TECHNICAL REPORT

ECDC roadmap for integration of molecular typing into European-level surveillance and epidemic preparedness

Version 1.2, 2013

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Abbreviations

CNRL	Community Network of Reference Laboratories for Human Influenza
EC	European Commission
EFSA	European Food Safety Authority
EPIS	Epidemic Intelligence Information System
EQA	External Quality Assessment
ESGEM	ESCMID Study Group for Epidemiological Markers
EU	European Union
FWD	ECDC Food- and Waterborne Diseases
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
IBD	Invasive Bacterial Diseases Network
MDR	Multidrug resistant
MIRU	Mycobacterial Interspersed Repetitive Units
MLST	Multi-Locus Sequence Typing
MLVA	Multiple Loci VNTR Analysis
MRSA	Meticillin-resistant *Staphylococcus aureus*
NGS	Next Generation Sequencing
PFGE	Pulsed Field Gel Electrophoresis
PHE	Public Health England
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (The National Institute for Public Health and the Environment), The Netherlands
RKI	Robert Koch-Institut, Germany
STEC	Shiga toxin-producing *E. coli*
TESSy	The European Surveillance system
VNTR	Variable Number Tandem Repeat
VRE	Vancomycin-resistant *Enterococcus faecium*
WGS	Whole Genome Sequencing
WHO	World Health Organisation
XDR	Extensively drug-resistant

Executive summary

This document presents the recommended priority options and implementation processes for the medium-term integration of molecular typing into EU-level surveillance and epidemic preparedness.

This roadmap Version 1.2, 2013 is meant to complement ECDC's Long-Term Surveillance Strategy [1] and guide the ECDC molecular typing activities over the next five years is based on the following inputs:

- ECDC concept paper on molecular surveillance and long-term surveillance strategy [1,7]
- Retrospective evidence of public health effectiveness and benefits of molecular typing for viral and bacterial pathogens identified in two commissioned systematic reviews
- Information on current molecular typing public health practices in the EU/EEA countries, EU disease networks and ECDC disease-specific surveillance strategies
- Capabilities and practical constraints identified from typing experiences in Member States and EU network capacity assessment projects
- Appraisal of technological advancements in comparative genomics likely to have an impact on future molecular epidemiological typing practices
- Expert guidance after two expert consultations and one on-line priority ranking exercise
- Experience from the ECDC-supported ongoing multistate typing pilot project.

In a meeting organised by ECDC in September 2012, essential feedback on the outline of this document was given by ECDC stakeholders. This feedback included the following key points:

- Rather than recommending a static list of pathogens for implementation over the next five years, the roadmap should propose a stepwise approach starting with a small number of priority pathogens with a plan for gradual expansion.
- The roadmap should focus on pathogens for which EU-integrated molecular typing data are absolutely needed for informed public health policies and interventions.
- The roadmap should build upon typing programmes that are already operational in Member States with minimal additional effort to achieve meaningful EU data sharing and analysis. This would allow for flexibility depending on shifting priorities in terms of emerging and resurgent diseases, drawing lessons from the experiences gained from the ongoing pilot projects, and allowing in-depth investigations of resources and capacities in Member States.
- Molecular typing data collection supported by ECDC should be well integrated with existing typing initiatives on an EU and/or global level.

A four-step development and implementation process is proposed for each new disease to be incorporated in the European Surveillance System (TESSy) molecular surveillance system. For pathogens shortlisted for potential implementation, there will first be development of a disease specific molecular surveillance and/or epidemic preparedness strategy followed by the preparation of a technical business case. The disease specific molecular surveillance and/or epidemic preparedness strategy must clearly describe the specific public health questions to be addressed through the typing data collection and analysis, and linked actions expected at the European level. For pathogens whose business cases are endorsed, an implementation phase will be followed by routine use of the system.

The molecular typing strategy and business case will be produced by ECDC in collaboration with a designated Disease Network Typing Reference Group. The main objectives of the molecular typing strategy and business case will be to validate findings and elaborate on the conclusions of the roadmap, and to make the necessary clarifications to permit practical implementation both from the Member State and ECDC perspective. Components to be addressed in the disease specific molecular surveillance business case include:

- refinement of public health objectives and benefits of molecular typing data integration into EU level surveillance and/or epidemic investigation
- definition of short, medium and long-term success indicators
- proposed sampling strategy suitable to the public health objectives
- verification of the molecular typing method(s) applied, including current capacities in Member States, need for standardisation, external quality assessments, standard operational procedures, training and other support
- data analysis and reporting aspects, including the need for advanced bioinformatics, and expert resources for data validation, management, and interpretation support
- data privacy, ethical or legal issues.

Advice will be sought from stakeholders and discussions with partner organisations will be needed to determine if molecular typing data should be stored and analysed within TESSy or within some other (pre-existing) external database whenever available.

The roadmap will be updated on an annual basis proposing molecular typing integration for additional pathogens. There will also be a bi-annual review and evaluation of performance and public health usefulness of all ongoing molecular surveillance components implemented. The purpose of this bi-annual review is to ensure that the ongoing data collection and analysis processes for each pathogen continue to meet their designated public health objectives.

Based on collected information presented in this report, ECDC proposes to start practical implementation of tools to support collection of molecular typing data for human influenza virus, with the aim to have a functional system to support the influenza community by the start of the 2014–2015 season. Molecular typing to support influenza surveillance was ranked as a top priority in two of the consultations executed. Characterisation of influenza viruses using standardised methods is well-established in nearly all Member States. A dedicated working group in the influenza network is in the process of producing a business case describing the background and needs for implementation, and describing how interactions with existing global influenza sequence initiatives should be conducted [3]. Implementation of this solution would significantly reduce the double reporting of sequence data for the users, and would lead to enhanced virological surveillance reporting in TESSy with a minimum of additional resources required from both ECDC and Member States.

Collection of sequence-based molecular typing data on human immunodeficiency virus for surveillance to support early detection and monitoring transmission of primary antiviral resistance, identification of transmission routes, and informing therapeutic guidance was identified by several expert consultations as being of public health importance and was considered timely for EU level surveillance to build upon systems piloted as research projects. HIV resistance testing through antiretroviral drug target regions sequence analysis typing is well-standardised, highly comparable across countries and performed in 14 Member States as part of standard clinical practice and resistance surveillance schemes, using various sampling strategies. Centralised collection and analysis of HIV sequence typing data was identified as having potential for public health benefit, but definition of sampling strategies and solutions to complex data privacy and confidentiality issues are needed before any such scheme can be implemented on a European level. ECDC concludes that development of a strategy and business case for HIV antiretroviral resistance surveillance could be explored in 2015.

The expert panels both gave high priority to *Neisseria meningitidis* typing, and the ECDC Vaccine-Preventable Diseases Programme has indicated a need for centralised data collection for surveillance purposes. The systematic review identified multilocus sequence typing (MLST) and *porA:fetA* sequence analysis as being the most useful methods for monitoring the genetic diversity of disease-causing strains, as was recommended by an expert panel commissioned in 2009 within the invasive bacterial disease (IBD) network for monitoring the reduction in invasive strains following introduction of new vaccine programmes. Building a molecular surveillance strategy to elaborate on the specific public health objectives that a system should support is therefore recommended in 2013. This would be followed by a business case in 2014 to analyse the resources available in Member States and draft the pilot testing phase methods and procedures for launch in 2015. There are already databases which collect MLST and *porA:fetA* data on *N. meningitidis*, which are managed and funded by the University of Oxford, UK and the University of Wurzburg, Germany. The business case needs to take into account interaction with the resources invested by these data managers, and their plans for how the database should be supported in the future.

Typing of *Legionella pneumophila* for outbreak studies and source attribution was ranked as a top priority both in the June consultation and in the discussions with Member States during the Joint Strategy Meeting in September 2012. The main method for *Legionella* typing is Sequence Based Typing (SBT, 7 loci MLST), for which there are agreed standards and protocols which are broadly applied at the European level. Typing of *L. pneumophila* has the potential to greatly enhance the resolution of cluster analysis and source identification as part of outbreak investigation and response. ECDC currently manages the epidemic intelligence information system - European surveillance network for travel-associated Legionnaires' disease- (EPIS-ELDSNet) for reporting and rapid communication of travel-associated *Legionella* cases, and has already supported capacity building for molecular typing of *Legionella* through numerous external quality assessments (EQAs) and training activities. Sequence-based typing (SBT) data is currently submitted, stored and analysed in a database managed and funded by Public Health England (PHE), UK. There are unresolved issues related to *L. pneumophila* typing that needs to be addressed in the *Legionella* specific roadmap and business case, such as the current infrequent use and application of existing resources for cross-border outbreak investigations.

Beyond the above listed diseases for which continuous molecular surveillance as performed across Member States is proposed for TESSy-based EU integration, there is an unmet public health need for molecular surveillance of transmissible clones and mobile genetic determinants of antimicrobial resistance of major epidemiologic significance. This includes epidemic/pandemic clones and plasmids harbouring drug resistance determinants encoding extensive resistance phenotypes and thereby associated with high risk of treatment and transmission control failures. These high risk resistant pathogens include cephalosporin resistant *Neisseria gonorrhoeae,* extensively-drug resistant (XDR) and carbapenem-resistant *Enterobacteriaceae,* and *Acinetobacter baumannii* and meticillin-resistant *Staphylococcus aureus* (MRSA). Both of the roadmap expert consultations highlighted the public health added value of molecular typing of both strains and antibiotic resistance genetic determinants for monitoring prevalence and risk factors associated with infections by multi-drug resistant (MDR)/XDR pathogens

and delineating cross-border and animal-to-human spread of epidemic strains and genes. Recent ECDC disease specific pilot projects on MDR *N. gonorrhoeae* [4] and MRSA [5] demonstrated the feasibility of pan-EU/EEA structured molecular epidemiology surveys of prevalence of MDR pathogens that provide new information for control strategies. Concordant expert consultations recommended that ECDC consider supporting the long-term surveillance of these issues through planning periodically repeated molecular prevalence surveys including also pathogens that are not typed as part of routine surveillance. Whereas these surveys have so far been performed using molecular databases hosted by national public health institutes and research laboratories, there is an opportunity to explore the pros and cons of their integration into a bioinformatics enabled TESSy platform to ensure integration with core surveillance, and provide data security and system sustainability.

Introduction

The inclusion of molecular typing into European level surveillance and epidemic preparedness has been debated among ECDC stakeholders since 2007. Based on these discussions, a concept paper for integration of EU level surveillance was developed in 2008 and was further updated in 2011 after internal consultation at ECDC [7]. This paper concluded that by combining molecular typing- and epidemiological data there is a potential to improve surveillance and epidemic preparedness, and that specific criteria should be fulfilled before implementing a typing scheme on a European level. Basic criteria for developing European or broader international collection of molecular typing are that there is a practical need for molecular typing data as information for public health action, the data meets specific surveillance and outbreak investigation objectives, the resources needed for implementation and management are proportional to the expected benefits and there are capacities in Member States for integrating molecular surveillance and epidemiological data for meaningful analysis at the EU level. On the technical side, methods need to be discriminatory in a meaningful way; data should be comparable between laboratories and the interpretation and assessments of the results done by experts with method and pathogen experience, including the involvement of specialists from Member States.

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 in the EU. To achieve this, ECDC collects, collates, evaluates and disseminates relevant scientific and technical data [14]. In its current long-term surveillance strategy, ECDC together with Member States has defined the principles of basic and enhanced surveillance components at the EU level [1]. The term 'basic surveillance' describes the common set of variables collected for all of the diseases under the remit of ECDC. In 'enhanced surveillance', the basic metadata set can be complemented with an additional set of disease-specific variables aligned with specific surveillance objectives. Here, detailed laboratory information can be included in the characterisation of disease agents. ECDC has supported a significant number of projects with molecular typing components. These projects have included setting up EQAs for specific typing methods, supporting the standardising of typing protocols, assessing the benefits of molecular typing for public health purposes and running transnational molecular typing projects. The experiences in terms of capability across the EU for a specific typing method, cost-benefit analysis and future expectations from the ECDC Disease Programmes and the disease networks is an important piece of information in the creation of this roadmap.

- ECDC has developed a communication platform tool, the Epidemic Intelligence Information System (EPIS) which allows Member State bodies to exchange information regarding current or emerging public health threats with a potential impact in the EU. This system is not a molecular typing platform *per se*, but has been used as a tool for sharing molecular typing information in outbreak situations.
- The current version of the European Surveillance system (TESSy) has been set up to accept inclusion of limited typing information as part of the case-based reporting. This information does not require any analytical functionality within TESSy to support interpretation and is submitted to TESSy as supplementary information rather than requested typing data. However, the data is not provided in a sufficiently routine or timely manner to be useful in for example outbreak investigations or cluster detection.
- TESSy v3 (launched for pilot testing in 2012) provides dedicated bioinformatic tools to support molecular typing data input, validation, normalisation, analysis and graphical report generation. This new data management module is suitable to molecular epidemiologic studies such as cluster detection and pattern matching search algorithms based on either sequence or fingerprint based analysis.

To get practical experience in setting up and administering molecular typing networks at a European level, including the routine use of the TESSy v3 technical platform for analysing the data, ECDC has initiated a molecular typing pilot study. A pilot project was limited to covers four different pathogens; *Salmonella enterica*, *Listeria monocytogenes* and Shiga toxin- producing *E. coli* (STEC) under ECDC's Food- and Waterborne Diseases (FWD) Programme and multidrug resistant *Mycobacterium tuberculosis* (MDR-TB). The experiences from the molecular typing pilot project was planned to feed into the further development of the molecular surveillance roadmap even more in the future

Introduction to molecular typing

Molecular typing refers to the application of laboratory methods capable of characterising, discriminating and indexing subtypes of microorganisms. Typing methods themselves can be classified into two main groups according to the basic principles of characterisation;

- phenotypic methods, including serological methods, focusing on observable or measurable morphological or biochemical properties of an organism
- genotypic methods strictly applying different methods for investigating the genetic code of the organism for characterisation purposes.

For the scope of this roadmap only genotypic typing methods are considered.

Molecular typing exploits the fact that due to imperfect replication of ribonucleic acid/deoxyribonucleic acid (DNA/RNA) or horizontal gene transfer, there is variation in the genome composition that accumulates and brings about diversity between different generations of microorganisms [7] [8]. By measuring this genomic divergence, there is an opportunity to conclude relatedness between individuals within a species. The speed of differentiation, sometimes referred to as the 'molecular clock' is different between different species and between different parts of the genome within a species. Molecular markers are well characterised areas within the genome of a species that can be targeted for molecular typing purposes. Different 'molecular clock rates' of these markers make them applicable for different purposes. For example, rapidly evolving markers where recent evolutionary events can separate two closely related samples from the rest of the population can be useful for investigating transmission within a hospital or source attribution in a food outbreak. Slowly evolving markers can on the other hand be useful for surveillance purposes when investigating long-term trends and transmission of strains.

There are multiple typing methods developed for most organisms. A simplified explanation for this observation is that advances in technology have allowed the development of faster and cheaper methods with higher resolution, and that these continuously replace older methods. This is only partly true and the reason for choosing a specific typing method is often multifactorial.

First, the choice of typing method is linked to the resolution needed to fulfil the objective of the investigation. The primary molecular typing objectives in the scope of this report are epidemiological objectives related to infectious disease surveillance and outbreak investigation. Both the molecular marker and the typing method itself need to be sufficiently validated in terms of reproducibility epidemiological concordance, typeability, etc. [8]

Second, the choice of typing method needs to be practically feasible for the intended use. Cost, ease of use, accessibility, and for the discussion of introducing molecular typing in the EU, capacity and capabilities across Member States to perform a specific method need to be acceptable given the objectives and potential benefits.

In recent years, nucleic acid sequencing using 'next generation' methods (also known as 'next generation sequencing' [NGS]) has emerged as a major tool for studying genetic diversity, short term evolution and characteristics of pathogenic microorganisms. This development has dramatically increased the amount of genetic information available for the human pathogens under the ECDC remit and is likely to directly or indirectly affect the use and rationale for typing, the targets for molecular typing and the laboratory method used.

Using molecular typing for public health

As was already described in the molecular surveillance concept paper [7], there are a number of potential public health objectives that molecular typing may contribute towards, but all these objectives are not relevant for all pathogens. One of the key aspects that need to be clarified before setting up any centralised collection of molecular typing for any pathogen is to define the set of specific public health objectives that the typing data collection will help to meet. Three main categories for achieving the potential objectives where molecular typing data may be beneficial can be discerned. However, the use of other types of sampling models or a combination of two or more sampling models should also be considered where appropriate, and will be defined in the disease specific molecular surveillance and/or epidemic preparedness strategies.

Benefits of molecular typing data for surveillance

Inclusion of molecular typing information into epidemiological surveillance has the potential to improve understanding of the mechanisms of disease transmission, and can be applied towards improving and better targeting infectious disease prevention and control measures. Some concrete benefits of molecular typing that may provide clear added public health value if applied to disease surveillance at an international level include:

- rapid detection of dispersed international outbreaks
- delineation of outbreaks, risk factors and epidemic source
- investigation of transmission chains across EU and globally
- detection of emergence and spread of new virulent types or drug resistant strains of human pathogens
- monitoring emergence of vaccine escape variants
- source attribution by linking to molecular monitoring of pathogen in animals, food and environment reservoirs.

Benefits of molecular typing data for outbreak detection and investigation

Molecular typing has become a standard part of laboratory investigations of outbreaks and epidemics of infectious diseases of all scales. It is required to identify and characterise epidemic strains, to refine the definition of confirmed epidemic cases based on identification of the epidemic strain(s), and therefore to delineate the exact magnitude of an outbreak. Furthermore, molecular typing is used to either generate or verify hypotheses

pertaining to probable modes of transmission and infection source of an outbreak. This is achieved by comparison of clonal relatedness between isolates from infected or colonised individuals over time and place and with isolates from the environment, animals, food and other contaminated products.

In addition, molecular typing can provide a powerful and sensitive tool for early outbreak detection [9,10]. To this end, it should be applied routinely as part of molecular surveillance of incident cases to continuously analyse cumulative data in real time for the early detection of single clone clusters of infected patients linked in time and/or place. This finding of molecular clusters generates the hypothesis that an outbreak is occurring, triggering verification by an epidemiological investigation. Because of the high resolution provided by this approach, many small clusters can be detected that would otherwise be masked by background 'noise' of endemic infection caused by multiple strains from independent sources.

Benefits of molecular typing data from structured surveys and point prevalence studies

A third identified area where there can be potential public health benefits and where ECDC frequently invest resources, are structured surveys and point prevalence studies with molecular typing components. The intention of these studies are not to reach implementation of comprehensive routine systems combining molecular typing and epidemiological data, but rather to do distinct efforts to get a snapshot of currently circulating strains of a pathogen in the population. Structured prevalence surveys are often conducted repeatedly to assess the impact of public health actions aimed at controlling transmission in a target population. Depending on the study, data from point prevalence studies have either been submitted through TESSy, or databases for collection of data from structured molecular epidemiology population surveys have been funded by ECDC and set up disconnected from TESSy as *ad hoc* microbiological database platforms hosted in Member States [11,12,13]. Potential benefits from the collection of molecular typing data in structured surveys and point prevalence studies include:

- Monitoring secular trends in pathogen type distribution causing disease over time and place within a population
- Identifying and delineate geographic extension of widespread epidemic/pandemic types
- Identifying high risk clones with particular public health importance, for example clones with particular virulence, antibiotic resistance profile etc.
- Distinguishing between recurrence and recrudescence within a population in an outbreak situation.
- Identifying shifts in strain distribution in target populations, altered by public health interventions aimed at controlling or eliminating disease transmission.

Roadmap methodology

ECDC's Microbiology Coordination Section drafted the ECDC roadmap - Version 1.2, 2013 taking into account the recommendations from stakeholders, ECDC Disease networks and Diseases programmes and was based on the following inputs:

- **Two systematic reviews and an expert guidance** on the public health effectiveness of molecular typing of viral and bacterial pathogens;
- **The two expert consultations**: The first consultation was during a face-to face meeting held on 14–15 June 2012, while the second was an online consultation. The experts independently ranked the expected disease specific impact of typing with regard to the public health objectives "Surveillance" and Outbreak detection". The ranking methodology applied the following criteria (weights of individual criteria in brackets):
    - need for EU surveillance to improve public health (15)
    - importance of EU-wide data collection and analysis for outbreak investigation purposes (15)
    - method sustainability for surveillance/outbreak investigation application (5)
    - affordability and accessibility of resources for surveillance/outbreak investigation (5)
    - Obstacles (legal, ethical, data protection) related to collection of data for surveillance/outbreak investigation purposes (5).
- **A survey of current typing activities** in the ECDC disease networks through the ECDC Disease Programmes: In May 2012, the ECDC Microbiology section conducted an internal survey among members of the ECDC Disease Programmes to investigate the current practices of molecular typing as applied to surveillance and outbreak investigation for 28 human pathogens. The survey covered five main areas:
    - public health objectives for molecular typing activities at national or higher level within the EU/EEA
    - sampling frames for molecular typing
    - current typing activities in Member States, European disease-specific networks, or other European wide projects
    - ECDC funded external quality assessment or training activities for molecular typing
    - expectations from the disease programmes for the future application of molecular typing.
- **A summary of supported ECDC activities:** Additional information on molecular typing activities within the ECDC disease networks was extracted from the ECDC Annual Microbiology Activity Reports (2010 and 2011) [28-29]. These reports summarise projects with microbiology components that have been outsourced by ECDC. The microbiology projects can span over multiple areas such as EQA, training, typing, capacity assessment, technical guidance. For the purpose of this roadmap we have extracted all information relevant for molecular typing. Reports or publications resulting from these outsourced projects were also analysed, if available.
- **Early experience from the pilot project**.

Table 1 summarises the collected evidence supporting implementation of EU molecular surveillance per disease and health issue.

Table 1. Summary of evidence supporting implementation of EU molecular surveillance per disease and health issue

Pathogen	Outbreak investigation				Surveillance										June consultation priority	September consultation priority
	Outbreak size delineation, refined case definition	Comparison of epidemic strains with previous strains	Source trace-back identification, confirmation, control	Epidemic case contact tracing	Monitoring strain distribution over place and time	Single strain cluster detection, early outbreak detection and alert	Source attribution of foodborne or waterborne disease	Transmission cluster/network identification for contact tracing	Emergence/detection/monitoring of epidemic (multi) drug resistance	Identification of epidemics of mobile element drug resistance	Monitoring of vaccine strain, prediction of preventability	Detection of novel hyper-pathogenic variant strain	Detection of diagnostic escape variant strain	Pandemic strain detection and monitoring		
Multidrug resistant *Acinetobacter baumannii*	SR / DP	SR / DP	SR / DP		SR / DP	SR			SR / DP					SR / DP	Low	Low
*Bacillus anthracis**	SR	SR			SR	SR								SR	Low	Medium
Bordetella pertussis		SR			SR			SR			SR / DP				Low	Low
*Clostridium botulinum**	SR	SR			SR	SR		SR							Low	Low
Clostridium difficile	SR / DP	SR / DP	SR		SR / DP	SR		DP	DP			DP		SR / DP	Medium	Medium
Corynebacterium diptheriae	SR	SR / DP	SR		SR						DP				Medium	Medium
Coxiella burnetii						DP									Low	Low
Vancomycin-resistant *Enterococcus faecium* (VRE)	DP	SR / DP			SR	SR		SR							Low	Low
Shiga-Toxin producing *E. coli*	SR / DP	SR / DP	SR / DP	SR / DP	SR / DP	SR / DP	SR / DP	SR / DP	DP	DP		DP			High	High
Hepatitis A virus		DP	SR / DP	DP			SR				SR				Medium	High
Hepatitis B virus						DP		SR	SR		SR	SR			Low	Medium
Hepatitis C virus				SR	SR			SR / DP	SR			SR	DP		Medium	Medium
Human Immunodeficiency Virus (HIV)		SR			SR	DP		SR	SR / DP						Medium	High
Influenza virus	SR	SR	SR		SR / DP	SR / DP					SR / DP	SR		SR / DP	High	Low
Multidrug resistant *Klebsiella pneumoniae*	SR / DP	SR / DP	SR	DP	SR	SR		SR	SR					SR	Medium	High
Legionella pneumophila	SR	SR / DP	SR / DP	SR	SR	SR									High	Low
Listeria monocytogenes	SR		SR / DP		SR / DP	DP	DP								Medium	Medium
Measles virus*		SR		SR	SR			SR			SR				High	High
Mumps virus*		SR			SR										Medium	Medium
Multidrug resistant *Mycobacterium tuberculosis* (MDR-TB)	SR	SR	SR / DP	SR / DP	SR / DP	SR		SR / DP	SR / DP		SR	DP			High	High
Neisseria gonorrhoeae	SR	SR / DP			SR	SR			DP			SR	SR	SR	Low	Low
Neisseria meningitidis	SR	SR			DP	DP					DP			SR	High	High
Norovirus	SR / DP	DP	SR / DP		SR / DP		SR	SR	DP			DP		DP	Low	Medium
Salmonella enterica	SR	SR	SR / DP	SR	SR	SR / DP	SR / DP	SR	SR					SR	High	High
SARS virus*						SR						SR			Low	Medium
Meticillin-resistant *Staphylococcus aureus*	DP	SR / DP	DP	DP	SR / DP				SR / DP			DP	DP	DP	High	High
Streptococcus pneumoniae					SR / DP				DP		SR			SR	Medium	Low
West Nile virus		SR / DP			SR			SR							High	High

SR (blue) indicates molecular typing objective identified as documented in the systematic reviews, DP (yellow) indicates molecular typing objective identified as applied in the EU by the survey of ECDC Disease Programmes, SR/DP (green) indicates molecular typing objective both identified in the systematic reviews and in the survey.

* indicates that no information was provided by the ECDC Disease Programmes about current application of typing of this pathogen for the public health sub-objective.

Roadmap priorities for disease specific genomic typing development and implementation, 2012-2015

Based on all the evidence, consultations and recommendations received, ECDC proposes the following roadmap V1.2 priorities for pathogen/disease specific genomic typing development and implementation, 2012-15. The next revision of the roadmap V1.2 implementation and priorities will take place in 2015-2016.

Pathogens already undergoing molecular typing data integration into TESSy (pilot project, 2012–2014)

Salmonella enterica

For *Salmonella enterica*, integration of molecular typing data collection using PFGE and MLVA (for *S.* Typhimurium only) has been implemented in TESSy as part of the molecular surveillance pilot project. The project will continue until May 2014, at which point it will be evaluated and a decision will be made about its future. The molecular surveillance pilot project is described in more detail in the molecular surveillance project description document [15].

Listeria monocytogenes

For *Listeria monocytogenes*, integration of molecular typing data collection using PFGE has been implemented in TESSy as part of the molecular surveillance pilot project. The project will continue until May 2014, at which point it will be evaluated and a decision will be made about its future. The molecular surveillance pilot project is described in more detail in the molecular surveillance project description document [15].

Shiga-Toxin producing *E. coli*

For STEC, integration of molecular typing data collection using PFGE has been implemented in TESSy as part of the molecular surveillance pilot project. The project will continue until May 2014, at which point it will be evaluated and a decision will be made about its future. The molecular surveillance pilot project is described in more detail in the molecular surveillance project description document [15].

Multidrug-resistant *Mycobacterium tuberculosis*

For MDR-TB, integration of molecular typing data collection using MIRU-VNTR and spoligotyping has been implemented in TESSy as part of the molecular surveillance pilot project. The project will continue until May 2014, at which point it will be evaluated and a decision will be made about its future. The molecular surveillance pilot project is described in more detail in the molecular surveillance project description document [15].

Pathogens proposed for strategic development and potential implementation in 2013–2015

The pathogens and health issues described in this section are those for which ECDC recommends the corresponding Disease Programmes and networks to develop disease-specific molecular surveillance strategies and business cases for, to be able to make a proposal for EU integration of molecular typing data into surveillance programmes in 2015–2016. The completion of a business case is in itself not a commitment that implementation will actually be performed, but sets the scene for subsequent informed decisions.

Influenza virus

Virological characterisation of influenza viruses using standardised methods (sequencing, antiviral susceptibility testing etc) is well-established in nearly all Member States. The influenza molecular surveillance roadmap, which was produced by ECDC and commented on by an expert group in 2011 [3], clearly describes the public health benefits of having access to sequence information of individual strains. The influenza roadmap also expresses a very clear wish that any actions taken on the European level shall be well integrated with the existing global influenza sequence initiatives. ECDC has therefore discussed a solution through which EU/EEA Member State virologists that are also nominated TESSy users can submit their data in an external database and flag it also for reporting to TESSy or vice versa. This data would then become available in TESSy and could be linked to the full epidemiological case data records that are submitted separately. Implementation of such a solution would reduce the double reporting of sequence data for the users, and would lead to increased virological reporting in TESSy with a minimum of additional resources required from both ECDC and the Member States.

For the 2013–2014 influenza season, ECDC and Member States are already planning to move from the existing reporting scheme of aggregated reporting to strain-based reporting. If the business case is then endorsed in 2013, a system for simplified molecular typing data handling including sequence data could be available by the start of the 2014–2015 influenza season.

Human immunodeficiency virus

The systematic review identified clear public health benefits from using sequence-based molecular typing on HIV for surveillance of transmitted drug resistance, transmission routes, and informing treatment guidance. A recent report (unpublished) made by a consortium of HIV antiviral drug resistance experts identified the potential for early detection and characterisation of antiviral resistance as the main public health benefit of HIV molecular typing. The report also concluded that HIV typing and sequence-based resistance monitoring are based on well standardised methods, provide comparable data across countries and were performed in 14 Member States as part of standard clinical practice and national surveillance programmes. There were previous EU research-sponsored projects dedicated to the collection and analysis of HIV typing data for resistance monitoring (SPREAD [FP5] and EuResist [FP6] projects) but since 2010 funding for these projects is no longer active. Although both the generic and HIV expert panels identified the centralised collection and analysis of HIV typing data as important, they also identified the need to agree representative sampling approaches and resolve the issue of data protection and access before any such scheme can be implemented.

As EU-wide collection and continuing analysis of HIV typing and drug resistance data was identified as technically mature and having potential for public health benefits, we recommend that development of a EU molecular surveillance HIV strategy and business case be considered in 2015, including solutions to data privacy and confidentiality issues.

Neisseria meningitidis

For *Neisseria meningitidis*, the systematic review identified MLST and surface protein gene *porA:fetA* sequencing schemes as being the most useful methods for identifying the genetic diversity of disease-causing strains as was recommended by an expert panel commissioned in 2009 within the IBD network. The expert panels both gave high priority to typing at the EU level and ECDC has indicated a need for centralised data collection for surveillance purposes, and in particular for monitoring the reduction in vaccine targeted invasive strains following introduction of new vaccine programmes. A molecular surveillance strategy to elaborate on the specific public health objectives that a system should support is therefore recommended in 2013. This would be followed by a business case in 2014 to analyse the resources available in Member States and draft the pilot testing phase methods and procedures for launch in 2015.

There are already databases which collects MLST and *porA:fetA* data on *N. meningitidis*, which are managed and funded by the University of Oxford, UK and University of Wurzburg, Germany. The business case needs to take into account interaction with the resources invested by these data managers, and their plans for how the database should be supported in the future.

Legionella pneumophila

The original systematic review did not identify any publications demonstrating public health utility of legionella typing. This was most likely due to the exclusion of local studies applied as part of the selection criteria of the systematic review. The May and June expert panels provided a number of relevant publications demonstrating the public health use of *L. pneumophila* typing for outbreak studies and source attribution. Molecular typing of *L. pneumophila* greatly enhances the resolution of cluster analysis and source identification as part of outbreak investigation and response. ECDC currently manages EPIS-ELDSNet for reporting and rapid communication of travel-associated legionella cases, and has actively supported capacity building for molecular typing of legionella through numerous EQAs and training activities, and will continue to do so through a new call for tender soon to be published. The main method for legionella typing is Sequence Based Typing (SBT; 7 loci MLST) for which there are agreed standards and protocols which are broadly applied on the European level. SBT data is currently submitted, stored and analysed in a database managed and funded by PHE, UK. In addition to SBT, PFGE is also commonly used for legionella typing.

Legionella surveillance was ranked as a top priority both in the June consultation and in discussions with Member States. However, there are unresolved issues related to *L. pneumophila* typing, such as infrequent use and application of existing resources for cross border outbreak investigations. The results from the activities included in the upcoming tender should form the basis for a disease-specific roadmap in 2014 and business case in 2015, where the public health objectives and practical challenges of using molecular typing data in *L. pneumophila* outbreak investigation are clearly described.

High-risk multidrug and extensively-drug resistant (MDR/XDR) pathogens

In Table 2 and 3 are listed the two pathogens and one health issue for which EU level molecular surveillance is recommended to monitor the cross- border dissemination of MDR and XDR strains of high public health risk, due to treatment failures and potentially untreatable infections [16]. Beyond the diseases for which continuous molecular surveillance as performed across Member States would permit integration in continuous EU-level surveillance, there is an unmet public health need for molecular surveillance of these transmissible antimicrobial resistant pathogens of major epidemiologic significance, caused by epidemic/pandemic clones and plasmids. These difficult-to-control resistant pathogens include MDR/cephalosporin resistant *Neisseria gonorrhoeae,* extensively-drug resistant (XDR) and carbapenem resistant *Enterobacteriaceae* and *Acinetobacter baumannii* as well as MRSA [17]. Both of the

roadmap expert consultations highlighted the public health added value of molecular typing of both strains (clonal strain typing methods) and antibiotic resistance genetic determinants (resistance gene profiling) for monitoring prevalence and risk factors associated with infections by MDR/XDR pathogens, and delineating cross-border and animal-to-human spread of epidemic resistant strains and epidemic resistance genes.

Recent ECDC disease specific pilot projects on MDR *N. gonorrhoeae* [4] and MRSA [5] demonstrated the feasibility of pan-EU/EEA structured molecular epidemiology surveys of prevalence of MDR pathogens that provided new information for control strategies. Concordant expert consultations recommended to ECDC to consider supporting the long-term surveillance of these issues through planning periodically repeated prevalence molecular surveys. Whereas these surveys have so far been performed using molecular databases hosted by national public health institutes and expert research laboratories, there is now an opportunity to explore the pros and cons of their integration into a bioinformatics-enabled TESSy platform to ensure integration with core surveillance, data security and system sustainability.

Neisseria gonorrhoeae

For *Neisseria gonorrhoeae,* there is recent evidence that supports public health benefits for molecular typing using the NG-MAST method, specifically identification and confirmation of outbreaks, transmission chains and monitoring emergence of antimicrobial resistance across Europe and globally. Although both expert panels have assigned a low priority to gonorrhoea typing, the 2011 ECDC-supported study of an EU-wide dataset revealed the emergence of multidrug resistant strains in Europe that may over time become a larger public health threat [4]. If European level collection of molecular typing data for gonorrhoea is to be implemented, it will most likely be to support investigations of the circulating multidrug resistant strains only. In addition, a number of areas (sampling strategy, period intervals, quality of epidemiological information, additional NG-MAST and other genetic markers evaluation, stability of associations between sequence type and antimicrobial resistance) require further investigation before implementation of molecular surveillance based on periodic prevalence surveys of (multidrug resistant) gonorrhoea can be established at the European level.

Extensively drug-resistant/carbapenem-resistant *Enterobacteriacaeae* (including *Klebsiella pneumoniae*) and *Acinetobacter baumannii*

For monitoring the XDR/carbapenem-resistant *Enterobacteriacaeae (K. pneumoniae, E. coli*, etc.) and *A. baumannii,* there are a number of strain typing methods available for outbreak and prevalence studies, including internationally validated, sequence based methods like the MLST schemes. In addition, there are established schemes for PCR or micro-array based profiling of XDR and carbapenemase encoding genes. However, definition of molecular antimicrobial resistance surveillance strategy and completion of pilot prevalence studies, as planned for 2013 [18], are still needed to identify which strain and drug resistance molecular markers could be monitored before a business case for EU molecular antimicrobial resistance surveillance scheme can be developed in 2014. Furthermore, discussion on joint molecular database management will be required with research groups and public health organisations currently administering global reference MLST databases and providing expert characterisation of carbapenemase/XDR genetic determinants.

Meticillin-resistant *Staphylococcus aureus*

For MRSA**,** the main public health objectives for typing are linked to monitoring the emergence and geographical spread of multiresistant clones in the community as spill-over from hospital sources or animal husbandry as well as a tool for targeting and evaluating control measures at Member State level. The systematic review identified many methods that have been used for *S. aureus* typing for public health purposes. These include PFGE, *spa* sequence based typing, MLVA and a combined MLST and SSC-*mec* analysis. Whole genome sequencing WGS is being developed for molecular typing of this pathogen. *spa* sequence based typing is currently recommended as the primary typing system that is fully operational across the EU, complemented by subset typing of resistance genes and SCC*mec* cassette.

Pathogens not proposed for molecular surveillance implementation by 2015

The summaries of collected evidence described in this section are for those pathogens for which ECDC does not currently see a need for implementation into an EU level molecular typing system within the next five years. This is however subject to change if public health priorities shift.

Bacillus anthracis

The systematic review did not identify any relevant studies for this pathogen. Two papers were subsequently added by the expert panels, and there are some data supporting the use of WGS for outbreak investigation. However, due to the low priority given by the two expert panels and the lack of requests for inclusion by ECDC anthrax is not considered for further studies by 2016.

Bordetella pertussis

The systematic review and June expert panel identified molecular typing as useful for identifying circulating strains and changing profiles over time, including before and after the introduction of the vaccine. At present, novel typing methods (MLVA, SNP typing) are emerging. Currently, the priority for European-wide molecular typing for this pathogen is low; however, this may change as a result of the increased prevalence observed in Europe.

Clostridium botulinum

The systematic review identified very few relevant studies for this pathogen, and the June expert panel concluded that further evidence for public health use and method standardisation efforts are required before application of molecular typing on the European level.

Clostridium difficile

The systematic review and the June expert panel identified a number of papers documenting well PCR ribotyping as useful for outbreak investigation as well as surveillance at the national level. MLVA is emerging as a useful and more discriminatory method that can be used to resolve common ribotypes during an outbreak investigation. Harmonisation between Member States of these typing methods and protocols is however needed. Provided that methods and protocols are agreed on within the network, ECDC foresees that collection of molecular typing data could be of value for surveillance purposes. The decision to implement a centralised data collection will depend on the national typing strategies and availability of resources within Member States. This will need to be further reviewed once agreement on methods and protocols has been reached.

Corynebacterium diptheriae

Evidence collected showed a link between molecular typing and public health intervention, especially for outbreak investigation purposes. At present, ribotyping (widely viewed as the gold standard) is being complemented/replaced by newer methods (MLVA, MLST) that are in a developmental and validation phase. Both expert panels assigned medium priority to the EU integration of typing data for this pathogen. Future steps for integration of this pathogen into an EU level molecular typing system need to include validation and standardisation of new typing methods as well as plans for interactions with existing databases for storing ribotyping data (currently hosted by PHE, UK) and MLST data (currently hosted by University of Oxford, UK). Due to the low annual case incidence and the small number of Member States currently able to perform diphtheria typing, a centralised typing service option could also be considered.

Coxiella burnetii

The evidence for public health usefulness for *Coxiella burnetii* molecular typing is limited. It was not deemed a high priority by any of the expert panels, and ECDC does not foresee any European-level molecular typing scheme by 2016.

Vancomycin-resistant *Enterococcus faecium* (VRE)

Both expert consultations (June and September) deemed the inclusion of typing data for VRE to be of low priority. ECDC also does not foresee any need for centralised collection of molecular typing data by 2016. However, if in the future TESSy will support the inclusion of data from point prevalence studies, this position should be reassessed.

Hepatitis A virus

The systematic review and June expert panel identified public health benefits from using sequencing (sub-genomic fragments and/or WGS) to trace HAV infection through risk groups, to link HAV outbreaks to food and environmental sources, and to monitor potential vaccine escape strains. The June expert panel recognised the need for a transnational sequence data sharing facility, and the September consultation also confirmed the importance of using molecular typing data in public health analyses. ECDC foresees the need to collect sequence data for outbreak investigation purposes; however, integration of HAV molecular typing data is not considered a priority in this version of the roadmap. Annual updates of the roadmap can however give HAV typing future priority. In addition, ECDC foresees the need to have a future network for foodborne viruses in place to provide information on Member State activities and feasibility aspects.

Hepatitis B virus

The systematic review and June expert panel identified public health benefits from using molecular typing (mainly sequencing) to track HBV transmission and for monitoring drug resistance and vaccine escape. In addition, there is good evidence in the literature for linking HBV genotyping to clinical presentation and disease progression monitoring.

The June expert panel recognised the need for a transnational sequence data sharing facility, such as The International Repository for Hepatitis B Virus Strain Data managed by PHE, UK [19]. However, due to the low priority given by the two expert panels it is not considered a priority for further studies until 2016.

Hepatitis C virus

The systematic review identified the potential use of HCV molecular typing data (mainly sequencing) for public health purposes such as surveillance of genetic diversity, drug resistance, treatment outcomes and disease severity, although much of the work done is still at a research stage and currently not directly applicable. Due to the relatively low priority given by the two expert panels and the lack of request for inclusion by ECDC, HCV is not considered a priority for further studies by 2016. However, there is a new, promising HCV vaccine currently under development; should that vaccine be routinely used in Member States then molecular typing to support monitoring of circulating strains etc. may be of higher priority.

Measles virus

The public health usefulness of measles molecular typing is well established, and both expert panels considered the sharing of measles typing data to be of high priority. However, there is no need for any additional efforts on the European level as this need is already met by the World Health Organization (WHO) global typing surveillance system.

Mumps virus

The evidence for public health usefulness of mumps molecular typing is limited. Mumps is part of the vaccine-preventable diseases monitored globally by WHO, and no additional action at the European level is needed.

Norovirus

The systematic review identified evidence for public health usefulness of typing of norovirus for outbreak investigation and source attribution purposes. In addition, monitoring of emerging strains is important for predicting population impact. The main method used is sequencing, and a global database for collection of norovirus sequences is managed and funded by RIVM, Netherlands [20].

Due to the relatively low priority given by the two expert panels and the existence of a global database norovirus is not considered a priority for further studies by 2016.

SARS virus

The evidence for public health usefulness for SARS molecular typing is limited and not seen as a priority by neither the expert panels nor ECDC. However, it should be acknowledged that molecular characterisation may play an important role when dealing with emerging pathogens.

Streptococcus pneumoniae

The systematic review and June expert panel identified molecular typing as useful for this pathogen, especially in relation to surveillance activities to support monitoring of vaccine programmes. However, it was not deemed a high priority by any of the expert panels, and ECDC does not foresee any European-level molecular typing scheme by 2016.

West Nile virus

The evidence identified in the systematic review for public health usefulness for West Nile virus molecular typing is limited, and ECDC does not foresee any European-level molecular typing scheme. The virus was however deemed a high priority by both expert panels, and given the emergence of disease in southern Europe and potential public health impact, European-level collection of molecular typing may need to be further investigated within the next few years.

Table 2 provides an overview of the pathogens proposals for implementation in terms of public health objectives and expected EU level risk assessment actions and risk management implications per proposed priority pathogen.

Table 2. Proposed priority pathogen, public health objectives and expected EU level risk assessment actions and risk management implications*

Pathogens/AMR issue proposed for implementation	Potential public health objectives*	Expected EU level risk assessment action(s)	Potential EU level risk management implication(s)
Salmonella enterica, Shiga-Toxin producing *E. coli* , *Listeria monocytogenes*	Outbreak investigation	ECDC rapid risk assessment, support to multicounty outbreak investigation and food source trace back (both jointly with food safety authorities)	RASFF notification, Food source control and vehicle recall
	Control-oriented surveillance	Early detection, verification and notification of dispersed cross-border clusters, Outbreak investigation	Coordinated outbreak control
	Strategy-oriented surveillance	Food source attribution of disease, Identifying population groups /food categories at increased risk of infection with specific strains	Targeted food chain decontamination interventions to eliminate the source of specific endemic strains
MDR *Mycobacterium tuberculosis*	Outbreak investigation	Support to multicounty outbreak investigation	Coordinated outbreak control
	Control-oriented surveillance	Identify and investigate high-risk strains ("super spreaders" and/or MDR/XDR-TB), Epidemic investigation	Coordinated epidemic control and revision of treatment guidance
	Strategy-oriented surveillance	Provide an overview of (MDR)-TB clusters and strain diversity in the EU, Identify high-risk geographical areas and/or population groups	Evaluation of TB control programme
Influenza virus	Outbreak investigation	Detection of potential pandemic influenza strains, virological Risk Assessment	Pandemic notification and public health response
	Control-oriented surveillance	Detection of novel influenza viruses,	Development of diagnostics and seed viruses for vaccines
	Strategy-oriented surveillance	Detection of genetic markers associated with antiviral resistance, Rapid epidemiological investigation Detection of genetic change in circulating influenza viruses,	Revision of treatment guidance Altering the strain selection for current vaccine (when combined with antigenic data)
Human Immunodeficiency Virus (HIV)	Strategy-oriented surveillance	Early detection and characterisation of antiretroviral resistant strains, Characterisation of transmission routes and risk groups	Revision of clinical treatment guidance, Evaluation of HIV prevention and control programme
Neisseria meningitidis	Outbreak investigation	Support to multicounty outbreak investigation	Coordinated outbreak control measures including strain-targeted vaccination
	Control-oriented surveillance	Identify cross-border epidemic strains	Coordinated epidemic control measures including strain-targeted vaccination
	Strategy-oriented surveillance	Monitoring of vaccine strain coverage and vaccine programme effectiveness	Revision of vaccine composition or national immunisation programmes
Legionella pneumophila	Outbreak investigation	Travel-associated cross-border outbreak investigation and source trace-back	Outbreak control through source elimination
Neisseria gonorrhoeae/MDR	Strategy-oriented surveillance	Monitoring of emergence and cross-border transmission of MDR strains, Identify high-risk geographical areas and/or population groups	Revision of clinical treatment guidance, Evaluation of gonorrhoea prevention and control programme
Enterobacteriaceae and *Acinetobacter baumannii* XDR/carbapenem-R	Strategy-oriented surveillance	Monitoring of emergence and cross-border transmission of XDR strains, Identify high-risk geographical areas and/or population groups	Evaluation and revision of healthcare infection control and prevention programmes including active surveillance of cross-border patient transfers
Staphylococcus aureus MDR/meticillin-R	Strategy-oriented surveillance	Monitoring of emergence and cross-border transmission of MDR strains, Identify high-risk geographical areas and/or population groups (including livestock contact)	Evaluation and revision of healthcare infection control and prevention programmes including active surveillance of cross-border patient transfers

RASFF: Rapid alert system for food and feed; * The public health objectives are categorised as described in Baker et al [6]

Table 3 lists the six pathogens/health issues proposed for strategy definition and business case studies in 2013–15, in addition to the four pathogens under pilot TESSy testing in 2012–2013. Indicative timelines are given for gradual development, testing and implementation.

The principles guiding the selection of priorities in this table are:

- disease or health issue for which EU molecular surveillance is necessary to inform public health policies and coordinated public health actions of Members States;
- pathogen with fully validated and portable (sequence-based) typing system in operation in a substantial proportion of Member States.

Table 3. Roadmap for 2012–2015 implementation of EU molecular surveillance per pathogen/antimicrobial resistance

Pathogens/AMR issue proposed for implementation	Typing method	EU public health objective/sampling design			Disease specific roadmap step/year			
		Surveillance, continuous	Surveillance, prevalence surveys	Multi-state outbreak investigations	Molecular surveillance strategy	Business case	System implement ation	Routine use from
Salmonella enterica	PFGE, MLVA	*		*	2011	2012	2013	2014
Shiga-Toxin producing *E. coli*	PFGE	*		*	2011	2012	2013	2014
Listeria monocytogenes	PFGE	*		*	2011	2012	2013	2014
MDR *Mycobacterium tuberculosis*	MIRU-VNTR, spoligotyping	*		*	2012	2012	2013	2014
Influenza virus	sequencing	*			2012	2013	2014	2015
Human Immunodeficiency Virus (HIV)	sequencing	*			2014	2015	2016	2017
Neisseria meningitidis	sequencing	*		*	2013	2014	2015	2016
Legionella pneumophila	sequencing			*	2014	2015	2016	2017
Neisseria gonorrhoeae/MDR	sequencing		*		2013	2015	2016	2016
Enterobacteriaceae and *Acinetobacter baumannii* XDR/carbapenem-R	PCR, sequencing		*		2014	2015	2016	2017
Staphylococcus aureus MDR/meticillin-R	PCR, sequencing		*		2014	2015	2016	2017

Pathogens in blue shaded rows are included in the TESSy molecular surveillance pilot project, 2012–2013

Pathogens in orange shaded rows are proposed as high risk multidrug and extensively-drug resistant (MDR/XDR) pathogens with prevalence surveys including molecular typing components planned

Roadmap implementation and evaluation

Roadmap implementation process

The implementation of molecular surveillance will be a four-step process for each pathogen or health issue shortlisted for potential implementation into EU wide molecular surveillance or epidemic preparedness system:

- ECDC will consult with disease network representatives to define a disease specific molecular surveillance and/or epidemic preparedness strategy. This includes validation and refinement of the public health objectives of molecular typing, expected benefits, and process and outcome success indicators.
- The ECDC Disease Programmes and disease networks will prepare a disease-specific business case describing the surveillance or outbreak sampling frame and study design, typing methods, data management resource and IT requirements, and a draft data collection and management protocol. On the basis of this report, ECDC will consult with Member States to get their feedback on the business case and a decision about implementation will be made.
- If the business case is endorsed, the implementation phase will take place.
- Following adjustments found necessary during the initial implementation phase, the technical system and any associated activities will become part of routine surveillance and epidemic intelligence/outbreak response support activities.

These four phases are described in more detail below:

The disease specific molecular surveillance and/or epidemic preparedness strategy

The definition of the disease specific molecular surveillance and/or epidemic preparedness strategy will include validation and refinement of the public health objectives and expected operational benefits of integrating molecular typing data for a particular pathogen and/or antimicrobial resistance issue. It will also propose process and outcome indicators of performance. The strategy will be drafted by the ECDC Disease Programmes in collaboration with a Disease Network either through an annual network meeting or an *ad hoc* meeting with a Typing Reference or Advisory Group. Some networks already have such groups whereas other networks may need to appoint them.

The business case

A template of topics to be covered in the business case report will be provided by ECDC. Key components will be:

- Strategy: Pathogen-specific public health objectives and benefits of TESSy integration of molecular typing data at the EU and Member State level
- Short, medium and long term process and outcome success indicators
- Description of the sampling strategy:
  - Assessment of whether the current sampling strategies are appropriate to fulfil the EU level public health objectives
  - Proposal of the sampling strategies and alternative strategies, e.g. repeated prevalence surveys
- Description of the molecular typing method(s) and nomenclature:
  - Availability of agreed typing method(s)
  - Description of the level of standardisation of the typing method(s)
  - Status of EQA/certification scheme(s) in EU and/or Member States
  - Recent/likely developments in terms of new typing methods
- Description of data management aspects that affect the choice of technical implementation:
  - Description of available EU or global typing databases:
    - Name, hosting organisation and funding
      - A description of what kind of collaboration/interaction is needed and/or desirable with this database
  - Data collection and analysis aspects:
    - Proposed list of variables (metadata)
    - Molecular data format: 'raw data' or 'normalised data'
    - A description of to what extent Member States are able to link their case-based epidemiological data to the isolate-based molecular typing data
    - Analysis plan and geospatial/bioinformatics functionalities:
      - A discussion on how the molecular typing data should be interpreted and used in an epidemiological context
    - A description of any integration needed with other systems and/or tools (i.e. EPIS platform)
- A description of the resource needs for molecular typing of this pathogen:
  - A review of the current practices of Member States in terms of overall availability of resources

    - List of twinning arrangements, centralised typing services etc. where relevant
    - If known, an indication of future availability of resources on the Member State level
  - A description of the resources needed at ECDC for supporting the management of the submitted typing data
    - Technical support
    - Analysis support
- Ethical and/or legal issues
  - Data protection and confidentiality aspects
  - Data ownership and intellectual property aspects
- Plans for technical training and expert meeting(s)

System implementation

If the business case is endorsed, the system implementation phase will commence. Based on the comments received during the business case consultation process, ECDC in collaboration with the disease network will define a system implementation plan. This plan will cover all technical aspects of and contain a clear timeline for the implementation phase. Some important areas that will require Member State involvement include:

Data curation, analysis and interpretation

Where appropriate, ECDC will follow up the needs identified in the business case with calls for tender for specific support services. One area where this is expected is the validation, analysis and interpretation of typing data, which will be of high importance.

EQA and certification scheme(s)

High data quality is a prerequisite for analysis and of vital importance for the success of any molecular typing data collection. Different typing methodologies will require different levels of EQA and other types of standardisation efforts. EQA schemes should also form the basis for certification and approval of direct acceptance of molecular typing data uploaded in TESSy where needed.

Through the ECDC Disease Programmes, EQA rounds covering key aspects of molecular typing capabilities will be executed within the relevant laboratories in the disease networks and Member States. The pathogen-specific molecular typing coordination group have an important role to play in terms of providing advice on the scope, content and frequency of EQA to support molecular typing capacity building within the network and ensure high quality outputs of the full system. For the certification process, the pathogen-specific molecular typing coordination group should support ECDC in presenting a practical solution that promotes maximum Member State participation while still ensuring the required data quality.

Training

Based on results from EQA or capacity analysis made as part of the feasibility study, ECDC will direct resources to support molecular typing training initiatives.

Upgrading and testing the system

If TESSy is proposed to be the system of choice for storing, integrating and analysing molecular typing data, ECDC will implement the necessary changes in the system. User acceptance testing and validation of the system, both internally and with a subset of Member State laboratories, will be executed as part of the implementation phase.

Routine molecular typing

As implementation phase ends, the technical system and any associated activities will become part of routine surveillance and epidemic intelligence/outbreak response support activities. All Member States will now be invited to contribute with data.

Timeline

Table contains a tentative timetable for the gradual roadmap implementation process. It is however important to note that each step may require either more or less time than indicated in the table, depending on disease-specific needs and issues. The timetable is of course also subject to revision if public health priorities shift.

Evaluation and revision

Revision of the roadmap

Due to the constantly changing nature of molecular typing for public health, it is anticipated that the roadmap document and associated list of priority pathogens is updated annually. This revision should incorporate input from the relevant disease networks and ECDC, previous evaluations of the roadmap implementation, as well as any strategic discussions on molecular typing held with stakeholder groups.

Evaluation of roadmap implementation

Regular reviews of the public health benefits of enhanced molecular typing data collection are very important, and therefore interim evaluations of typing data collection for each new pathogen are foreseen after about one year of operation. While this is clearly not enough time to be able to assess the success of any long-term public health objectives, a review of the short term public health objectives and success indicators as well as the functionality of the system, resource needs and associated activities (training, external quality assessment [EQA] etc.) will enable ECDC and Member State users to learn from the first year of operation and agree on minor changes needed to make the system more efficient towards reaching its long-term goals. This evaluation should be made by ECDC and the disease network with contributions on specific issues from relevant stakeholders, and should cover the following areas:

- review of how well the short term public health objectives addressed and success indicators have been achieved
- evaluation of the performance of the technical system
- summary of the data volume, coverage and quality
  - review of resources used so far, and expected future needs
- follow-up of related activities:
  - curation
  - training
  - EQA/certification

In addition, ECDC foresees a bi-annual evaluation of the public health added value from molecular typing data collection. This exercise will cover all pathogens for which molecular typing data has been implemented and will be led by the ECDC Microbiology Coordination Section and will summarise the ongoing molecular typing data collection managed by ECDC, review its short- and long-term public health objectives, evaluate the use of molecular typing data in ECDC's daily work, summarise resource needs (current and future) based on information gathered from the disease networks, and assess the 'value for money' aspect per pathogen.

General considerations for the development of disease-specific molecular surveillance strategies and business cases

Molecular typing data collation at the EU level

Need for EU-level data integration and analysis

The critical precondition for developing an ECDC system is to provide molecular typing analysis output that is needed for EU-level public health action and that is not available from any other place. Such information may include information regarding incidence and spread on a European level of a specific molecular type found in the Member State, and of baseline epidemiological data reported for cases with the specific molecular type. The cluster analysis tool included in the molecular typing pilot project is one attempt at providing this kind of information to the users, as is the future integration of structured molecular typing data in EPIS FWD v2. It is essential that a dialogue between ECDC and the Member States ensures that TESSy is making maximum use of the shared molecular typing data submitted and is delivering feedback of practical value for disease prevention and control to the users.

Resources and funding issues

Although the potential of molecular typing for improving public health is well established, it is clear that this can only be realised if adequate resources are available for producing and analysing the data.

As has already been described in the sections above, it is impossible to separate the discussion of resources from the discussion of data generation, analysis, use etc. The issue of resources cuts across all other issues, and must be addressed at several different levels and from different angles.

From the financial perspective, it is clear that any collection of molecular typing data at the European level will rely heavily on Member States using their own funds for the actual typing. It is however unrealistic to expect all Member States to have funds to type (or have someone else type) all pathogens, as Member States will naturally focus their resources for molecular typing on pathogens where typing has a maximum impact on their national public health efforts. It is critical that the pathogen-specific situation for each Member State is clear before any decision can be made about whether to collect typing data for that pathogen at the EU level.

Resource management also includes providing the people working in the field with the expertise needed as a result of new developments. The expert meeting 'Molecular epidemiology of human pathogens: how to translate breakthroughs into public health practice' which was organised by ECDC in November 2011 clearly recognised the need for advanced training and capacity building, especially in the field of bioinformatics, and for developing cross-disciplinary competences of public health professionals, as provided by programmes such as the European Programme for Public Health Microbiology (EUPHEM).

Point prevalence studies: alternative sampling design for molecular surveillance schemes

The potential benefits of the collection of molecular typing data through point prevalence studies have been described above. There are many potential advantages with also integrating molecular typing data from point prevalence studies in TESSy:

- Compliance with the ECDC mandate to manage EU-level surveillance systems for communicable diseases
- Sustainability: Ensures comparability of recurrent prevalence studies in a systematic manner
- Possibility of combining epidemiological and microbiological data.

When the first pathogen for which molecular typing data is collected through point prevalence studies is proposed for implementation, an evaluation of the concept of gathering molecular typing data through point prevalence studies in TESSy (or elsewhere) will be carried out.

Reporting frequency

The reporting frequencies needed for making efficient use of typing data for public health purposes depend on the pathogen investigated and the typing method(s) applied, but most of all on the public health objectives the typing data collection seeks to fulfil. Generally, for many surveillance-related objectives less frequent reporting is perfectly acceptable, whereas if typing data is to be used for outbreak investigation a near real-time reporting frequency is

needed. In this context, the term real-time is also disease dependent. For example, data needs to be more rapidly collected to be useful in an STEC outbreak investigation compared to a tuberculosis investigation.

It is imperative that the required periodicity of reporting is discussed and described together with the specific public health objectives. Before any decision is made to collect the data at the EU level, Member States (and ECDC) must assess whether their national systems would allow them to meet those reporting requirements, and what kind of resources would be required. Data collection of typing data for a specific pathogen should not be initiated unless a majority of Member States agree that the reporting frequency required for the data to be useful at the EU level can be upheld.

Where needed, ECDC should seek to support Member States in meeting these more frequent reporting needs. One way of doing this (described in the ECDC molecular surveillance concept paper [7]) would be through supporting Member States to establish automated ('machine-to-machine') reporting to TESSy, which in the longer term would require less resources on the Member State level. In parallel, ECDC should also ensure that other tools (templates, scripts etc.) to facilitate easy TESSy reporting are available to Member State users for whom automated reporting is not an option.

Cross-border strategies for generating data

In ECDC, various initiatives for increasing technical proficiency for or outsourced access to molecular typing are supported. These initiatives can include EQA, training, technique assessments etc. However, given the difference in Member States' laboratory systems, financial situation, disease burden and priorities, it is neither realistic nor cost-effective that all Member States should have the technical capacity to carry out typing for all the different human pathogens within their national reference public health laboratories. To increase access to the required typing services in a cost-efficient manner, ECDC should focus on establishing alternative ways of making molecular typing of relevant pathogens possible for all Member States.

One model is for ECDC to support and/or actively organise twinning arrangements between Member States. Such arrangements, which are currently organised directly by Member States on a bilateral basis, involve two Member States agreeing that one of them will process certain samples from the other Member State under some agreed terms of collaboration.

In the food and animal health sector, the European Commission has established a network of EU-wide reference laboratories, whose tasks also include typing of relevant samples from National Reference Laboratories in all Member States. ECDC and the EC are currently investigating the feasibility of EU-level reference laboratories for human samples [21].

Molecular typing data sharing at the EU and global level

It is important that the ECDC initiative to collect molecular typing data at the EU level is not carried out in isolation. Many human infectious diseases increasingly have a global reach. In addition to the severe acute respiratory syndrome and influenza $A(H1N1)_{pdm09}$ pandemics, pandemics in poultry of highly-pathogenic avian influenza (H5N1) virus, the intercontinental transmission of *Vibrio cholerae*, and the global spread of emergent antimicrobial resistance are exacerbated by global trade and travel. Local infectious disease events have the potential to develop rapidly into international public health emergencies. The specific epidemiologic profile of infectious agents varies because infectious agents can readily transfer between distinct reservoirs and exchange genetic material. Rapid detection, analysis and timely public health response to infectious diseases worldwide are crucial for the prevention and global spread of infectious diseases [22].

Open vs. closed sharing of typing data, data privacy and confidentiality aspects

The sharing of epidemiological data through TESSy has traditionally been restricted to a closed community, where access rights are role- and disease-specific and granted by Member States through a formal nomination process. The exact rules that control the data access in TESSy are described in the TESSy Data Access policy [23] which is periodically revised and approved by Member States. The molecular typing pilot project in 2012–2014 elected to follow the TESSy data access policy also for the typing data, and specific nominations have been received from Member States to grant specific users specific access to the data.

As typing data becomes increasingly complex, the data storage and analysis needs are also such that a collaborative approach is needed to make best use of the global resources and to get the most use out of the data. Next-generation sequencing techniques, which are expected to replace and/or complement existing molecular typing methods within a relatively short time period [24], are a particularly good example of where the management and analysis of the data will require such highly specialised and qualified expertise that it is simply not feasible that all public health institutions shall have this competence in-house. The expert meeting 'Molecular epidemiology of human pathogens: how to translate breakthroughs into public health practice' which was

organised by ECDC in November 2011 identified the central storage and curation of data is needed for public health application of NGS, and proposed that ECDC should (in global collaboration) be involved in setting the design and accreditation standards for genome-based surveillance databases.

An overall goal of the ECDC molecular surveillance initiative should thus be to strive towards maximum openness and sharing of molecular typing data while still respecting data privacy and confidentiality regulations. In the long-term, ECDC should work together with Member States to identify ways of sharing typing data also with the wider scientific community, without compromising Member States' data privacy and confidentiality restrictions. In addition, the TESSy Data Access Policy needs to better describe some issues where there are specific concerns related to the collection of molecular typing data. These include:

- data ownership
- user access and nomination
- scientific publication policy
- management of multinational clusters.

A revised TESSy data access policy document was reviewed and approved by ECDC's Management Board in November 2015.

Collaboration with existing typing initiatives on an EU or global level

Molecular typing data collection supported by ECDC should be well integrated with existing typing initiatives on an EU and/or global level. There is no added value from the EU side to create a competing molecular surveillance system where a functional one already exists, so every effort should be made to link in collaboration with these initiatives. However, it is ECDC's responsibility to ensure that all data collected through these initiatives are submitted by a user that has been nominated by an appropriate Competent Body. Specific agreements will need to be made between ECDC and each initiative to ensure that Member States' reporting requirement to ECDC can be adequately met by submission through a system external to ECDC.

Focus should be on simplifying data submission for the Member States, and on minimising reporting to more than one system and reporting of the same data in more than one format ('double reporting'). It may be that some double reporting is needed for a limited period of time (for example during a pilot phase where it is tested which is the preferred way of the Member States for sending molecular typing data), but then a plan is needed for ensuring that this double reporting is monitored and ultimately resolved.

Staying current – how to support and harmonise the use of new (state-of-the art) typing technologies

In a fast-developing research world, new typing methods for specific pathogens frequently become available. While it may be tempting from the scientific perspective to use the most advanced method available for typing, the requirements from the public health side are rather more conservative. In public health, molecular typing data, like epidemiological data, is primarily important as information for public health action. Therefore there are often high requirements that the data has been generated in a standardised and comparable way and that data is robust enough to be used for public health action. Data generated using a non-standardised and/or non-validated protocol may well be interesting from a research perspective, but usually cannot form the basis of a decision for public health action without further verification. Any sharing of molecular typing data at the EU level must therefore ensure that standardisation and validation issues are adequately addressed for the pathogens and methods used, and that there is a broad consensus amongst the data providers and users on these topics.

Supporting typing method standardisation and validating public health use

A molecular epidemiology expert consultation held in 2011 identified that ECDC has role in keeping Member States informed of developments, and in supporting activities that lead to validation of newer typing methods and bioinformatic tools that are customised for public health use [22]. In addition, an expert panel organised by ECDC in June 2012 considered that ECDC has a role in supporting international validation studies of new methods, and in developing consensus definition and co-ordination of the use of standard methods that have demonstrated epidemiological utility.

There was a strong consensus that clear definitions are lacking for these terms, especially for clinical and public health laboratories processing samples from human origin. The concept of standardisation is particularly difficult for bacterial pathogens where there is often a wide range of methods available which vary greatly in terms of need for standardisation and resources already invested to make data comparable between laboratories. The bacterial field cover highly standardised methods (PFGE for *Salmonella enterica, Listeria monocytogenes* and STEC; MLVA for *Salmonella* Typhimurium; MIRU-VNTR for *Mycobacterium tuberculosis* etc.) with community-agreed laboratory

protocols and comprehensive EQA schemes in place, to laboratory methods not standardised at all. For the viral pathogens, the frequent use of sequence-based methods implies a certain inherent level of standardisation, but there are still large differences in the levels of laboratory protocol harmonisation and data sharing. The expert panel concluded that the lack of validation studies published is likely due to the difficulty of having articles on this topic accepted and published in mainstream peer reviewed journals, and partly due to a lack of resources and/or interest at the local/regional/national/EU level to publish results of completed validation studies.

It is clear that the concepts of standardisation and validation are key issues to review in-depth for each pathogen-method combination considered for EU-wide data collection. It is also to be expected that additional efforts are needed for international validation studies of new methods and/or methods agreed to be applied at the European level.

Transitioning to next generation methods

Recent technological advances in molecular biology, specifically in sequencing and mass spectrometry, are likely to transform detection and typing methodologies applied in clinical and public health microbiology laboratories. The use of new mass spectrometry instruments and applications is likely to have the highest impact in clinical laboratories, especially as replacement of culture-based bacterial characterisation. This can influence the number of samples available for secondary testing that can be used for public health purposes. Next generation sequencing applications are predicted to first influence molecular typing as used for surveillance and outbreak investigations [22,25].

In November 2011, ECDC hosted the meeting 'Molecular epidemiology of human pathogens: how to translate breakthroughs into public health practice' to discuss with European experts the opportunities and challenges related to the introduction of these new methods into laboratory microbiology. Here it was concluded that next generation sequencing-based typing technologies represent a window of opportunity for public health application in the coming years. However, in spite of its great potential there are a number of hurdles for implementation of NGS for public health purposes. Without either well-utilised local instrumentation or contact with larger sequencing institutes, many public health institutes are excluded from the possibility of using NGS routinely. The time from sampling to results is generally too long and a major unresolved question is how genome data should be best analysed for epidemiological application. Harmonisation of quality parameters for NGS data production is needed. Routine removal of genetic regions with high variation gave rise to concerns that too much information is left out of analyses – a concern for the analysis of mobile genetic elements that are of particular interest for public health. To stimulate developments, collaborations in research networks, combining human and veterinary bacteriology, virology and epidemiology with genomic bioinformatics, molecular biology and population genetics should be facilitated and ECDC has a role to play to as information broker, capacity builder and natural hub for many of the involved laboratories [22].

In general, the sequencing community advocates for free sharing of genomic data, but it is acknowledged that translation into public health practice highlights several issues including ownership of data, confidentiality and national policies. During the November meeting, it was proposed that these aspects should be kept in mind when developing new bioinformatic tools and systems with adjustable access levels, depending on the users and purposes of using the information could be one solution to mitigate these obstacles [22]. ECDC is also participating actively in the initiative on use of genomics for global surveillance and outbreak detection and diagnostics, which is led jointly by the Danish Technical University (DTU), and the Food and Drug Administration (FDA), US. This initiative aims to create a global solution for NGS data sharing and analysis for multiple scientific disciplines, and it remains to be seen to what extent its tools and solutions will be suitable from the European public health perspective [24].

To further explore opportunities and address barriers within this field, ECDC will present a separate framework for NGS data handling for public health purposes at the European level by 2016. This date may however be brought forward if ECDC Disease Programmes or networks decide to support one or more projects where solutions to this issue are needed on a disease-specific basis.

Coordination with typing activities in food, feed and animal sectors

The European Commission in collaboration with ECDC and the European Food Safety Authority (EFSA) are leading an initiative to encourage the collation of data on molecular testing so that the linkage of molecular typing data from humans to similar types of data from food and animals is possible. Given that the data is provided in a timely manner, this would enable rapid detection of clusters and outbreaks, and suggest links with potential sources. Such linking would allow the speeding-up of epidemiological investigations, a better evaluation of the importance of certain foods and animal populations as sources of food-borne infections and outbreaks through case-by-case source attribution studies, and a better understanding of the epidemiology of food-borne infections.

Maximum use should be made from the existing networks (EURLs, ECDC FWD network, EFSA Task Force) and data collection systems so that any duplication is avoided, and both management and handling of information is under mandate of appropriate authorities/institutes. This is part of the EU follow-up/lessons learnt of the *E. coli* O104:H4 outbreak. The added value of a centralised molecular typing data sharing and analysis resource was clearly illustrated in the recent coordinated cross-border and inter-sectoral investigation of a *Salmonella* Stanley foodborne outbreak affecting ten Member States [26].

Due to the potentially very large financial and societal impact of the results of these types of joint analyses, the information access must be regulated between and monitored by the involved parties. An agreement on the data availability and use must be clearly established before any information is published.

Molecular surveillance of antimicrobial resistance

For several bacterial pathogens covered in this review, only multidrug- resistant strains have been included. The reason for this is that they have a public health impact related to the increased morbidity, mortality and costs associated with difficult to treat infections. In addition, multidrug resistance is amplified in Gram-negative pathogens such as *Klebsiella pneumoniae, E. coli, A. baumannii* both by epidemic dissemination of resistant strains but also by horizontal spread of plasmid and other transferable genetic elements between pathogens. During the expert consultations and Joint Strategy Meeting workshop, a suggestion was made to consider approaching the issue from the resistance marker perspective rather than from an individual pathogen point of view. ECDC has followed such an approach in its risk assessment recommendation for enhanced surveillance and early warning of extremely resistant pathogens of high public health impact such as carbapenemase-producing/carbapenem-resistant *Enterobacteriaceae* and *A. baumannii* [16].

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