemic or epizootic, to participate actively, in close collaboration with the body responsible for epidemiological surveillance of this disease, in documenting isolates of the implicated pathogens, in order to confirm that outbreak cases have a single aetiology and if necessary to differentiate them from endemic cases, to monitor any possible microbiological changes (for example the acquisition of resistance to antivirals, antibiotics, etc.) and especially to characterise them with sufficient precision to enable the source of the outbreak to be identified with certainty. This last point is especially important in the case of foodborne human illnesses, for which it is essential to identify the source of the outbreak in order to implement the appropriate public health measures. This last aspect, which is particularly important in terms of public health, is in fact intensely operational. It therefore requires a sound working relationship and mutual confidence, often on a daily basis, between the microbiology reference laboratory (or laboratories if several are involved, sometimes reporting to different ministries such as those responsible for health, agriculture, the environment, etc.) and the body responsible for epidemiological surveillance. The participation of microbiology reference laboratories in epidemiological investigations (which in France, for example, is inscribed in the mission of the NRCs) is one way of developing a common approach to this work.

The interactions characterising the relationships between the two types of investigator concerned with epidemiological disease surveillance – microbiologists and epidemiologists – are ***regular*** (for spatio-temporal tendencies, the adoption of new laboratory techniques or epidemiological methods, etc.), ***intense*** (during health emergencies, outbreaks, etc.) and ***organised*** (in order to have a clear view of the role of each participant, particularly during investigations of outbreaks). A sound relationship between these two types of partner with scientific cultural backgrounds that are different but necessarily complementary facilitates and vastly improves the results achieved in terms of public health.

Molecular diagnostics, a challenge to the role of microbiology reference laboratories in the monitoring of the way strains circulate

There can no longer be any doubt about the importance of molecular epidemiology in the activities of microbiology reference laboratories, whether for finding the source of contamination and the incriminated foodstuff in foodborne illnesses or, in animal or human health, for determining the origin of the clone of a pathogen implicated in a nosocomial infection, for finding the source of an emerging viral disease in Europe, or for determining the virulence of a given population of pathogenic bacteria. In all such cases, it is essential for microbiologists to work side-by-side with epidemiologists.

For microbiology reference laboratories, molecular diagnostics, especially if performed as a first-line response, which is the case increasingly often, will become a considerable challenge in the future. For most pathogens, molecular diagnostics seems bound to replace traditional methods involving the culture and isolation of strains of bacteria, viruses and fungi, thus bringing about a considerable change in the range of tools available to us for characterising the phenotype and genotype of pathogen isolates, while also progressively reducing the nature and memory of our collections and limiting the possibilities for retrospective historical analysis. This is important not only on an epidemiological and clinical level but also more fundamentally, especially as it will limit the possibility of studying the evolution of pathogens.

However, this is not particularly new. For several years now we have been faced with pathogenic microorganisms that

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were impossible or difficult to cultivate. The hepatitis viruses are an example of this, and especially hepatitis E virus (HEV). For this latter case, which cannot be cultivated routinely, microbiologists have nonetheless developed a comprehensive system of diagnosis and molecular typing (Baylis, 2011) performed directly on biological samples supplied by clinics (faeces, serum) or even from water samples. Targeted PCR followed by sequencing of the amplified strand enables the virus to be classified in one of four described genotypes, and then subtyped and located in the phylogenetic tree of HEVs. The same type of approach can now be extended to other genera of virus, irrespective of whether they are difficult to cultivate (Kroneman, 2011; Ren, 2013).

Molecular typing has also often been used on bacteria in place of traditional serotyping, which can be long and laborious (Doumith, 2004). Although certain techniques of molecular typing can theoretically be used on bacteria without requiring the traditional bacterial culture phase, at least when a sample is potentially mono-microbial, such as Variable Number Tandem Repeat (VNTR) typing, Single Locus Sequence Typing (SLST), typing the gene of the A protein of *Staphylococcus aureus*, or even Multi-Locus Sequence Typing (MLST), in practice techniques for the molecular typing of bacteria are carried out after traditional cultivation and isolation. This is the case of the most widely used typing techniques such as MLST and VNTR typing, and of course macro-restriction of DNA by pulsed-field gel electrophoresis (PFGE) for which considerable quantities of DNA are necessary. Easier access to whole sequences of bacterial genomes (Whole Genome Sequence, WGS) or viral genomes for molecular epidemiology by Next Generation Sequencing (NGS) will provide information of such quality and quantity, of use to both epidemiologists and physicians specialising in infectious diseases, that we may very well see these techniques becoming mainstream in the not too distant future. The knowledge that Whole Gene Sequencing will bring to the virulome, the "toxome" (the full set of all genes encoding toxins) and the resistome (Wright, 2007) of one or more clinical isolates could be essential for providing the patient with appropriate care, and also for decision-making in matters of public health. In addition, Whole Gene Sequencing, which currently requires DNA obtained from a pure culture, could also be performed, at least theoretically, by Whole Genome Amplification (WGA) based on Multiple Displacement Amplification (MDA) using DNA-polymerase of the phage Phi29 and random primers (Lasken, 2003). WGA kits are already on the market and can be used to obtain between 40 and 50 µg of DNA after reaction from 10 ng of DNA, which is enough from which to obtain a whole sequence. The method has also been adapted to enable the detection and amplification of very small quantities of DNA in pathological samples, such as for bacteria of the species *Chlamydia trachomatis* (Seth-Smith, 2013).

When molecular diagnostics is carried out in clinical microbiology laboratories it cannot be done in a single step: before the actual amplification phase, the phases involving the dilution of potential inhibitors and the concentration of DNA and RNA also provide essential sources of biological matter. In fact, only a few µL are generally used for diagnostic PCR, the remainder being stored for at least a few weeks and used at the request of reference laboratories to characterise the genotype or for molecular epidemiology, or alternatively for research purposes.

Lastly, the TYPENED experiment in the Netherlands (Niesters, 2013) provides another response to this challenge as a way of encouraging clinical microbiologists and infection specialists to take an interest in data from molecular epidemiology. The concept exploits a shared database which compiles clinical, microbiological (sequences) and epidemiological data. All participating laboratories, whether clinical or reference, have access to all the data in the base, thus allowing real-time comparison between the data obtained by a diagnostics laboratory and those obtained by other laboratories at the same period for example, or having the same clinical expression, the same therapeutic response, etc. Clinicians, public health epidemiologists and microbiologists from reference laboratories thus all benefit.

The outlook for the development of these systems seems very promising, as they open the door to real improvements in the monitoring of infectious diseases at a global level, both for clinicians specialising in infectious diseases and for microbiologists, epidemiologists and risk managers. With or without the traditional pathogen cultivation stage and after a few technical improvements in instrumentation, it will be possible to obtain complete sequences for each pathogen implicated in a disease at reasonable cost. Apart from the improved therapies that molecular microbiology will provide, we will achieve faster real-time integration of all the available information on the patient or patients, the pathogens and the epidemiological data. After all, molecular data can be transmitted and exchanged with incomparably greater ease than the isolates of bacterial, viral, fungal or parasitic pathogens. As long as these data are shared, we have an opportunity to create a global system of interlinked databases for the genetic characterisation of microorganisms isolated from patients, both human and animal, and the potential sources of contamination (hospital samples, foods, drinking water, etc.). Such integrated monitoring (Aarestrup, 2012) will enable public health authorities to provide better-coordinated responses, including across borders when necessary, which are also better adapted to real threats to public health.

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