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Abbreviations

AF	Advisory Forum
BIOHAZ panel	Scientific Panel on Biological Hazards
CB	Competent Body
DG RTD	Directorate-General for Research and Innovation
DG SANCO	Directorate-General for Health and Consumers
DSN	Dedicated Surveillance Network
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EQA	External quality assessment
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
ESGEM	ESCMID Study Group on Epidemiology Markers
EU	European Union
MDR-TB	Multidrug-resistant tuberculosis
MLST	Multilocus sequence typing
MLVA	Multiple-loci VNTR analysis
MRSA	Methicillin-resistant *Staphylococcus aureus*
NMFP	National Microbiology Focal Point
PMEN1	Pneumococcal Molecular Epidemiology Network 1
TESSy	The European Surveillance System
VNTR	Variable-number tandem repeat
WHO	World Health Organization

1 Document purpose

The European Centre for Disease Prevention and Control (ECDC) has proposed a concept on how to integrate molecular typing data into disease surveillance at the EU level by taking into account the structures developed by the former dedicated surveillance networks (DSNs) and other networks, in line with the overall ECDC public health microbiology strategy.

The first version of this paper underwent a wide consultation process in 2008. This document provides an updated version of the principles for gradual integration of agreed molecular typing data and methods into the European Surveillance System (TESSy). It also aims to serve as a general guidance for the key stakeholders in the Member States, EC, and ECDC.

2 Introduction

ECDC has a mandate to 'foster the development of sufficient capacity for diagnosis, detection, identification and characterisation of infectious agents which may threaten public health' [1]. To achieve this, ECDC shall 'collect, collate, evaluate and disseminate relevant scientific and technical data'. Furthermore, ECDC has a mandate to maintain the database(s) for such epidemiological surveillance [1].

The ECDC long-term surveillance strategy [2] has defined the principle of having basic and enhanced surveillance components at the EU level. This division is still valid and has been taken into account in this concept paper.

Basic surveillance defines the frame for routine epidemiological surveillance consisting of a common set of variables for all diseases with reporting frequencies defined for each disease, based on its surveillance objectives and the agreement with the Competent Bodies in the Member States.

Enhanced surveillance means that for priority diseases – which have been decided and agreed upon jointly with Member States – basic surveillance is complemented by additional information according to the disease-specific surveillance objectives, e.g. more detailed microbiological information is included in the set of variables to be collected. At the time of producing this concept paper, the priority diseases were, in practice, those diseases for which an EU-funded dedicated surveillance network (transferred to ECDC between 2007 and 2010) was in place. Enhanced surveillance objectives were also defined for a few other diseases, covering additional sets of variables.

Before the existence of ECDC, surveillance of communicable diseases on the European level was to a large extent based on networking of expertise in the EU Member States through DG SANCO-funded DSNs [3]. It has been crucial to take these existing structures and developments into account; thus, the evaluation and assessment of 17 DSNs served as a natural starting point to review what kind of surveillance activities existed and how these data should be integrated into future surveillance activities at the EU level. The objectives for surveillance were formulated by the DSNs in different ways – and consequently varied in detail, focus and comprehensiveness. Some of the DSNs had strong laboratory components, while there were none in other DSNs. Most of the DSNs with laboratory components have had molecular typing activities, which were outsourced when basic DSN surveillance activities were transferred to ECDC.

3 Towards a consensus on molecular typing data integration

On 16 and 17 July 2007, ECDC invited a small number of experts with comprehensive experience on molecular typing for surveillance of different pathogens from various angles for a brainstorming meeting at ECDC in Stockholm. The outcome of that meeting was used to draft the first version of a concept paper on integrating molecular typing data as a part of EU-level surveillance. Subsequently, the draft concept paper was discussed with the expert group, then with the ECDC Advisory Forum (AF), before it was issued for wider consultations (including NMFPs and ESGEM) to ensure that the views of leading surveillance and microbiology experts in the EU were incorporated. The wide consultation among leading molecular typing experts took place in mid-2008, and the final revisions based on these consultations were discussed during the AF meeting in November 2008. In addition, the core content of the concept paper was revisited and discussed in the AF working group in May 2011.

This concept paper is linked to two essential development steps in ECDC. Firstly, a prerequisite for this paper is to agree on the future surveillance objectives for diseases or disease groups. These are part of the long-term surveillance strategy which outlines the further development of disease-specific surveillance objectives in close collaboration with the Member States [2]. In addition, high-quality microbiological reference level laboratory capacity at the national level, or, alternatively, easy access to these services will be necessary for the successful implementation of those surveillance objectives that are tied to the systematic collection of molecular typing data. The 'General strategy and framework of actions for ECDC cooperation with microbiology laboratories and research institutes in the EU (2007–2013)' established a forum of NMFPs, nominated through the Management Board members by the national authorities [4]. This forum performed a survey of national reference laboratory systems for communicable diseases in the Member States and developed a consensus definition of public health microbiology and core public health functions of national reference laboratories [5].

The long-term surveillance strategy addresses the integration of epidemiological and laboratory data in the Member States. While EU Member States have a good capacity to diagnose and characterise most of the common communicable disease agents, a concept was needed to define how the integration of advanced molecular typing data with epidemiological data at the national level could be performed in order to implement molecular surveillance that can support the control of communicable diseases in the Member States and at the EU level.

In this document, molecular typing refers to any technique and method that is used to characterise microorganisms at the nucleotide level. Phenotypic information is to some extent already captured through TESSy, and it is envisioned that the links between genotypic and phenotypic information will be enhanced in the future.

4 The role of molecular typing in public health

Molecular typing of pathogens that cause infectious diseases complements the traditional epidemiological surveillance by providing appropriate discriminatory analyses to foster the rapid and early detection of dispersed international clusters/outbreaks, to detect and investigate transmission chains and the relatedness of strains, and to detect the emergence of antimicrobial resistance and new evolving pathogenic strains. It also supports studies to trace-back the source of an outbreak and identify new risk factors, as strains can be linked more accurately to epidemiological and clinical data.

The analysis of molecular typing data can also aid the study of pathogen characteristics and the pathogen's behaviour in a community of hosts, for example its spread over time and space, its disease transmission dynamics, its genetic factors (including mutations which influence the recurrence or virulence of infections), antigenic drifts/shifts of strains over time, and the development of drug resistance across multiple generations of strains [6-8].

This information offers help in understanding the disease mechanisms and can be applied towards improving and better targeting existing infectious disease prevention and control measures and thus presents a clear and immense benefit for public health and public health policies.

Laboratory techniques have developed quickly in the past years, and an increasing number of pathogens can be, and will be, further characterised by advanced laboratory methodologies. Furthermore, an increasing number of diseases may be diagnosed without culturing, using molecular typing techniques only, for example invasive meningococcal disease [9]. However, molecular typing for diagnostic purposes cannot at present completely override the need to isolate the pathogens of the diseases, which is still necessary for strain collections and in particular phenotypic testing, e.g. for antimicrobial resistance.

The 'endpoint' of typing techniques is sequencing a whole genome of a pathogen which has the highest discriminatory power [6-8]. However, for epidemiological purposes, methods with lower discriminatory power are sufficient for many diseases as far as public health is concerned.

The section below describes the public health benefits that have been identified both at the national and at the international level. They are also illustrated by some concrete examples of the roles of molecular typing in public health.

4.1 Surveillance of the spreading of strains

- Molecular typing enables the monitoring of the spread of clones and strains (geographically and in time).
  - Example: Multilocus sequence typing (MLST) is a well-established and robust method that can be used for the global surveillance of meningococcal disease [10].
  - Example: Molecular serotyping is an increasingly used method for many bacterial pathogens. It has been recently developed for all *Streptococcus pneumoniae* serogroups [11] and for some common *Salmonella* serotypes [12], thus allowing rapid monitoring of disease spread.
  - Example: Serosubtyping of *Neisseria meningitidis*, where subtypes are defined by sequencing the variable regions in the outer membrane protein PorA gene. Serosubtyping has become an important part of the characterisation scheme for *Neisseria meningitidis* [13].

4.2 Prediction of strain characteristics

Molecular typing enables the monitoring of strain characteristics such as:

- Epidemic potential
  - Example: Antigenic variation of norovirus strains may result in rapidly evolving epidemic strains that spread effectively globally across countries [14].
- Vaccine preventability (important when a vaccine does not cover all strains)
  - Examples: Meningococcal disease, invasive *Haemophilus influenzae* and *Streptococcus pneumoniae* disease.
- Virulence
  - Example: A single nucleotide mutation can have a remarkable effect on virulence. For example, a point mutation in one Group A streptococcus (GAS) strain altered the maturation of specific protease responsible for tissue damage and resulted in decreased necrotizing fasciitis effect [15]. However,

other virulence factors like transmissibility and the capability to cause bacteraemia remained the same.
  - Example: Whole genome sequencing has elucidated – through phylogenomic and biogeographic analysis – the evolution of virulence, the acquisition of antimicrobial drug resistance, antigenic shift, and the local and global dissemination of pathogenic lineages of *Streptococcus pneumoniae*, *Clostridium difficile* and *Staphylococcus aureus* [7].
  - Example: The global increase of *Clostridium difficile* infection and diarrheal disease has been linked to the transcontinental spread of successful clones. Phylogeographic analysis has revealed that these clones have emerged by large-scale intergenomic transfer and recombination of pathogenicity islands in multiple lineages [8].

4.3 Monitoring of vaccine efficacy and detection of vaccine-escape mutants

- Molecular typing enables monitoring the variability of possible vaccine targets.
  - Example: Antigenic drift in influenza virus is regularly followed on a global level to determine the content of vaccines for the upcoming season.
  - Example: Phylogeographic analysis has documented the capsule-switching recombination events that have enabled the population shift of multidrug-resistant vaccine-escape serotype 19A *Streptococcus pneumoniae* following immunisation in the USA with conjugate polysaccharide vaccine [6].

4.4 Monitoring of antimicrobial resistance

- Molecular typing enables monitoring the acquisition and spread of genes that determine resistance to antimicrobial agents (also including antiviral, antifungal and antiparasitic agents).
  - Example: Molecular typing of MDR-TB provides information on incidence and prevalence of international strain genotypes (Haarlem, Beijing, etc.), transmission dynamics, and transmission routes. It also allows the identification of clusters within the genotypes. One of the most important results of molecular surveillance of MDR-TB in the EU was the identification of Cluster E0051. It involved 12 countries and 175 cases, 165 of which originated from former Soviet Union countries [16].
  - Example: Phylogeographic whole genome analysis has shown how the multidrug-resistant clone PMEN1 of *Streptococcus pneumoniae* has diversified by mutation and recombination as it disseminated worldwide over the past four decades since its emergence in Europe [8].

4.5 Detection of outbreaks

- If applied routinely in real time, molecular typing could allow for an early detection of national and international clusters/outbreaks.
  - Example: In the field of nosocomial infections, molecular typing provides substantial support to the surveillance of hospital-acquired infections and guides infection control practices [17].
  - Example: The sequence-based single locus typing (SLT) of *S. aureus* protein A (*spa*) typing, which can be useful for local surveillance and infection control purposes [18] as well as national surveillance [19].

4.6 Linking isolates from different sources (human, animal, food, feed)

- For food-borne pathogens, molecular typing enables linking pathogen data from human, food, animal, and environmental sources and thus facilitates the early identification of potential sources of outbreaks/clusters.
  - Example: A multinational *Salmonella* Agona outbreak in humans was linked through pulsed-field-gel-electrophoresis (PFGE) to pre-cooked meat products which were produced in one country and traced back to other European countries through various food outlet chains [20].

4.7 Meaningful global surveillance

- Molecular typing enables linking to global surveillance.
  - Example: National shigellosis outbreaks detected at the same time in Denmark and Australia could be linked through PFGE typing to a baby corn packing house in Thailand in 2007 [21].
  - Example: Antigenic drift in influenza virus is regularly followed on a global level; see section 4.3 above.

All the public health benefits mentioned above may provide clear added value at the international level and thus are important elements for surveillance objectives at EU level. None of the benefits mentioned above are possible without epidemiologically meaningful discriminatory molecular typing methods for pathogens, without sustainable and high-quality online databases that aggregate comparable typing results, and without global networking.

5 Criteria for molecular typing methods

Comprehensive guidelines identifying the important criteria for validation and application of typing methods in bacterial epidemiology have already been published in 2007 [22]. The criteria for integrating the various typing methods into a surveillance approach at the EU-level are defined below. The typing methods should:

- be driven by practical needs for surveillance as 'information for action';
- improve surveillance and control of a disease/pathogen in at least one of the following public health areas:
  - surveillance of potential preventability by vaccination
  - epidemiological monitoring of vaccine-preventable diseases as regards to changes in distribution of strains of increased epidemic capacity or increased virulence
  - early outbreak detection, including dispersed cross-border or international clusters of infection
  - outbreak investigation, including common source tracing
  - tracking the emergence and spread of genetic determinants of antimicrobial resistance or virulence markers in pathogens of public health relevance
  - population biology of public health relevance if needed for risk assessment or policy development
  - other areas of public health relevance
- be discriminatory in a meaningful way according to the needs of disease-specific surveillance or outbreak investigation;
- be cost-efficient, i.e. the resources needed for the implementation and the management of molecular surveillance must be available and proportional to their expected benefits;
- produce quality-assured, comparable and valid data between laboratories across countries;
- be interpreted and assessed by epidemiologists and molecular typing experts of the pathogen in order to guide public health response and decision-making;
- give unambiguous typing data resulting in a single and standard nomenclature of clonal genotypes;
- provide data which facilitate the electronic intra- and inter-laboratory exchange of typing data; and
- be epidemiologically validated and accepted by the relevant national authorities (for example reference laboratories for the specific diseases).

6 Principles for integrating molecular typing data into surveillance

The key principle is that the typing results should be interpreted in the context of all available clinical and epidemiological information. However, duplication of data collection should be avoided.

ECDC will support centrally managed storage and aid with the management of molecular typing data that are based on agreed methods. Where no disease-specific molecular typing activities exist and a need is identified through relevant expert groups or through ECDC external networks, specific studies may be performed before a decision is made on addition of new molecular typing data to surveillance of that disease. If the methods and expertise exist in laboratories outside the EU, these should be consulted as well before specific studies are undertaken in order to avoid duplication of the work and to promote international collaboration. Collaborations with the Directorate-General Research & Innovation and Directorate-General for Health and Consumers are highly desirable to foster synergy and avoid duplication of activities.

ECDC will facilitate the collection of high-quality and valid data. To enhance international comparability and ensure the good quality of collected data, the following principles will be applied:

- Methods and techniques are to be harmonised or standardised to a level which is necessary and feasible.
- Interpretation and normalisation of raw molecular typing data (where needed) as well interpretation of cluster analysis should be done
  - by typing experts that have appropriate microbiological expertise and experience in the methodology and the pathogen; and/or
  - by automatic curation, which has been implemented recently for *spa*-typing of *S. aureus*[1]; and/or
  - by epidemiologists involved in the epidemiological or clinical investigations.
- EQA schemes and reference material for the agreed molecular typing methods need to be available to the national reference laboratories to enable the laboratories to assess their own performance.
- All Member States should have access – if appropriate – to the use of an agreed molecular typing method for every pathogen, either by building up the capacity on their own or by getting services from those Member States that have developed the capacity.
- Good technical and microbiological support will be made available for the Member States.
- Training in the agreed methods will be provided for the national reference laboratories in the Member States.
- Linkages between human, food, animal and environmental data will be promoted where relevant and appropriate.
- Data integration facilitates links to global surveillance.
- Molecular typing databases should be centrally managed to ensure common methods in the validation of data; also, a reference code should be assigned to each submitted molecular typing result. Where needed, these activities must be performed manually by curators. The (automated) reference type assignment function of these databases must also be publicly available and accessible online, so public users can have a reference code assigned to their molecular typing result.
- Molecular surveillance within TESSy should be organised in a way that ensures that both the National Public Health Reference Laboratories and the National Surveillance Centres are involved in deciding which laboratory has the data submission and user rights and if and how the molecular typing data can be linked to epidemiological data in TESSy.
- Data collection should, to the greatest extent possible, follow the TESSy principles for user and data access collaboration, as defined in the TESSy User Guide [23]. Wherever possible, data collection should be done through automated procedures because of the potentially complex nature of typing data and in order to receive results timely and efficiently.

[1] www.seqnet.org

7 Process for decisions regarding priority settings for molecular surveillance targets and methods

The methods of choice for surveillance and public health purposes need to be agreed with the Member States.

The performance of the molecular typing surveillance needs to build upon the achievements by the DSNs[2].

- If a DSN has agreed on molecular typing activities, this should be considered as a starting point, provided that the criteria established under section 6 above are met.
- If there is no DSN in place, but there are research-based networks that have developed and agreed on molecular typing activities at the European level, these networks could serve as potential starting points, provided that the criteria established under section 6 above are met.

Typing expert groups may be needed to lead the disease/pathogen-specific work to propose suitable molecular typing techniques/disease(s) for EU level surveillance, taking into account surveillance objectives and priorities.

- Expert groups can be formed by combining representatives from the nominated disease-specific experts (laboratory and epidemiology).
- Expert groups may invite external experts from appropriate research groups, e.g. learned societies and active typing network members, ESGEM or experts from other stakeholder groups like EFSA's BIOHAZ Panel working groups, European Union reference laboratories, WHO collaborating centres, etc.
- It should be ascertained that the guidance outputs of these expert groups are in line with the EU's and ECDC's strategic documents on surveillance, epidemic intelligence and public health microbiology.

Proposals on new typing methods/diseases need to be discussed within ECDC's external networks and consulted with relevant stakeholders such as the European Union reference laboratories (where appropriate), the EU and other European institutions, relevant research groups, NMFPs and the network of national surveillance contact points before final approval by ECDC and subsequent integration into EU-level molecular surveillance.

Multidisciplinary discussions will be promoted with the goal of arriving at a procedure for standardisation of typing methods between public health, food, feed, animal, and environmental reference laboratories when possible and appropriate.

Examples of tasks for the typing expert groups:

- Proposing appropriate methods/techniques for disease-specific molecular surveillance
- Assessing EQA needs
- Identifying and proposing feasibility and validation study needs
- Proposing molecular typing algorithms
- Assessing standardisation/harmonisation needs of typing methods
- Identifying training needs
- Proposing organisation of typing data collection (considering surveillance objectives and already existing systems)

[2] This includes other current ECDC-supported, disease-specific laboratory networks.

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