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**TECHNICAL** REPORT

# ECDC strategic framework for the integration of molecular and genomic typing into European surveillance and multi-country outbreak investigations

**2019–2021**

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# ECDC strategic framework for the integration of molecular and genomic typing into European surveillance and multi-country outbreak investigations

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# Contents



# Figures



# Tables

# Abbreviations

| | |
|---|---|
| AMR | Antimicrobial resistance |
| ARHAI | Antimicrobial Resistance and Healthcare-Associated Infections (disease programme at ECDC) |
| BIGSdb | Bacterial Isolate Genome Sequence Database |
| CARD | The comprehensive antibiotic resistance database |
| CCB | Competent Coordinating Body |
| CDI | *Clostridium (Clostridioides) difficile* infections |
| C/CRE | Carbapenem- and/or colistin-resistant Enterobacteriaceae |
| cgMLST | Core genome multi-locus sequence typing |
| CPE | Carbapenemase-producing Enterobacteriaceae |
| CRAB | Carbapenem-resistant *Acinetobacter baumannii* |
| CRE | Carbapenem-resistant Enterobacteriaceae |
| DR | Drug resistance |
| EFSA | European Food Safety Authority |
| EEA | European Economic Area |
| ELDSNet | European Legionnaires' disease Surveillance network |
| ELiTE project | European *Listeria* Typing Exercise |
| EMERT | European Meningococcal Epidemiology in Real Time |
| ENA | European Nucleotide Archive |
| EPIS | Epidemic Intelligence Information System |
| EQA | External Quality Assessment |
| ERLTB-Net | European Reference Laboratory Network for TB |
| ESCMID | European Society of Clinical Microbiology and Infectious Diseases |
| ESGLI | ESCMID Study Group on *Legionella* Infections |
| ETMS | Event and Threat Management Solution |
| EU | European Union |
| EULabCap | EU Laboratory Capability Monitoring System |
| EURGen-Net | European WGS-based surveillance of carbapenem- and/or colistin-resistant Enterobacteriaceae network |
| EURL | European Union Reference Laboratory |
| EuSCAPE | European Survey on Carbapenemase-producing Enterobacteriaceae |
| Euro-GASP | European Gonococcal Antimicrobial Surveillance Programme |
| EVD | Emerging and Vector-Borne Diseases (disease programme at ECDC) |
| EWGLI | European Working Group on *Legionella* infections |
| FWD | Food- and Waterborne Diseases (disease programme at ECDC) |
| HA | Influenza virus haemagglutinin gene |
| HAV | Hepatitis A virus |
| HCV | Hepatitis C virus |
| HIV | Human immunodeficiency virus |

| | |
|---|---|
| HSH | HIV, AIDS, STIs and Viral Hepatitis (disease programme at ECDC) |
| IBD-LabNet | Invasive Bacterial Diseases Laboratory Network |
| IPD | Invasive pneumococcal disease |
| IRV | Influenza and other Respiratory Viruses (disease programme at ECDC) |
| MDR TB | Multidrug-resistant tuberculosis |
| MIRU-VNTR | Mycobacterial interspersed repetitive units – variable number tandem repeat |
| MLST | Multi-locus sequence typing |
| MLVA | Multiple loci VNTR analysis |
| MRSA | Meticillin-resistant *Staphylococcus aureus* |
| MSM | Men who have sex with men |
| MTS | Molecular typing system |
| NGS | Next generation sequencing |
| NMFP | National Microbiology Focal Point |
| NSFP | National Surveillance Focal Point |
| PCR | Polymerase chain reaction |
| PFGE | Pulsed-field gel electrophoresis |
| RIVM | Dutch National Institute for Public Health and the Environment |
| RRA | Rapid Risk Assessment |
| ROA | Rapid Outbreak Assessment |
| SBT | Sequence-based typing |
| SFTP | Secure file transfer protocol |
| SNP | Single-nucleotide polymorphism |
| SNV | Single-nucleotide variant |
| SRA | Short read archive |
| SSR | The Surveillance System Reengineering Project |
| ST | Sequence type |
| STEC | Shiga toxin-producing *E. coli* |
| TATFAR | Transatlantic Taskforce on Antimicrobial Resistance |
| TB | Tuberculosis |
| TB | Tuberculosis (disease programme at ECDC) |
| TESSy | The European Surveillance System |
| US CDC | Centers for Disease Control and Prevention of the United States |
| VNTR | Variable Number Tandem Repeat |
| VPD | Vaccine-Preventable Diseases (disease programme at ECDC) |
| WGS | Whole genome sequencing |
| WHO | World Health Organization |
| WNF | West Nile fever |
| WNND | West Nile neuroinvasive disease |
| WNV | West Nile virus |
| XDR | Extensively drug-resistant |

# Executive summary

Two ECDC publications are particularly relevant for the state-of-the-art integration of microbiology/microbiological data into outbreak investigations, public health surveillance, and improved disease control and prevention: the Centre's *Public health microbiology strategy 2018–2022* and the 2016 *Expert opinion on whole genome sequencing* (WGS). To add value to these publications, this strategic framework document presents a proposed list of priority pathogens/diseases and outlines technical implementation options for the medium-term integration (2019–2021) of molecular/genomic typing information into EU-level surveillance and multi-country outbreak investigations. This framework is intended to consolidate ECDC activities in this area that were initiated in 2012 to support EU/EEA Member States. It builds upon progress made earlier and draws on ECDC molecular/genomic surveillance roadmaps, the opinions of ECDC disease programme experts, and recent evidence for the public health benefits of molecular/genomic typing in surveillance and outbreak investigations under a One Health approach.

WGS is a fast-moving technology. The pace of change and operational capacities vary between pathogens/diseases and countries. ECDC therefore proposes to prioritise the implementation of WGS, depending on the disease and public health application. The following criteria were applied in this context:

- Public health priority and potential added-value for infection control offered by integrating molecular typing data into epidemiological investigations (e.g. early cross-border outbreak detection, accurate outbreak investigations, improved understanding of the determinants of incidence, transmission dynamics, or prediction of pathogen virulence and drug resistance).
- Feasibility of realising the potential added-value based on standardised and validated typing schemes and establishing fluid, semi-automated data management and analysis workflows.
- Proportionality of pooled typing resource capacity available at Member State and ECDC level required to realise the added value, including potential efficiencies to be achieved across systems and health sectors.
- Potential synergies and interoperability of information systems with those of the European Food Safety Authority (EFSA), the World Health Organization and national public health partners at EU and international levels.

Information related to these criteria was provided by literature reviews and a vast body of experience accrued within ECDC disease networks and molecular typing operations that conducted cross-border outbreak investigations and carried out European surveillance proof-of-concept studies in the past three years. In addition, a series of surveys among National Microbiology Focal Points (NMFP) was performed in 2015–2017 to map progress in the capabilities of national public health reference laboratories with regard to WGS for national surveillance and outbreak studies. Following the European Commission's mandate for One Health genomic surveillance of foodborne pathogens, these EU capacity mapping surveys were done in collaboration with concomitant surveys performed by EFSA among national reference laboratories for food safety. The strategic framework was revised based on the comments received from consultations with the National Focal Points for Microbiology and Surveillance (October 2018) and the ECDC Advisory Forum (February 2019).

Based on the above-mentioned criteria and in consideration of the evidence and partner consultations, ECDC proposes in this strategic framework a number of priorities for 2019–2021. In general, Member States should receive support for the gradual use of sequence-based typing so they can participate in joint response and surveillance operations with EU/EEA Member States. The following applications and pathogens (depending on preparedness for reporting of high-quality sequence data) are particularly relevant:

- **Outbreak investigation objective; support to multi-country outbreak investigations through sequence-based typing:** *Campylobacter* spp., *Clostridium difficile*, hepatitis A virus, *Legionella* spp., *Listeria monocytogenes*, multidrug-resistant *Mycobacterium tuberculosis* (MDR TB), *Neisseria meningitidis,* outbreaks of emerging multi- or extensively drug-resistant (MDR or XDR) bacteria, outbreaks of new pathogens or new modes of transmission of healthcare-associated or community pathogens, *Salmonella enterica*, Shiga-toxin producing *E. coli* and West Nile virus.
- **Control and strategy-oriented objectives; EU-wide sequence-based continuous surveillance:** influenza virus, *Listeria monocytogenes*, MDR TB*, Neisseria meningitidis, Salmonella enterica* and Shiga-toxin producing *E. coli*.
- **Strategy-oriented objective; sentinel surveillance or surveys**: antibiotic-resistant *Neisseria gonorrhoeae*, *Bordetella pertussis*, carbapenem- or colistin-resistant Enterobacteriaceae, carbapenem-resistant *Acinetobacter baumannii*, HIV-transmitted drug resistance, and *Streptococcus pneumoniae*.

It is proposed to explore jointly with EFSA the added value and feasibility of sequence-based surveillance for *Campylobacter jejuni/coli, Clostridium difficile* and hepatitis A virus, for which EU-wide implementation should be postponed until further review in 2021.

To implement the proposed operations, ECDC is developing a set of digital applications available in-house and/or externally that will be used to share, store and analyse sequence-based or WGS typing data. Applications will be

able to produce graphical representations of the genomic and epidemiological data analysis for risk assessment. These digital applications encompass the following functionalities: Data providers submit sequences as well as related descriptive and epidemiological data directly to the application; this implies machine-to-machine communication. The WGS data submission process should ensure that every isolate submitted to the WGS application contains corresponding epidemiological data, which can be retrieved from The European Surveillance System (TESSy). Depending on Member State policies for public WGS data access, WGS data could be uploaded to a protected, access-controlled, long-term storage solution and made public (for example after an initial embargo period). Associated epidemiological and WGS data will be jointly analysed with a high level of automation to identify and visualise transmission signals and patterns.

Implementation of the proposed actions will be subject to the availability of technical staff and operational resources as allocated in the ECDC Single Programming Document. Monitoring and evaluation will be carried out in accordance with the Centre's annual work plan. All outcome and impact indicators will be included in the Agency's annual reports. Detailed reporting will use indicators published in the Centre's *Public health microbiology strategy 2018–2022* and the *Surveillance strategy 2013–2020*.

Issues of optimised epidemiological and microbiological data linkage and timely reporting for control-oriented surveillance and outbreak investigations as well as re-definitions of outbreak and outbreak cases will be resolved through pragmatic problem solving and multidisciplinary discussions in a disease-by-disease manner.

ECDC will continue to liaise and collaborate with EFSA, EU research and development projects on applied pathogen genomics, WHO, and national public health partners at EU and international levels. These collaborations aim at developing international surveillance standards and sequence-based strain type nomenclature to ensure strategic coherence and operational coordination for cost efficient delivery of the strategic framework in the global context. ECDC will further collaborate with the Member States and provide support for managing the transition to genome-based typing methods by sharing technical guidance and providing multidisciplinary training in applied genomic epidemiology.

# Introduction

The inclusion of molecular typing information into European Union (EU)-level surveillance and multi-country outbreak investigations was proposed by ECDC in a concept paper [1], followed by strategic roadmaps on implementation in 2013 [2] and 2016 [3]. The rapid advances in state-of-the-art technology demonstrate the importance of regularly revising the priority list of pathogens/diseases: new public health needs lead to changing practices in molecular typing for public health and the increasing use of WGS for routine surveillance and outbreak studies.

At a joint strategy meeting in 2015, the ECDC Advisory Forum, the National Focal Points for Microbiology (NMFPs), and the National Surveillance Focal Points (NSFPs) recommended that ECDC consolidate ongoing molecular typing for surveillance projects and prioritise those diseases where it was possible to demonstrate convincing EU public health benefits. They advised that molecular typing for EU surveillance should undergo a step-wise transition to WGS. Essential in this context, the joint strategy meeting stated, were standardised methodology, external quality assessments, training, and validation and assessment of WGS added-value, along with an assessment of different technological options. It was recommended that ECDC should monitor the technological and the scientific knowledge developments and guide Member States by providing continuous updates on WGS cost, performance, and validation. In addition, the public health value of WGS should be assessed while national capacities should be mapped.

In 2016, an ECDC Expert Opinion on WGS for public health surveillance – a strategy to harness WGS to strengthen EU outbreak investigations and public health surveillance [4] – claimed that WGS-based typing would become the primary microbial typing method for the investigation of multi-country outbreaks and disease and antimicrobial surveillance in the EU by 2020, at least for bacterial pathogens. Consultations on this opinion lead to a number of requests for ECDC involvement:

- Multidisciplinary interpretation of information arising from the combination of epidemiological data and pathogen sequence characterisation to guide public health action.
- Contributing to a global agreement on WGS analytical approaches, epidemiological interpretation criteria and genomic strain nomenclature by pathogen and surveillance objective.
- Supporting a multistate evaluation of the public health effectiveness of WGS-based typing in disease surveillance programmes by measuring outcomes in disease prevention or outbreak size, before and after implementation.
- Training efforts to further develop the new, integrative field of genomic epidemiology; additional efforts to build a common knowledge level through continuous professional development.

In 2018, ECDC updated its genomic roadmap to a strategic framework: ECDC gathered input from the NMFPs and NSFPs, relevant disease networks, ECDC Disease Programmes and molecular surveillance operation groups; joint work was carried out together with the European Food Safety Authority (EFSA) and the European Commission (EC); strategic discussions were held with ECDC's Advisory Forum. At the level of EU/EEA countries, national plans were assessed, as were national WGS capacities in outbreak investigations and public health surveillance.

This document summarises the progress made in the areas of EU-wide enhanced molecular typing for disease surveillance, genomic surveillance, and multi-country outbreak support. It also documents how Member States use WGS in public health operations and reviews the current evidence for the effectiveness of WGS. Finally, this document proposes a strategic framework that sets out a revised list of priority pathogens/diseases for preparatory work and/or implementation in 2019–2021 (EU surveillance and support of multi-country outbreak investigations) and outlines the technological steps that are necessary to develop an integrated analytical framework that combines sequence-based typing data with epidemiological data at the European level.

# Strategic framework development: methodology

ECDC developed a molecular and genomic strategic framework based on a review of the ECDC roadmap 2.0 [3], taking into consideration the following inputs:

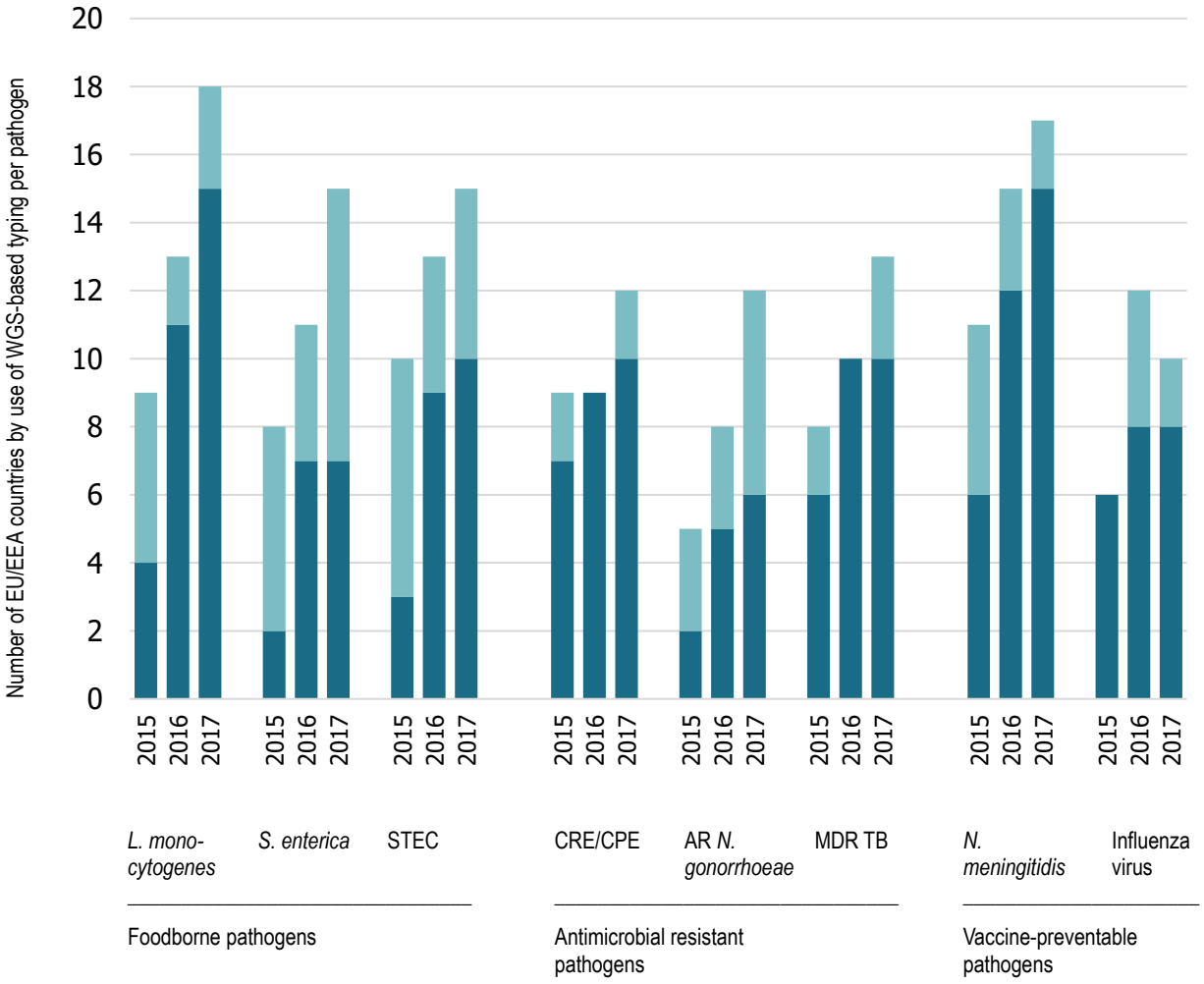
- ECDC updated disease-specific proposals for integration of molecular and genomic typing into EU-level surveillance and cross-border outbreak investigation: based on new public health needs and the development of new technologies, ECDC's Disease Programmes worked with the ECDC Microbiology Coordination Section to revise the list of priority pathogens to be considered for development or implementation in 2019–2021. The proposed typing methods and the public health objectives were also updated where necessary.
- ECDC conducted an internal evaluation of progress towards the previous roadmap implementation of EU-wide enhanced molecular typing and genomic surveillance as well as multi-country outbreak support. This included technical reports, publications, and data/indicators from the EU Laboratory Capability Monitoring System (EULabCap) (including the number of Member States reporting molecular typing data to ECDC through The European Surveillance system (TESSy) and the EU/EEA typing fraction expressed as notified case coverage in 2016).
- Annual ECDC-NMFP surveys on national plans and national capacity for WGS use in outbreak investigation and public health surveillance were conducted; disease-specific capacities and methods used at the EU/EEA country level in 2017 (and planned for 2019) were assessed [5].
- Inputs on the draft strategic framework document were received from consultations with the ECDC Advisory Forum and the National Focal Points for Microbiology and for Surveillance.

# Roadmap 2.1 implementation: progress report

## WGS for national surveillance and outbreak investigations in the EU/EEA

As recommended by the ECDC Competent Bodies and the ECDC Advisory Forum, ECDC mapped the use of WGS by national reference laboratories for specific public health applications (disease surveillance and outbreak investigations) in EU/EEA countries through annual surveys (2015–2017) directed at the NMFPs [5,6]. WGS-based typing use for routine surveillance of at least one human pathogen markedly increased from 0 countries in 2013 to 20 countries in 2017. In addition, the latest survey results indicate that by 2019, 29 Member States intend to use WGS-based typing for public health surveillance for at least one pathogen [5]. WGS-based typing was rapidly deployed across Europe for Roadmap 2 priority pathogens (Figure 1). Implementation was most extensive for *Listeria monocytogenes* and *Neisseria meningitidis*.

**Figure 1. Number of EU/EEA countries using WGS-based typing for routine surveillance and outbreak investigations (■) or only for outbreak investigations (■), by year and pathogen, 2015–2017**




*AR: Antibiotic-resistant; C/CRE: Carbapenem-and/or colistin-resistant Enterobacteriaceae; MDR TB: Multidrug-resistant* M. tuberculosis*; STEC: Shiga toxin-producing* E. coli. *The surveillance systems apply diverse sampling frames that range from survey-based to sentinel and comprehensive longitudinal sampling. These categories are not mutually exclusive, as several Member States have reported both current use and plans for further/expanded use of WGS.*

Considering the fact that the disease sampling fraction is a key predictor of sensitivity for outbreak detection by molecular typing in a population, WGS analysis of a substantial fraction of incident cases of a disease is more likely

to allow detection of clusters of linked cases in the EU/EEA. Data from the 2017 WGS survey suggest that this fraction is over 50% of notified cases for at least three diseases under national genomic surveillance (namely listeriosis, STEC infection and invasive meningococcal disease), based on a conservative estimate limited to countries reporting a comprehensive national sampling frame for WGS typing at that time (Table 1).

**Table 1. Pooled national genomic-based surveillance capacity, estimated as proportion of population covered and sampling fraction of cases notified by countries using comprehensive WGS-based surveillance, EU/EEA 2017**

| Pathogen/disease | % of EU/EEA population covered by WGS-based surveillance | % of EU/EEA notified cases tested by WGS-typing |
|---|---|---|
| *L. monocytogenes* | 47 | 52 |
| STEC | 33 | 51 |
| *Salmonella enterica* | 44 | 35 |
| MDR tuberculosis | 25 | 12 |
| Invasive meningococcal disease | 57 | 69 |

# ECDC Roadmap 2.1: implementation of activities

The lessons learnt after 32 months of implementation of ECDC Roadmap 2.1 differed markedly by type of public health operation: real-time support to cross-border outbreak investigation, continuous real-time surveillance, and multi-annual genomic epidemiology surveys.

**Outbreak investigations.** From November 2015 to June 2018, ECDC facilitated the investigation of 41 presumptive multi-country foodborne outbreaks caused by *S. enterica*, *L. monocytogenes* or STEC. Data were shared in a timely fashion, and a comparative analysis of WGS data was conducted, using patient epidemiological data (Annex 1). As part of these investigations, over 2 000 bacterial genomes were sequenced; about half of the investigations/analyses were funded by ECDC. While multicentre WGS data collation and phylogenetic clustering analyses were initially done by bioinformatics experts in the affected Member States, the management of these activities was increasingly taken over by an ECDC molecular operations team. Investigations confirmed 31 multi-country outbreaks and identified the food source for 12 of these outbreaks (see Annex 1 on ECDC Rapid Risk Assessments and Joint Rapid Outbreak Assessments with EFSA).

Apart from foodborne bacterial outbreaks, multi-country outbreaks caused by other pathogens were investigated jointly by ECDC and affected Member States, using WGS or gene sequence analysis. Outbreaks included both person-to-person and foodborne cross-border outbreaks of hepatitis A virus. Outbreak detection was supported by gene sequence typing and the application of an international genotype nomenclature (HAVNet platform, managed by RIVM) [7]. Likewise, two multi-country outbreaks of drug-resistant tuberculosis were detected in Europe in 2017 and investigated, with ECDC supporting WGS-typing data production, exchange and phylogeographic/epidemiologic analysis [8,9]. Both investigations helped identify the likely place of strain transmission and guided case-finding for treatment and control [8,9]. Finally, in June 2018, a travel-associated, multi-country outbreak of carbapenem-resistant *Klebsiella pneumoniae* ST-392 was detected by analysis of epidemiological and WGS data among patients returning to Sweden and Norway from a hospital in Spain [10]. These successful investigations highlight the need for EU-wide sharing of viral sequence or bacterial WGS data in near real-time together with epidemiological data for delineation and resolution of international outbreaks in both community and healthcare settings. These public health applications of sequencing technology clearly demonstrate the value of versatile sequence data management ; they also show a clear need for ECDC to provide scalable response support to forthcoming multi-country outbreaks.

**EU surveillance**. Several countries prepared for the transition from molecular to genomic typing integration and continuous, real-time surveillance at the EU level for outbreak detection of diseases as prioritised by the Member States (i.e. listeriosis and invasive meningococcal disease). Recent surveys of EU/EEA national surveillance methods indicate that although data analysis and storage is mostly performed in-house, there is a tendency in bioinformatics to use standardised approaches, e.g. the use of core genome MLST (cgMLST) for clustering clonal types or querying international databases for resistome prediction. ECDC work has focussed on developing and testing practical, high-throughput and secure data sharing platforms and building consensus on international nomenclature with experts from academia, EU disease networks, EFSA, and international public health partners (see below under Disease Programmes).

In order to monitor trends in multidrug-resistant gonococci and Enterobacteriaceae, several pilot tests for pan-European cross-sectional surveys have established powerful genomic epidemiology methodologies, landscapes of resistance genes, and high-risk clones genome libraries as baseline references for future surveys (see below under Disease Programmes).

Progress with Roadmap 2.1 [3] action implementation during the period January 2016–June 2018 is summarised in Table 2; the text below describes the state of play by ECDC Disease Programme and target pathogen.

**Table 2. Mid-term implementation of ECDC Roadmap 2.1 proposed actions for molecular typing integration into European surveillance and multi-country outbreak investigations**

| Pathogen | Action | Status |
|---|---|---|
| **ECDC programme: Antimicrobial Resistance and Healthcare-Associated Infections (ARHAI)** | | |
| *Clostridium difficile* | Evaluation of EU surveillance feasibility and utility with PCR-ribotyping (2016–18) | |
| | WGS: monitor progress of international validation of WGS-based typing | |
| Carbapenem-/colistin-resistant Enterobacteriaceae | Business case based on EuSCAPE 2014 survey (2016) | |
| | Development of protocol for WGS-based EU survey (2016) | |
| | First WGS-based EU survey (2017–18) | |
| Meticillin-resistant *Staphylococcus aureus* | *spa*-typing pilots (Staphylococcus Reference Laboratory project) | |
| | Development of genomic typing business case | |
| **ECDC programme: Food- and Waterborne Diseases and Zoonoses (FWD)** | | |
| *Listeria monocytogenes* | Integration of PFGE/WGS quality assurance. Maintenance of PFGE EU data collection and analysis system extended to EFSA collaboration (2016–18) | |
| | Development and testing of WGS-based surveillance, including quality control, core genome allele nomenclature and epidemiological validation [11-13] (2015–18) | |
| | Implementation of WGS-enhanced surveillance (2018–19) | |
| *Salmonella enterica* | Integration of PFGE/MLVA/WGS quality assurance and EU data collection and analysis system extended to EFSA collaboration (2016–18) | |
| | WGS: focus on data sharing and analysis for outbreak investigation including testing of open access analysis platforms (2016–19) | |
| | Contribution to design and testing of international allele and strain WGS nomenclature | |
| Shiga toxin-producing *E. coli* (STEC) | Integration of PFGE/WGS quality assurance. Maintenance of PFGE EU data collection and analysis system extended to EFSA collaboration (2016–18) | |
| | WGS: focus on data sharing and analysis for outbreak investigation including testing of open access analysis platforms (2016–19) | |
| | Contribution to design and testing of international allele and strain WGS nomenclature [14] | |
| **ECDC programme: Emerging and Vector-Borne Diseases (EVD)** | | |
| West Nile virus | Development of molecular typing strategy | |
| **ECDC programme: HIV, AIDS, STIs and Viral Hepatitis (HSH)** | | |
| Antibiotic resistant *Neisseria gonorrhoeae* | Development of business case based on pilot Euro-GASP WGS survey 2014 (2016) | |
| | Pilot WGS-based EU survey based on Euro-GASP 2014 (2017–18) | |
| HIV drug resistance | Map capacity among EU/EEA countries | |
| | Development of antiviral resistance surveillance strategy | |
| Hepatitis C virus | Map capacity | |
| | Development of antiviral resistance surveillance strategy | |
| **ECDC programme: Influenza and other Respiratory Viruses (IRV)** | | |
| Influenza virus | Implementation of business case in relation to surveillance for emerging pandemic (including zoonotic) strain detection and drug susceptibility monitoring | |
| | Implementation of sequence-based surveillance | |
| **ECDC programme: Tuberculosis (TB)** | | |
| Multidrug-resistant *Mycobacterium tuberculosis* | Maintenance of MIRU-VNTR external quality assessment and EU data collection and analysis (2016–18) | |
| | WGS: monitoring of progress of international validation of WGS-based identification, drug resistance prediction and typing nomenclature (2016–19) | |
| **ECDC programme: Vaccine-Preventable Diseases (VPD)** | | |
| *Neisseria meningitidis* | Development of business case based on strategy for TESSy-EMERT integrated analysis and pilot survey (2016) | |
| | Implementation of case based-WGS data linkage (2017–18) | |
| | Implementation of WGS based surveillance (2018–19) | |

*Legend: Actions fully achieved ( ), in progress ( ), not implemented ( ).*

# Antimicrobial Resistance and Healthcare-Associated Infections Programme

## Carbapenem-/colistin-resistant Enterobacteriaceae

In 2016–2017, as proposed in the Roadmap 2.1, ECDC developed – in consultation with external experts, the ECDC Advisory Forum and the Competent Bodies – a business case and protocol on genomic surveillance of carbapenem- and/or colistin-resistant Enterobacteriaceae (C/CRE) based on repeated European, multi-centre sentinel surveys following the EuSCAPE study model [15]. The objective of the surveys is to determine the occurrence, geographic distribution and population dynamics of high-risk C/CRE clones, and/or transmissible resistance/genetic elements within the European healthcare setting to inform risk assessment and control policies. In 2017, the European Antimicrobial Resistance Genes Surveillance Network (EURGen-Net), which is composed of National Technical Coordinators from 37 EU, EEA and EU enlargement countries, was set up for the recruitment of representative hospitals and the systematic collection of strains and epidemiological information. ECDC revised the draft EURGenCCRE survey protocol in consultation with EURGen-Net experts [16]. The next survey will be conducted in 2019.

## *Clostridium difficile*

As proposed in the Roadmap 2.1, the feasibility of implementing molecular surveillance of *Clostridium (Clostridioides) difficile* infection (CDI) by PCR ribotyping was established in 2016–18. Based on the latest European capacity survey, 21 EU/EEA countries had capacity for either conventional or capillary PCR ribotyping of *C. difficile* in 2014 [17]. As planned, the ECDC-funded project 'Microbiological Support to European Surveillance of *C. difficile* infections' (2016–2020), hosted a laboratory workshop in 2017 for EU/EEA countries that finalised an ECDC standard operating procedure for capillary PCR ribotyping [18]. A recent external quality assessment showed acceptable reproducibility among 20 reference laboratories for identifying the most common *C. difficile* ribotypes in Europe according to standard ribotype nomenclature (technical report in preparation).

ECDC started collecting surveillance data for CDI from EU/EEA countries in January 2016 [19]. Hospital participation is voluntary, using either of three surveillance modules for at least a quarter each year. In the enhanced surveillance module, 10 consecutive *C. difficile* clinical isolates per hospital are PCR ribotyped, and results are reported to ECDC quarterly. In 2017, 11 EU/EEA countries reported this information on nearly a third of all reported cases. Data analysis and reporting of hospital/national results is done semi-annually by ECDC, including analysis of local exceedance of case/type incidence per hospital.

To confirm clonality of *C. difficile* strains that share a ribotype (for example during outbreak investigations), second-line typing with a higher discrimination method can be performed either by multilocus variable-number tandem-repeat analysis (MLVA) or WGS typing [20]. A standard core genome MLST nomenclature has recently been proposed for *C. difficile* [21]. WGS-based typing was seldom used for national CDI surveillance in Europe in 2015 [5]. A mapping survey of molecular and WGS-based typing practice within the European CDI surveillance network was conducted in 2018 and results will be published in 2019.

## Meticillin-resistant *Staphylococcus aureus*

After the European *S. aureus spa* typing study [22] no further work was undertaken by ECDC on the development of a business case or a survey protocol for WGS-enhanced MRSA surveillance or surveys at the European level due to the stabilising incidence of MRSA invasive infections across a large part of the EU/EEA [23]. This change of plan was due to prioritising available resources in order to address the more pressing issues related to epidemic spread and rising incidence of infections caused by multidrug-resistant gram-negative bacteria in Europe.

# Food- and Waterborne Diseases and Zoonoses Programme

## *Listeria monocytogenes*

In 2016, 18 EU/EEA countries genotyped *L. monocytogenes* by PFGE or WGS for more than 80% of the reported cases [24], and 13 countries submitted PFGE data to ECDC. This number decreased to three countries in 2017 as PFGE was replaced by WGS for surveillance of listeriosis and/or outbreak investigations in 18 countries [5].

Currently, the joint ECDC–EFSA molecular typing database supports PFGE but not WGS data. However, a joint ECDC–EFSA working group is assessing various options for setting up and running an EFSA–ECDC pipeline for collecting and analysing WGS data. The report is expected for April 2019. Twenty EU/EEA countries were engaged in the joint ECDC–EFSA database and contributed to the upload of PFGE data on 2 907 clinical isolates of *L. monocytogenes* during the period Nov 2012–Sep 2017. As of 2017, 22% of the *L. monocytogenes* isolates were part of multi-country clusters. To foster the detection of probable/possible food, feed, animal and environmental *L. monocytogenes* isolates in a WGS-confirmed multi-country outbreak, ECDC aims to ensure that an outbreak PFGE profile, defined by the two enzymes *Apa*I and *Asc*I, is made available for comparison in the national databases, European Union Reference Laboratories (EURLs) and in the ECDC–EFSA joint molecular typing database.

ECDC's operational surveillance objectives and the Centre's guidelines on the use of molecular typing data were redefined in 2017. For outbreak investigation, ECDC initiated ad hoc WGS data collection and analysis using the BioNumerics software [11]. The European *Listeria* Typing Exercise (ELiTE) II validation study demonstrated that collection of assembled genomes using national pipelines yields acceptable data for centralised cgMLST analysis for outbreak investigations [13]. The TESSy molecular typing platform has been enabling raw or assembled WGS data sharing and analysis since June 2018. A ring trial to assess the reproducibility of *Listeria* genome assembly practices in Member States was executed in 2018, and data collection followed by launching comprehensive WGS-based surveillance in January 2019. In addition, an international vision on the integration of WGS to global surveillance of foodborne diseases has been agreed in the PulseNet International community [14].

### *Salmonella enterica*

ECDC continued to support the reporting of PFGE and MLVA type to TESSy. As of 2017, 37% of the >20 000 *Salmonella enterica* isolates with typing data available in TESSy were part of 903 multi-country clusters. These signals triggered 304 cluster investigations. In 2016, a total of 11 (~450 isolates) and 9 (~ 2 200 isolates) EU/EEA countries submitted PFGE and MLVA data to TESSy, respectively. A drop was recorded in 2017, with only 4 (~180) and 7 (~1 800) EU/EEA countries submitting PFGE and MLVA data to TESSy, respectively. At the same time, the use of WGS for salmonellosis surveillance increased from two to seven EU/EEA countries (Fig. 1).

ECDC's operational objectives for the collection and analysis of molecular typing data for salmonella surveillance and outbreak investigations were redefined and operationalised in 2017, based on routine weekly analysis of MLVA and PFGE clusters, followed by WGS verification and investigation of confirmed clusters. ECDC initiated ad hoc WGS data collection and analysed the data with BioNumerics software. Since November 2015, ECDC has supported WGS-based assessment of 31 signals of *S. enterica* outbreaks and investigated seven multi-country outbreaks of *S.* Enteritidis and four of other serotypes (Annex 1).

In addition, WGS-based prediction of salmonella resistance phenotypes (and molecular determinants of resistance) is increasingly used by reference laboratories in the EU Member States [5]. The collection of these types of data will be discussed with the disease network, with the aim to include these data into the EU protocol for harmonised monitoring of antimicrobial resistance in human salmonella and campylobacter isolates [25].

### Shiga toxin-producing *Escherichia coli* (STEC)

PFGE data were reported by 13 countries during the period, but very few outbreaks were detected. As of 2017, 4% of 2 015 STEC isolates with typing data reported to TESSy were part of 16 multi-country clusters. The last external quality assessment performed in 2016 demonstrated that the majority of participating laboratories were able to produce comparable PFGE typing results [26]. While in 2016 a total of eight EU/EEA countries submitted PFGE data, participation in 2017 dropped to three EU/EEA countries. At the same time, the use of WGS for STEC surveillance increased from three to nine EU/EEA countries (Figure 1).

The operational objectives for the molecular typing collection was redefined in 2017. WGS became the primary method for outbreak verification and support to outbreak investigation, with a total of 15 EU/EEA countries routinely using WGS for outbreak investigations [5]. WGS was applied to investigate two outbreak signals and proved valuable for outbreak confirmation/rejection purposes.

## Emerging and Vector-Borne Diseases Programme

### West Nile virus

Roadmap 2.1 proposed that the EVD Disease Programmes postpone the development of operationalisation of human West Nile virus (WNV) due to fact that the virus only affected a limited number of EU/EEA countries, with low health impact and low incidence [3]. However, over the last decade, significant outbreaks of West Nile fever (WNF) and West Nile neuroinvasive disease (WNND) were observed in humans and animals in Europe [27,28]. In 2018, a particularly remarkable increase of human and animal WNF and WNND was reported in the EU and surrounding countries (approximately fourfold increase compared to the previous years) [29]. Preliminary genetic studies indicate that the more severe infections were caused by lineage-2 strains [30]. Monitoring the circulating strains is therefore of public health value for preparedness and response. WNV infections in humans can remain asymptomatic, and asymptomatic people may transmit the virus via blood donation [31-33]. Contaminated blood poses a public health risk, which is why blood safety measures are taken in WNV-affected areas [34]. ECDC implemented weekly monitoring of WNV in Europe to identify affected areas [35].

In light of the 2018 increase in cases, ECDC discussed the need to develop a plan for EU sequence-based surveillance and coordinated multi-country outbreak investigations of human WNV infections with experts from EVD-LabNet (Emerging and Vector-Borne Diseases laboratory network) during the 2018 annual network meeting.

In Europe, different genetic lineages of WNV are circulating, although some of them (lineage 3, 4 and 7) were only detected in mosquitoes [36]. Lineage 1 has been endemic in certain regions for decades [37]. Viruses of genetic lineage 2 emerged in the last 15 years and have spread in Central Europe and the Mediterranean Basin [38,39]. Therefore, cross-border outbreak investigation, including sequencing of viruses, is proposed. Two EQAs showed

that the detection of lineage-2 WNV can be challenging for some laboratories [40,41]; ECDC will therefore consider to continue its WNV EQA activities for molecular detection and genotyping.

# HIV/AIDS, Sexually Transmitted Infections and Viral Hepatitis Programme

## Antibiotic-resistant *Neisseria gonorrhoeae*

As proposed in the Roadmap 2.1 [3], ECDC, in consultation with the Advisory Forum and the Competent Bodies, developed a business case for surveillance of antibiotic-resistant *N. gonorrhoeae* by repeated genomic epidemiology surveys in 2016. In this context, ECDC also took preparatory steps for TESSy data integration and reporting. In 2017, ECDC co-funded a pilot study for typing methodology evaluation using the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) 2013 survey isolates, which were – together with epidemiological information – collected from 1 054 patients in 21 EU/EEA countries. This work was done in collaboration with the Centre for Genomic Pathogen Surveillance, the Wellcome Sanger Institute, Public Health England, and Örebro University Hospital. The results demonstrated the superior resolution and more robust delineation of genomic lineages and clustering of strains by WGS-based phylogenomic analysis as compared to the previously used methods, multi-antigen sequence (NG-MAST) typing and multilocus sequence typing [42,43]. WGS analysis provided enhanced and more detailed understanding of the emergence, distribution and persistence of multidrug-resistant gonococcal strains in various risk groups, both nationally and internationally.

The study data were used to provide a baseline genome library for future molecular surveillance for *N. gonorrhoeae* in the EU/EEA. The authors also proposed a new web application to add new genomic data that would enable phylogenetic analysis and antimicrobial resistance prediction [44]. The next WGS-based typing survey will be based on the Euro-GASP isolates collected in autumn 2018.

## Human immunodeficiency virus (HIV)

Based on a capacity analysis performed in 2016, 19 of 21 responding countries reported that HIV sequence data were used at the national level for monitoring HIV drug resistance (HIVDR) [45]. In addition, 19 countries use HIV sequence data at the national level for the assessment of HIV subtype, and 15 countries use phylogenetic analysis of transmission events. Of 21 responding countries, 13 reported using HIV sequence data (subtype and/or DR) for surveillance purposes at the national level. Of those, nine stated that clinical, epidemiological and sequence data were routinely linked for analysis [45]. A pilot study was conducted in 2017 to assess the Member States' capabilities to report HIVDR data and test different reporting options (case-based data with sequence, interpretation of susceptibility, mutation codes or aggregated data) [46]. In the pilot study, the population of interest was newly diagnosed treatment-naïve HIV patients tested prior to initiating HIV treatment for susceptibility to any of the 22 available ARV drugs in the four main drug classes before initiating HIV treatment. Patients transferring care who had previously been on treatment were excluded. Pre-exposure prophylaxis (PrEP) was not considered as treatment, and cases exposed to PrEP were included. The pilot study showed that standard reporting of HIVDR data was feasible in the participating nine countries. Legal barriers for data sharing and standardisation of interpretation algorithms remain to be clarified in the process of developing an EU-wide HIVDR surveillance system [46]. Discussions are being held with WHO to see if they can host a common data platform to avoid certain data sharing issues. In the meantime, data on transmitted HIVDR will be collected in a simplified aggregated format.

## Hepatitis C virus (HCV)

ECDC performed a capacity mapping of HCV molecular typing activities in 2016. Half of the 16 countries that participated in the survey reported that they monitor HCV genotypes, mostly for specific clinical indications [47]. At the moment, the laboratory capacity for HCV genotype surveillance in the EU/EEA is still limited and therefore no further actions were planned. Data ownership issues, human resources and legislation issues are all significant challenges that would need to be addressed at the national level before HCV genotypes could be widely reported in the EU/EEA [47]. A variable for 'genotype' was recently added to TESSy, but completeness of reporting for this variable is still very low (<5%), which makes it likely that this variable will be discontinued. It is proposed to cancel further work on molecular typing for European HCV surveillance.

# Influenza and other Respiratory Viruses Programme

## Influenza virus

During influenza seasons 2009–2010 to 2017–2018, 25 EU/EEA Member States reported more than 39 000 haemagglutinin (HA) sequence identifier (ID) numbers in TESSy. All sequences are stored in the EpiFlu influenza sequence database, which is publically accessible and hosted by GISAID, the Global Initiative on Sharing All Influenza Data (https://www.gisaid.org/). Sequences can be retrieved by reported identifier numbers for analysis.

Routine weekly sequence-based data collection on influenza viruses has been established in TESSy, and virus characterisation reports are published weekly [48]. In addition to HA, data are available on neuraminidase gene sequence and $IC_{50}$ phenotypic testing [48]. Detailed genetic analyses (on a seasonal basis) were prepared on the relationship of European influenza viruses to global and regional patterns [49-51]. Influenza virus characterisation reports are provided jointly by ECDC and the WHO Regional Office for Europe to WHO Headquarters twice a year for northern and southern hemisphere influenza vaccine composition consultation; quarterly avian influenza summary reports are produced together with EFSA [52].

# Tuberculosis Programme

## Multidrug-resistant *Mycobacterium tuberculosis*

The Roadmap 2.1 proposes maintaining molecular surveillance of MDR TB (based on MIRU-VNTR typing) at the EU level. It supports the development of technical solutions for WGS-based typing until the required technical capacity was met at the EU level and more evidence on its added value for routine surveillance was obtained. In 2016 and 2017, rapid progress was noted in both areas. The number of EU/EEA countries applying WGS-based typing for MDR TB surveillance increased from 6 to 10 countries between 2015 and 2017 (Fig. 1). Pilot studies showed the added value of WGS for diagnosing TB as well as for detecting and tracing TB transmission events [53-56]. Several international MDR TB outbreaks were detected and resolved using WGS technology. Between 2015 and 2017, MIRU-VNTR was phased out as the gold standard typing method and replaced by WGS in several Member States. As part of the ongoing reference mycobacteriology quality support within the European Reference Laboratory Network for TB (ERLTB-Net), an EQA scheme for *M. tuberculosis* was developed for WGS-based typing. A pilot test was conducted in 2015, and further development took place in 2016 and 2017: 13 laboratories participated, and all reported comparable results; the inter-laboratory reproducibility of the WGS genotyping results for a core set of genes was excellent. This was also shown in the cross-border TB outbreak investigations supported by ECDC in 2017 [9].

In 2017, ECDC launched a pilot genomic TB surveillance development and validation project called EUSeqMyTB [57]. The project aims to standardise WGS analysis, build capacities, establish a digital data integration platform and collect data for a baseline EU/EEA *M. tuberculosis* genome library, including from EU/EEA Member States that do not yet have capacity for *M. tuberculosis* WGS typing.

# Vaccine-Preventable Diseases Programme

## *Neisseria meningitidis*

As outlined in Roadmap 1.0 and 2.1, genomic surveillance of invasive *N. meningitidis* disease was prioritised as a lead pilot application, thanks to the availability of validated WGS-based phylogenomic-typing and capsular/protein antigen typing schemes, standard allelic based genomic type nomenclature and curated database [58]. The extensive EU/EEA capacity for WGS-based surveillance of meningococcal infections was confirmed in 15 countries that applied WGS routinely on close to 70% of all notified invasive cases in the EU/EEA in 2017 (Figure 1 and Table 1).

As proposed, a business case was discussed with the Invasive Bacterial Disease Laboratory Network (IBD-LabNet) to link WGS-derived data to case-based epidemiological data at the Member State level; this included an interface between TESSy and EMERT (European Meningococcal Epidemiology in Real Time), a protected workspace within the PubMLST.org/neisseria database, using the BIGSdb analytical tools for integrated genomic epidemiological surveillance. In 2018, this project was initiated in collaboration with the IBD-LabNet consortium to enable Member States to submit data to EMERT and to ECDC. An essential baseline surveillance resource was established by IBD-LabNet with the publication of the open access European meningoccocal strain collection genome library, which is composed of 799 assembled and annotated genomes and 1 605 annotated core genome loci of all invasive meningococcal cases collected in 16 EU/EEA countries in the epidemiological year 2011–2012 [59]. The study confirmed the possibility to determine in a single WGS analysis the genetic lineage, MLST and multiple antigen type, the vaccine-related capsular and subcapsular protein antigen gene repertoire and markers of finer genotypic diversity such as cgMLST. A capacity building workshop on bacterial genomics was organised for 14 EU/EEA IBD-LabNet members in 2018 to transfer methodology on how to use the tools, the libraries and the database.

Pilot studies and collaborative initiatives undertaken by ECDC in the past three years on molecular typing for pathogens and diseases that are not prioritised in the current ECDC implementation framework are described in Annex 2.

# Strategic framework for EU integration of disease-specific molecular and genomic typing data into public health operations, 2019–2021

## Public health objectives

Following the previous ECDC Roadmaps, the public health operations to be enhanced by integration at European cross-national level of molecular and genomic information are categorised into three distinct applications by objective: **outbreak investigations**, **control-oriented surveillance** aiming at early outbreak detection and **strategy-oriented surveillance** to monitor longer term disease trends and effectiveness of prevention programmes.

ECDC Disease Programmes were consulted in 2018 regarding the need for updating the disease-specific objectives for EU molecular or genomic surveillance and for considering new pathogens or diseases for implementation during 2019–21. Based on new public health priorities, technology developments, expanded operational capacity across Europe and lessons learned from these operations, the ECDC Disease Programmes proposed eight new diseases or health threats to consider in addition to those in Roadmap 2.1 and added new objectives for five priority diseases.

The updated public health objectives to be addressed for inclusion in the present implementation framework are summarised by pathogen in Table 3. Objectives relating to operations to be further postponed are mentioned as 'not included' for this implementation phase and will be reviewed in 2021.

**Table 3. Public health objectives for EU molecular or genomic-based operations by pathogen, as applicable for mid-term (2019–2021) implementation**

| Disease programme and pathogen | Objectives for | | |
|---|---|---|---|
| | Outbreak investigation | Control-oriented surveillance | Strategy-oriented surveillance |
| ECDC programme: Antimicrobial Resistance and Healthcare-Associated Infections | | | |
| Carbapenem- and/or colistin-resistant Enterobacteriaceae (C/CRE) | NEW<br>1. Early confirmation of multi-country dimension of outbreaks<br>2. Identification of genetic vector/modes/sources of transmission<br>3. targeting of control measures<br>4. Assessment of effect of the control measures | Not included | 1. Detection/delineation of cross-region or cross-border dissemination of high-risk clones/plasmids<br>2. Identification of high-prevalence geographical areas associated with the spread of specific high-risk clones<br>3. Detection and genotypic identification of high-risk clones/plasmids<br>4. Monitoring time trends in the frequency of occurrence of particular genotypes in the population and identification of high-prevalence population groups<br>5. Impact assessment of prevention and control programmes<br>6. Targeting high-risk populations, geographical areas and dissemination pathways |

| Disease programme and pathogen | Objectives for | | |
|---|---|---|---|
| | Outbreak investigation | Control-oriented surveillance | Strategy-oriented surveillance |
| NEW Carbapenem-resistant *Acinetobacter baumannii* (CRAB) | 1. Early confirmation of multi-country dimension of outbreaks<br>2. Identification of genetic vector/modes/sources of transmission<br>3. Targeting of control measures<br>4. Assessment of effect of the control measures | Not included | 1. Detection and genotypic identification of high-risk clones<br>2. Identification of high-prevalence geographical areas associated with the spread of specific high-risk clones<br>3. Detection/delineation of cross-region or cross-border dissemination of high-risk clones<br>4. Monitoring time trends in the frequency of occurrence of particular genotypes in the population and identification of high-prevalence population groups<br>5. Impact assessment of prevention and control programmes<br>6. Targeting high-risk populations, geographical areas and dissemination pathways |
| *Clostridium difficile* | NEW<br>1. Early confirmation of multi-country dimension of outbreaks<br>2. Identification modes/sources of transmission between hospitals and across national borders<br>3. Targeting of control measures<br>4. Assessment of effect of the control measures | Not included | Not included |
| Meticillin-resistant *Staphylococcus aureus* (MRSA) | NEW<br>1. Early confirmation of multi-country dimension of outbreaks<br>2. Identification of genetic vector/modes/sources of transmission<br>3. Targeting of control measures<br>4. Assessment of effect of the control measures | Not included | Not included |
| NEW Outbreaks of emerging multidrug resistant pathogens/clones/plasmids | 1. Early confirmation of new AMR health threats.<br>2. Assessment of multi-country dimension of outbreaks<br>3. Identification of genetic vector/modes/sources of transmission<br>4. Targeting of control measures<br>5. Assessment of effect of the control measures | Not included | Not included |

| Disease programme and pathogen | Objectives for | | |
|---|---|---|---|
| | Outbreak investigation | Control-oriented surveillance | Strategy-oriented surveillance |
| NEW Outbreaks of newly emerging pathogens/ new modes of transmission of known healthcare-associated pathogens | 1. Assessment of multi-country dimension of outbreaks<br>2. Identification of modes/sources of transmission<br>3. Targeting of control measures | Not included | Not included |
| ECDC programme: Emerging and Vector-Borne Diseases | | | |
| West Nile virus | NEW<br>1. Assessment and early confirmation of multi-country dimension of outbreaks<br>2. Identification of outbreak lineages and their relationship to other circulating lineages<br>3. Support of blood safety control measures<br>4. Identification of potential genetic markers of neurovirulence | Not included | Not included |
| ECDC programme: Food- and Waterborne Diseases and Zoonoses | | | |
| NEW *Campylobacter jejuni/C. coli* | 1. Verification of multi-country outbreaks<br>2. Support to investigation of outbreak sources/vehicles jointly with EFSA | Not included | Not included |
| NEW Hepatitis A virus | 1. Verification of multi-country outbreaks<br>2. Support to investigation of outbreak sources/vehicles jointly with EFSA<br>3. Assessment of the effectiveness of control measures | Not included | Not included |
| NEW *Legionella* spp. | 1. Support to assessment of dimension of outbreaks, including travel-related cases reported through EU/EEA surveillance<br>2. Support to investigation of outbreak sources<br>3. Assessment of the effectiveness of control measures | Not included | Not included |
| *Listeria monocytogenes* | 1. Verification of multi-country outbreaks (WGS)<br>2. Investigation of outbreak sources/vehicles jointly with EFSA (PFGE, WGS) | Outbreak detection (WGS) | 1. Monitoring trends in the frequency of occurrence of particular genotypes in the population.<br>2. Identification of persistent strains |

| Disease programme and pathogen | Objectives for | | |
|---|---|---|---|
| | Outbreak investigation | Control-oriented surveillance | Strategy-oriented surveillance |
| *Salmonella* enterica | 1. Verification of multi-country outbreaks (WGS)<br>2. Investigation of outbreak sources/vehicles jointly with EFSA (PFGE, MLVA, WGS)<br>3. Use predicted antimicrobial resistance patterns to characterise human clinical isolates, i.e. as an epidemiological marker, to support identification of outbreaks and related cases | 1. Outbreak detection (MLVA, possibly WGS)<br>2. Identify and monitor, in human clinical isolates, genetic determinants of resistance that are important for public health e.g. to aid recognition of epidemic cross-border spread of multi-drug resistant strains | 1. Monitor, in human clinical isolates, trends in the occurrence of predicted resistance to antimicrobial agents relevant for treatment of human Salmonella infections, including comparison with food/animal isolates<br>2. Monitor, in human clinical isolates, trends in the occurrence of predicted resistance to other antimicrobial agents of public and animal health importance, including comparison with food/animal isolates<br>3. Monitor, in human clinical isolates, trends in the occurrence of predicted resistance to antimicrobial agents that may be needed for future therapeutic use.<br>4. Monitor, in human clinical isolates, the prevalence of ESBL, plasmid-encoded Ambler class C β-lactamases (pAmpC) and carbapenemase genootypes |
| Shiga toxin-producing *E. coli* (STEC) | 1. Verification of multi-country outbreaks (WGS)<br>2. Investigation of outbreak sources/vehicles jointly with EFSA (PFGE, WGS) | Outbreak detection (possibly WGS) | Monitoring trends in the frequency of occurrence of particular genotypes in the population. |

| Disease programme and pathogen | Objectives for | | |
|---|---|---|---|
| | Outbreak investigation | Control-oriented surveillance | Strategy-oriented surveillance |
| ECDC programme: HIV, AIDS, STIs and Viral Hepatitis | | | |
| Antibiotic-resistant<br>*Neisseria gonorrhoeae* | NEW<br>1. Investigation and delineation of multi-country outbreaks | Not included | 1. Detection/delineation of emergence and cross-region/cross-border dissemination of public health relevant strains.<br>2. Genotypic identification and characterisation of highly virulent, multidrug resistant and/or transmission-successful strains.<br>3. Understanding the genetic and phenotypic stability of public health relevant strains<br>4. Identification of high-risk patient population groups associated with the spreading of specific strains.<br>5. Monitoring trends in the frequency of occurrence of particular genotypes in the population.<br>6. Understanding the dynamics of antimicrobial resistance in the context of antibiotic stewardship intervention policies.<br>7. Reporting emerging trends in resistance to learned societies and professional associations to help guide their treatment protocol updates. |
| HIV transmitted drug resistance | Not included | Not included | 1. Monitoring the prevalence of and trends of transmitted HIV drug resistance in newly diagnosed HIV patients, when they start antiretroviral treatment (ART) for the first time, to inform national treatment policies in the EU/EEA Member States.<br>2. Identification of at risk populations.<br>3. Identification of high-risk geographical areas and/or risk groups for HIV drug resistance transmission.<br>4. Reporting emerging trends in resistance to learned societies and professional associations to help guide their treatment protocol updates. |

| Disease programme and pathogen | Objectives for | | |
|---|---|---|---|
| | Outbreak investigation | Control-oriented surveillance | Strategy-oriented surveillance |
| ECDC programme: Influenza and other Respiratory Viruses | | | |
| Influenza virus | Not included | Detection of potential pandemic influenza strains | 1. Identification of locally circulating virus types and subtypes and their relationship to global and regional patterns.<br>2. Monitoring antiviral susceptibility.<br>3. Facilitating vaccine strain selection.<br>4. Describing the antigenic and genetic characteristics of circulating viruses. |
| ECDC programme: Tuberculosis | | | |
| Multidrug-resistant tuberculosis | NEW<br>Provide information to support the investigation of international outbreaks to:<br>- characterise drug resistance mutations of epidemic M. tuberculosis strains<br>- delineate and map the spread of epidemic strains<br>- identify the source(s)<br>- identify risk factors and transmission patterns between individuals/communities | 1. NEW. Detection of multi-country outbreaks<br>2. Identification and investigation of high-risk strains ('super spreaders' and/or MDR/XDR TB). | 1. Monitoring the distribution of MDR TB strain diversity in the EU/EEA.<br>2. Identification of high-risk geographical areas and/or population groups.<br>3. Evaluating the TB control programmes |
| ECDC programme: Vaccine-Preventable Diseases (VPD) | | | |
| NEW *Bordetella pertussis* | Not included | Not included | 1. Determine the impact of current vaccines/immunisation on vaccine escape mutants, and spread of successful clones and of antibiotic resistance.<br>2. Provide evidence for guidance on immunisation strategies of current and new vaccines. |
| *Neisseria meningitidis* | Support the investigation of national and international outbreaks:<br>- establishing the spread of particular strains locally or regionally<br>- identification of risk factors, epidemic origin and transmission patterns between individuals | 1. Detection, verification and description of national and international outbreaks<br>2. Identification and monitoring of prevalence of vaccine escape variants, based on analysis of outer-membrane protein vaccine targets | 1. Detect the emergence and spread of new virulent or epidemiologically successful sequence types<br>2. Assessment of vaccine strain antigen match as predictor of effectiveness<br>3. Assess antibiotic susceptibility and genes conferring susceptibility/resistance patterns |

| Disease programme and pathogen | Objectives for | | |
|---|---|---|---|
| | Outbreak investigation | Control-oriented surveillance | Strategy-oriented surveillance |
| NEW *Streptococcus pneumoniae* | Not included | Not included | 1. Determine the impact of current vaccines/immunisation on type-replacement, capsular switch, and spread of successful clones and of antibiotic resistance.<br>2. Provide evidence for guidance on life-course immunisation strategies of:<br>-current vaccines<br>-new vaccines that are not only based on capsule antigens |

*Note: Objectives not in the scope of the current strategic framework are marked 'not included'. New pathogens or objectives that are not included in Roadmap 2.1 are labelled* NEW

# Priorities for preparation and/or implementation 2019–2021

For the period 2019–2021, ECDC proposes to gradually prepare for, and/or implement, the integration of sequence-based typing data workflows for joint EU/EEA response and surveillance operations for the following applications and pathogens (subject to availability of resources and methodology agreements with disease networks):

- **Outbreak investigation objective: support to multi-country outbreak investigations through sequence-based typing:** *Campylobacter* spp., *Clostridium difficile,* hepatitis A virus, *Legionella* spp., *Listeria monocytogenes*, multidrug-resistant *Mycobacterium tuberculosis* (MDR TB), *Neisseria meningitidis,* outbreaks of emerging multi- or extensively drug-resistant (MDR or XDR) bacteria, outbreaks of new pathogens or new modes of transmission of healthcare-associated or community pathogens, *Salmonella enterica*, Shiga-toxin producing *E. coli* and West Nile virus.
- **Control and strategy-oriented objectives: EU-wide sequence-based continuous surveillance:** influenza virus, *Listeria monocytogenes*, MDR TB and *Neisseria meningitidis,* possibly *Salmonella enterica* and Shiga-toxin producing *E. coli* (depending on preparedness of having high quality data processing and sufficient national and EU level resources/capacity).
- **Strategy-oriented objective: sentinel surveillance or surveys**: antibiotic-resistant *Neisseria gonorrhoeae*, *Bordetella pertussis*, carbapenem- or colistin-resistant Enterobacteriaceae, carbapenem-resistant *Acinetobacter baumannii*, HIV-transmitted drug resistance, and *Streptococcus pneumoniae*.

ECDC proposes to prepare for and/or implement the following operations over the period 2019–21 for: a) support to multi-country outbreak investigations, b) continuous EU surveillance, and c) sentinel surveillance.

## A. Support to multi-country outbreak investigations

To confirm, delineate and investigate signals of multi-country clusters of genomically-related cases and putative outbreaks, ECDC shall further expand its operational and expert support services to facilitate near real-time (whole genome or gene) sequence-based data production, data sharing, and integrative epidemiological analysis with the EU/EEA Member States, and where relevant, EFSA or other agencies or technical partners.

The extension of these response operations to various threats will depend on the pathogen versatility of IT solutions to be adopted for efficient management of sequence and epidemiological data workflows (see proposed models in below section) and the allocation of genomic epidemiology and risk assessment expert resources both at ECDC and among relevant disease network participants.

### *Campylobacter jejuni/Campylobacter coli*

**Method:** cgMLST/wgMLST, SNP phylogenomic analysis

**International molecular/genomic typing schemes and resources:** cgMLST allele nomenclature and global genome library (http://pubmlst.org/campylobacter) – curated by the University of Oxford (United Kingdom) – or equivalent commercial/open access reference databases for allele and/or strain nomenclature, subject to further evaluation.

**Rationale:** There is not yet evidence of human cross-border outbreaks of *C. jejuni* or *C. coli* infections in Europe. This may be related to under-ascertainment as molecular surveillance is limited. Countries that have applied whole

genome MLST on *Campylobacter* infections have found that cases often cluster in time and space [60]; they also detected persistent outbreaks across states [61].

**Priorities for preparation and/or implementation in 2019–21:** EU/EEA country capacities for, and practice of, WGS-based typing of *C. jejuni and C. coli* will be mapped in 2019. To explore the existence of multi-country foodborne campylobacteriosis outbreaks, ECDC will offer WGS support during the high season (summer months) to assess the existence/absence of possible cross-border events.

### *Clostridium difficile*

**Method:** First line: PCR ribotyping; second line: WGS

**International molecular/genomic typing scheme and resources:** capillary PCR-ribotype (standard scheme and public reference nomenclature database available); WGS-based cgMLST scheme or SNP mapping.
**Rationale:** *C. difficile* is a major healthcare-associated pathogen and an emerging community pathogen in the EU. International epidemics are illustrated by the pandemic spread of multidrug-resistant hyper-virulent clones associated with ribotype 027 from North America to other continents in the early 2000s [62]. There is growing evidence of zoonotic transmission of *C. difficile* from animal and environmental reservoirs to humans [63]. Findings of a recent pan-European genomic cross-sectional survey of *C. difficile* infection (CDI) in 20 countries indicate frequent national spread of epidemic *C. difficile* strains within care networks as well as multi-country dissemination of clades causing community-associated infection, raising the hypothesis of foodborne origin [64].
WGS-based typing was used for CDI outbreak investigation in eight EU/EEA countries in 2015 (NMFP survey, 2015, unpublished). Current national WGS-based typing capacity is unknown and under review in the surveillance network. First-line typing by PCR ribotype is available in 20 EU/EEA countries, 11 of which reported ribotype data on nearly a third of their reported cases in 2017 (quarterly reporting to TESSy). ECDC conducts a semi-annual data analysis. Limited access to culture-based *C. difficile* isolates can constrain both ribotyping and WGS typing because only a minority of diagnosed CDI cases was culture confirmed in 2014 [17].
**Priorities for preparation and/or implementation in 2019–21:** Based on the 2018 survey of capacity for ribotype and WGS-based typing, it is proposed that ECDC and the CDI surveillance network discuss how to further optimise routine CDI diagnostics and improve reporting of ribotype data for CDI cases through TESSy in 2019. ECDC will perform biannual ribotype cluster detection analysis. In collaboration with the consortium for 'Microbiological Support to European Surveillance of *C. difficile* infections' (2016–2020), ECDC will encourage the development of a standard WGS-based typing scheme/algorithm for second-line typing in support of outbreak investigations. A training curriculum and standard operating procedures for typing will be offered when standard molecular techniques have been agreed upon in 2020–2021. ECDC will then prepare the integration of *C. difficile* WGS or add derived type data to its TESSy genomic platform in order to support multi-country CDI outbreak investigations by 2021 (using, for example, operational model 2 or 3; see below).

### Hepatitis A virus

**Method:** Sequencing of the VP1/2a region

**International molecular/genomic typing scheme and resources:** HAVNet sequencing protocol, genotype nomenclature and global reference database [65].

**Rationale:** Hepatitis A has caused several large multi-country outbreaks in the EU, including foodborne outbreaks linked to frozen berries in 2013–2014 [66] and person-to-person outbreaks among men who have sex with men (MSM) communities in 2016–2017 [67]. These outbreaks were confirmed and resolved by comparing HAV VP1/2a region sequence data from outbreak cases and the HAVNet database with a sequencing protocol based on an ECDC expert consultation [65]. Experts at the Dutch National Institute for Public Health and the Environment (RIVM) support ECDC and affected countries with the assessment of HAV sequences related to outbreak investigations.

In 2016, 17 EU/EEA countries performed HAV sequencing, and 16 of them compared their data with data from the HAVNet database. Reference laboratories used diverse protocols and amplicon lengths, indicating the need for further standardisation [68]. The majority of countries that reported collaboration with HAVNet in 2016 (9 out of 15) submitted sequences to the database or used it for sequence comparison. Of the countries submitting sequences, only three indicated regular sequence submission. The remaining countries only submitted irregularly or when an outbreak was suspected.

**Priorities for preparation and/or implementation in 2019–21:** ECDC will collaborate with HAVNet and the FWD network to promote HAV sequencing for outbreak investigations, using the HAVNet protocol. ECDC will consult with HAVNet and the FWD surveillance network about the need for updating the existing protocol.

### *Legionella* species

**Method:** First line: Sequence-based typing (SBT); second line: WGS

**International molecular/genomic typing scheme and resources:** schemes available for SBT, MLST, cgMLST and extended MLST; SBT nomenclature, public database and automated annotation available (Public Health England, United Kingdom);

**Rationale:** Current diagnostic methodologies allow *Legionella* species and serogroup identification. SBT of *Legionella pneumophila* isolates was developed by the European Working Group on *Legionella* infections (EWGLI) and has been referenced for use in surveillance reporting by the European Legionnaires' Disease Surveillance network (ELDSNet) since 2010, both for annual and travel-associated Legionnaires' disease surveillance systems. The SBT scheme uses a combination of seven housekeeping and virulence genes and can discriminate over 2 000 sequence types (STs) [69].

However, since a large proportion of cases are caused by a few common STs, the SBT method can lack discriminatory power. Recent investigations have highlighted the superior value of WGS for matching environmental and clinical strains in identifying the source in outbreaks and guiding control measures [70-74]. A study of *L. pneumophila* genomes indicate recent emergence of hyper-virulent clones [75]. An extended WGS-based MLST scheme with ~50 genes provides optimal epidemiological concordance while substantially improving the discrimination of SBT and maintaining backwards nomenclature compatibility [76].

Current WGS-based typing capacity is unknown. In 2015, only one EU country reported routine use of WGS for *L. pneumophila* surveillance; two EU countries used WGS for outbreak investigations and nine more countries were planning its use for outbreak analysis within the next three years (NMFP survey, 2015, unpublished). Although direct typing on clinical or environmental samples is to some degree possible, limited access to culture-based *L. pneumophila* isolates is a major hurdle for WGS because only 12 EU/EEA countries managed to have at least 10% of LD cases confirmed by culture in 2016 [24]. In 2016, only 12% of all cases were diagnosed by culture, and 34% of the culture-confirmed *L. pneumophila* cases were reported with ST type (six countries). Results are expected for a 2018 survey coordinated by Public Health England on the usefulness of the current SBT database held at PHE. This survey was part of the activities of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Study Group for Legionella Infections (ESGLI).

**Priorities for preparation and/or implementation in 2019–21:** It is proposed that ECDC surveys European capacity for SBT and WGS-based typing of *L. pneumophila* in the ELDSNet network and EU enlargement countries in 2019. ECDC will also discuss with the ELDSNet network how TESSy reporting of molecular data can be improved for cases of travel-associated Legionnaires' disease (TALD). ECDC will encourage the development of a standard WGS-based typing scheme/algorithm and work to facilitate access to a nomenclature database in collaboration with ELDSNet and ESGLI [77]. Training courses for Member States will be offered when standard molecular techniques have been agreed upon.

ECDC will then prepare the integration of sequence or derived type data to its platform in order to support outbreak investigations involving TALD cases (e.g. by applying operational model 2, see below). ECDC will extend its work with EU/EEA participants in the LD surveillance network to obtain more information on feasibility and added value of routine WGS-enhanced surveillance, either as first line or second-line method combined with SBT. Follow-up risk assessments, risk communication, and risk management on detected national/cross-border SBT and/or WGS-based clusters of TALD will be agreed among Member States participating in ELDSNet.

### *Listeria monocytogenes*

**Method:** WGS

**International molecular/genomic typing schemes and resources:** cgMLST/wgMLST, SNP phylogenomic analysis. BIGSdb-*Lm* cgMLST database or equivalent commercial/open access reference databases for allele and/or strain nomenclature, subject to further evaluation.

**Rationale:** EU Member States want to prioritise WGS for both outbreak investigations (18 countries in 2017) and surveillance (15 countries in 2017, with eight planning to use WGS by 2019), taking into account the severity of invasive listeriosis, the preventability of the disease through food safety control interventions, and the availability of mature WGS-based typing standards and allele nomenclature. Combined WGS typing capacity for this pathogen was estimated between 50 and 80% of the total EU case burden in 2017. WGS-based typing was used by ECDC and the affected countries for confirming and assessing eight multi-country outbreaks in 2016–18. *L. monocytogenes* raw reads and assembled draft genome data can be uploaded to TESSy with BioNumerics software (machine-to-machine communication; operational model 1, see below).

**Priorities for preparation and/or implementation in 2019–21:** Based on ELiTE WGS study baseline data, response support activity will likely expand to up to 15 listeriosis multi-country clusters to be investigated annually after launching real-time WGS data sharing and the initiation of weekly analysis for routine EU surveillance of listeriosis in January 2019. ECDC will support sequencing of *L. monocytogenes* isolates from countries with no or insufficient WGS capacity. Upload of either short reads or assemblies to TESSy will be facilitated by a user-friendly uploader interface within operational model 1 (see section below). A ring trial will further validate the portability of

national WGS assembly pipelines. In collaboration with EU and other international partners, ECDC will contribute to the selection and adoption of WGS-based strain nomenclature for *L. monocytogenes*.

## Multidrug-resistant *Mycobacterium tuberculosis*

**Method:** WGS

**International molecular/genomic typing schemes and resources:** TB phylogenetic lineage and cgMLST nomenclature, antibiotic resistance mutation nomenclature and phenotype prediction software [78].

**Rationale:** Practical experience gained with investigation of several multi-country outbreaks of MDR and XDR TB in the EU in 2016–2017 established the feasibility and added value of real-time sharing and combined analysis of WGS-based typing and resistance profiling with epidemiological data (see progress report section). EU/EEA overall capacity for WGS-based typing increased almost two-fold between 2015 and 2017, with 13 countries applying WGS for MDR TB outbreak investigations.

**Priorities for preparation and/or implementation in 2019–21:** The EUSeqMyTB consortium will benchmark, validate and develop standards for WGS-based typing of MDR TB in 2019–2020 [57]. Sequencing will be provided through the project to support affected countries lacking the technical capacity for WGS typing (operational model 3, see below). In 2021, standard laboratory protocols, bioinformatics tools and the TB European genome library will be transferred to ECDC (operational model to be defined in 2020).

## *Neisseria meningitidis*

**Method:** WGS

**International molecular/genomic typing schemes and resources:** cgMLST for the type nomenclature and to confirm clusters of fine type genotypes; MLST and The European Meningococcal and Haemophilus Disease Society (EMGM) antigen fine type schemes for historical comparisons, capsular and outer membrane protein vaccine antigen matching profile for vaccine effectiveness prediction, antimicrobial resistance gene databases and detection tools (ResFinder, The Comprehensive Antibiotic Resistance Database (CARD)) available. PubMLST.org/neisseria database is available, including global and European meningococcal strain collection genome library.

**Rationale:** There is some evidence for the occasional occurrence of multi-country outbreaks of invasive meningococcal disease in Europe in recent years [79]. EU/EEA overall capacity for WGS-based typing has rapidly increased between 2015 and 2017, with 17 countries using WGS for outbreak investigations in 2017.

**Priorities for preparation and/or implementation in 2019–21:** ECDC will assist Member States to submit sequenced-based data using EMERT, a protected workspace within the PubMLST.org/neisseria database that contains the BIGSdb analytical tools for integrated genomic epidemiological surveillance, outbreak detection and investigations. Work in this area will be conducted in close collaboration with the IBD-LabNet consortium and the University of Oxford (United Kingdom), the curator of the PubMLST.org/neisseria cgMLST database. Eventually, this will enable Member States to submit their data to ECDC for analysis and output reporting (operational model 2, see below). Integration with Event and Threat Management Solution (ETMS, the successor to the EPIS-VPD platform) will be established as part of the ECDC Surveillance System Reengineering project (SSR).

## Outbreaks of emerging multi- or extensively drug-resistant bacteria

**Method:** WGS

**International molecular/genomic typing scheme and resources**: MLST, cgMLST typing schemes, single-nucleotide polymorphism (SNP) phylogenomic analysis of clonality; characterisation of resistome and mobilome using international nomenclature and automated sequence-based identification tools for drug resistance gene/mutation, Inc and pMLST plasmid typing, *IS* and *Tn* elements.

**Rationale:** Both the EU and the WHO action plans on antimicrobial resistance call on improving the timeliness and resolution of surveillance of priority antimicrobial-resistant pathogens to help detect and interrupt international dissemination. Increasing EU/EEA capacity is illustrated by 12 countries using WGS for C/CRE and MDR gonococcal outbreak investigations in 2017 (Figure 1). Multi-country outbreaks of emerging MDR or XDR pathogens benefit from ECDC-coordinated real-time sharing and analysis of WGS and epidemiological data. This was demonstrated in 2018 when the first multi-country outbreak of carbapenem-resistant *K. pneumoniae* in Europe was detected thanks to comparative WGS data analysis [10]. MDR or XDR pathogens can include C/CRE, CRAB, MRSA, glycopeptide-resistant enterococci and other pathogens (Tables 3 and 4).

WGS analysis will permit to delineate these outbreaks and trace their origin and mode of spread at an early stage when control measures are easier to implement and more likely to be successful. This would support the strategic priorities of ECDC's Antimicrobial Resistance and Healthcare-Associated Infections disease programme (ARHAI) to 'improve the effectiveness of and capacity for rapid response to AMR and HAI threats and outbreaks at the EU and Member State levels'. The Transatlantic Taskforce on Antimicrobial Resistance (TATFAR) collaboration programme with US and Canadian public health agencies has prioritised enabling international WGS information exchange on

emerging AMR threats, supported by mapping of the WGS-based antimicrobial resistance (AMR) surveillance and alert systems across partners in 2018–2020.

**Priorities for preparation and/or implementation in 2019–21:** As per TATFAR plan, mapping of the WGS-based AMR surveillance and alert systems across partners will be conducted across the EU in 2018–19; modes of international WGS information exchange on emerging AMR threats will be explored. ECDC's TESSy genomic platform and a protected workspace for Member State WGS data submission should become available in 2020, making it possible to report and analyse epidemic MDR/XDR bacterial pathogens (operational model 1, see below). Close collaboration with the Centers for Disease Control and Prevention of the United States (US CDC) on technical standards and tools used for genomic surveillance of high-impact AMR threats will be sought. Integration with ETMS (the successor to the EPIS-ARHAI platform) will be established as part of the surveillance system reengineering process.

## Outbreaks with new pathogens or new modes of transmission of healthcare-associated or community pathogens

**Method:** WGS; other molecular identification/typing and virulence characterisation methods as applicable. International molecular/genomic typing scheme and resources: to be developed by pathogen, as applicable.

**Rationale:** Investigation of multi-country outbreaks of healthcare-associated or community pathogens with new modes of transmission would benefit from ECDC support for WGS typing, sharing of epidemiological data, and coordinated comparisons of data from affected EU/EEA countries, including those without domestic WGS capacity. Recent examples in which WGS added value for national and EU-wide control include the global healthcare-associated outbreaks of *Candida auris* [80] and *Mycobacterium chimaera* infections [81]. WGS-based epidemiological analysis of newly emerging healthcare-associated or community threats is essential for a coordinated European approach in preventing EU-wide spread. However, there often is an incomplete understanding of the epidemiology and biology of emerging or epidemic pathogens, as well as a lack of standardised genotyping tools. This would likely require ECDC to collaborate with specialised scientists to help solve knowledge and technical gaps during the urgent investigation phase.

**Priorities for preparation and/or implementation in 2019–21:** Considering the limited resources of ECDC, it is proposed to explore a longer-term strategy to broaden the ECDC framework and budgeting scheme across Disease Programmes for WGS-based support to multi-country outbreaks caused by diverse epidemic–prone microbial pathogens based on public health impact of the emerging health threat.

## *Salmonella enterica* and Shiga toxin-producing *Escherichia coli*

**Method:** WGS to confirm and investigate clusters of MLVA and/or WGS genotypes, as applicable.

**International molecular/genomic typing schemes and resources:**
Core genome MLST, wgMLST, MLST and CRISPR or equivalent commercial/open access reference databases for allele and/or strain nomenclature, or SNP analysis, subject to further evaluation.

**Rationale:** In 2016–17, use of WGS-based typing for routine surveillance of *Salmonella* and STEC remained stable (seven EU/EEA countries). WGS use for outbreak investigations increased to 15 countries in 2017 (Figure 1).

**Priorities for preparation and/or implementation in 2019–21:** Given the high health impact and the preventable nature of salmonellosis and STEC infection, frequent occurrence of confirmed cross-border *Salmonella* outbreaks as well as increasing EU capacity for WGS-based typing, it is proposed to discontinue first-line PFGE typing for surveillance in 2019 but continue the existing molecular surveillance by MLVA and use WGS for outbreak verification and investigation. It is foreseen that the joint ECDC–EFSA molecular typing database will be upgraded to cover also management and analyses, with WGS data to support outbreak investigations. ECDC will assist Member States by supporting ad hoc comparative WGS-based typing for the coordinated investigation; the Centre will also team up with EFSA for joint assessments of EU/EEA multistate outbreaks of salmonellosis or STEC infections. Inter-laboratory reproducibility ring trials with dry-lab WGS analysis practices (including assembly, mapping, allele calling and single-nucleotide variant calling) in the Member States may be needed; this depends on the results of the existing EQA schemes, which include WGS cluster analysis.

## West Nile virus

**Method:** WGS

**International molecular/genomic typing schemes and resources:** Molecular typing strategy will be based on phylogenetic analysis of complete genome sequences for comparison of lineages. Potential virulence markers will be identified on the basis of nucleotide or putative amino acid sequence alignments compared to reference sequences with potential virulence-associated substitutions [82].

**Rationale:** Molecular methods are used to detect WNV outbreaks early and to detect WNV in asymptomatic blood donors to prevent transfusion-related infection. Phylogenetic analysis is used to assess the multi-country dimension of the outbreaks, give indication of the source of infection, and find neurovirulence markers in the viral genome. In

addition, the analysis can be used to compare the outbreak lineages to other circulating strains from human cases, mosquitos, amplifying hosts (birds) and other sensitive hosts (equids).

**Priorities for preparation and/or implementation in 2019–21:** ECDC will launch a call for a pilot study on the genetic characterisation of outbreak WNV strains in EU/EEA countries. The complete genome sequences of WNVs in the collaborating European countries will be determined and analysed. In preparation for a more routine outbreak assessment and response, the study will advise on possible options for data sharing and storage. A standardised sequencing protocol will be developed. ECDC will continue to support EQAs for the molecular detection of WNV and develop a scheme for quality assessment of WGS data. The pilot study will also advise ECDC on the resources required for data collection and production of relevant outputs for public health. In order to compare the sequences from humans, vectors and animals, One Health cooperation needs to be established [83]. The study will provide information about the usefulness of collected data for the linking multi-country outbreaks to risk groups for transmission (blood donors), geographic risk areas, and determinants for disease severity.

## B. EU-wide sequence-based continuous surveillance

It is proposed that ECDC work with the EU/EEA Member States and where relevant, EFSA, WHO or other external institutes, to operationalise EU-wide gene sequence or WGS-based surveillance of the following pathogens in 2019–21:

### Influenza virus

**Method:** Haemagglutinin and neuraminidase gene sequencing or WGS

**International molecular/genomic typing scheme and resources:** Haemagglutinin (for phylogenetic analysis) and/or neuraminidase (for antiviral susceptibility) gene sequencing [84]; WHO nomenclature for virus strains and clades.

**Rationale:** The rationale is to detect potentially pandemic strains, identify locally circulating virus sub-/types and their relationship to global and regional patterns, monitor antiviral susceptibility, facilitate vaccine strain selection, and describe the antigenic and genetic characteristics of circulating viruses [85].

**Priorities for preparation and/or implementation in 2019–21:** By the end of this period, surveillance harmonisation for influenza strain detection and resistance monitoring will be established, and options for genomic surveillance will be explored [50]. It is proposed to continue the sequence-based monitoring of influenza viruses. ECDC will continue to develop automation tools for data analysis. This will improve the integration of the Member State sequences reported to the GISAID EpiFlu database, the virus characterisation and epidemiological data reported to TESSy. Another advantage is that all data can be analysed in a timely fashion and shared at the twice-yearly WHO vaccine composition consultations [51]. In addition, support tools for Member States to facilitate their sequence analyses will be provided. ECDC and EFSA will continue to report jointly and regularly on the avian influenza; this includes phylogenetic analysis of the circulating strains [52]. A new open web-based bioinformatics tool (INSaFLU) for the analysis of primary next generation sequencing read data (which also delivers influenza (sub)type, sequence alignment and phylogenetic tree) was published, giving the Member States the option to use it routinely [86]. As earlier, ECDC will continue to provide web-based and wet-laboratory training; in addition, EQAs will be conducted, covering a wide range of laboratory techniques.

### *Listeria monocytogenes*

**Method:** WGS

**International molecular/genomic typing scheme and resources:** WGS- based cgMLST and SNP mapping

**Rationale:** EU Member States want to prioritise WGS for comprehensive surveillance, taking into account the severity of invasive listeriosis, the preventability of the disease through food safety control interventions, and the availability of mature WGS-based typing standards. The national capacity increased from 2016 to 2017, with 15 EU/EEA countries using WGS-based typing for listeriosis surveillance in 2017 and eight more countries planning to do so by 2019 (Figure 1) [5]. Combined WGS typing capacity for this pathogen was estimated between 50 and 80% of EU total case burden in 2017. A retrospective WGS-based typing study (ELiTE) showed good portability of WGS data from different sequencers and bioinformatics pipelines; the study also found a high frequency of clustering of listeriosis cases within and across EU national borders over a four-year period [13].

**Priorities for preparation and/or implementation in 2019–21:** ECDC and EFSA have been mandated by the European Commission to define technical options and formats for the collection of comparable WGS data on foodborne bacterial pathogens (including *L. monocytogenes*); the mandate also specifies support for integrated cross-sectoral data analysis using the joint ECDC–EFSA molecular typing database. A new joint ECDC–EFSA working group was established to define these options; the report is expected by April 2019.

On the public health side, ECDC will start implementation and evaluation of WGS-based surveillance of *L. monocytogenes* in March 2019. ECDC will provide training and support sequencing of *L. monocytogenes* isolates

from countries with no or insufficient WGS capacity. Upload of either short reads or assemblies to TESSy will be facilitated by a user-friendly interface within operational model 1 (see section below).

The ELiTE WGS dataset is almost in its entirety available as baseline genome library for use in routine surveillance. In addition, isolates sequenced with ECDC funding will be gradually made available through public repositories, with accompanying descriptive data following the embargo period agreed with the country in question. Collaboration with EU and international partner agencies will be continued in order to agree upon a global WGS-type (strain) nomenclature and establish a publically accessible WGS-type (strain) nomenclature database.

### Multidrug-resistant *Mycobacterium tuberculosis*

**Method:** WGS

**International molecular/genomic typing schemes and resources:** TB phylogenetic lineage and (multiple) cgMLST nomenclature(s), antibiotic resistance mutation nomenclature and automated phenotype prediction software tool; WGS data not backward compatible with MIRU-VNTR scheme.

**Rationale:** TB and multidrug-resistant TB remain a public health problem within the EU/EEA. Enhanced surveillance for rapid identification of emerging M/XDR TB clusters and accurate tracing of MDR TB transmission are critical to inform TB control and prevention strategies. The superiority of WGS analysis over conventional methods for rapid *M. tuberculosis* identification, antibiotic resistance prediction, outbreak detection and high-resolution tracing of transmission networks is now well established [87]. Multi-country outbreaks were recently revealed by WGS analysis (see MDR TB progress report and outbreak support sections above). EU/EEA overall capacity for WGS-based typing for national MDR TB surveillance increased almost two-fold over 2015–17, with 10 countries using WGS. Capacity, however, was limited in high-incidence countries hence the EU combined national WGS-based surveillance capacity was estimated to cover only 12 to 16% of the total EU MDR TB case burden in 2017.

**Priorities for preparation and/or implementation in 2019–21:** The EUSeqMyTB consortium will develop standards during 2019–2020 to establish EU-wide surveillance with WGS-based typing protocols and data management tools [57]. The project will provide sequencing to support countries which lack sufficient technical capacity (operational model 3, see below). In 2021, standard laboratory protocols, bioinformatics tools, and the European TB genome library will be transferred to ECDC. The Centre will then manage MDR TB genomic surveillance at the EU/EEA level (operational model to be defined in 2020).

### *Neisseria meningitidis*

**Method:** WGS

**International molecular/genomic typing schemes and resources:** cgMLST for the type nomenclature and to confirm clusters of fine type genotypes; MLST and EMGM antigen fine type for historical comparisons, capsular and outer membrane protein vaccine antigen matching profile for vaccine effectiveness prediction, antimicrobial resistance gene databases and detection tools (ResFinder, CARD) available. PubMLST.org/neisseria cgMLST database available, including global and European meningoccocal strain collection genome libraries.

**Rationale:** Invasive meningococcal disease remains an important public health threat in the EU, but there are new vaccines against *N. meningitidis* for prevention. EU/EEA overall capacity for surveillance using WGS-based typing of *N. meningitidis* increased almost two-fold between 2015 and 2017, with 15 countries applying WGS for national surveillance of invasive meningococcal infections in 2017 and nine more planning to do so by 2019. The combined national WGS-based surveillance capacity was estimated to cover approximately 70% of total EU case burden in 2017 thereby presenting a strong surveillance population sampling frame for outbreak detection and monitoring vaccine match of circulating strains in the EU/EEA.

**Priorities for preparation and/or implementation in 2019–21:** As explained above for outbreak support, ECDC will assist Member States to submit sequenced-based data for EU surveillance using EMERT, a protected workspace within the PubMLST.org/neisseria database which contains the BIGSdb analytical tools for both integrated genomic epidemiological surveillance and outbreak investigations (operational model 2, see below).

### *Salmonella enterica* and Shiga toxin-producing *Escherichia coli*

**Method:** WGS

**International molecular/genomic typing schemes and resources:** EnteroBase (containing cgMLST, wgMLST, MLST and CRISPR) or equivalent commercial/open access reference databases for genotype nomenclature, subject to further evaluation. Antimicrobial resistance gene databases and online detection tools (ResFinder, CARD, AMR Finder) available.

**Rationale:** In 2016–17, WGS-based typing was used for surveillance of salmonella and STEC in seven and nine EU/EEA countries, respectively (Figure 1). This represents a minimum coverage of 35% and 51% of total EU disease burden, respectively (Table 1). WGS-based prediction of salmonella resistance phenotypes (and molecular determinants of resistance) is increasingly used by reference laboratories in EU Member States instead of standard

antimicrobial susceptibility testing. The revised EU-2018 surveillance case definitions require Member States to perform AST for a representative subset of salmonella isolates. Countries are increasingly reporting isolate-based quantitative AMR data to TESSy in accordance with the EU harmonised AMR monitoring protocol. The revised EU-2018 surveillance case definitions also include a genetic definition of AMR determinants. Given the high health impact and the preventable nature of salmonellosis and STEC infection through food safety interventions, and in spite of still limited EU capacity for WGS-based surveillance, it is proposed to gradually replace first-line PFGE and MLVA typing for surveillance and offer Member States the possibility to upload WGS data on a voluntary basis. EU-level surveillance analysis for cross-border cluster detection will be introduced gradually, along with the preparedness for processing high-quality sequence data for EU-wide surveillance purposes.

**Priorities for preparation and/or implementation in 2019–21:** ECDC will assist the Member States by supporting the implementation and evaluation of WGS-enhanced surveillance of salmonella and STEC using operational model 1 (see below and Figure 3). Whether and when to participate in these genomic surveillance activities and what sequence data should be shared through the ECDC platform for European surveillance decided by the Member States, based on expected benefits from cross-country comparisons weighed against the burden of uploading substantial amounts of data. Thresholds of sequence relatedness within cluster signals from genomic surveillance analysis that are considered adequate for undertaking further epidemiological cluster investigations will be agreed at the operational expert level with Member States experts from the FWD network.

EQAs on *in silico* analysis practices (including assembly, mapping, allele calling and SNV calling) in the Member States may be organised, depending on the results of existing EQA schemes that include WGS cluster analysis. Quality assessment for MLVA data will be continued in 2019. In collaboration with EU and international partners, ECDC will promote the adoption of WGS-based strain nomenclature for *S. enterica* and STEC.

ECDC and the FWD network will revise the EU protocol for harmonised monitoring of antimicrobial resistance in human *Salmonella* isolates, in accordance with the new EU case definition of genotypic antimicrobial resistance [88]. This will enable reporting of WGS-predicted salmonella resistance genotypes (and molecular determinants/sequence data) by using phenotype prediction tools and knowledge bases compatible with those used by EFSA and the EU Reference Laboratory for Antimicrobial Resistance for the analysis of resistance in food isolates.

## C. Sentinel surveillance or surveys

### Antibiotic-resistant *Neisseria gonorrhoeae*

**Method:** WGS

**International molecular/genomic typing scheme:** WGS phylogenomic typing scheme; MLST; NG-MAST; resistome

**Rationale:** The increasing threat of multidrug- and extensively drug-resistant *N. gonorrhoeae* and its potential public health impact, together with the increasing number of reported *N. gonorrhoeae* infections in the EU/EEA, requires careful monitoring of the impact of current control policies, particularly among high-risk groups. In addition, the surveillance network has demonstrated an optimal typing feasibility for *N. gonorrhoeae*. Results from the 2013 typing survey showed WGS to be more discriminative to delineate transmission networks and better suited to answering public health questions than sequence typing (NG-MAST) [89]. Member State capabilities for using WGS in surveillance of antibiotic-resistant *N. gonorrhoeae* is already significant and will be supplemented by centralised ECDC-supported testing.

**Priorities for preparation and/or implementation in 2019–21:** The next WGS analysis is planned for 2019, based on the Euro-GASP 2018 survey collection. A scientific collaboration with a sequencing facility will allow for sequencing of a larger number of isolates and provision of expertise and tools developed for the analysis and visualisation of WGS data as previously done for the 2013 survey [44]. It is proposed that ECDC prepares the integration of *N. gonorrhoeae* WGS to its TESSy genomic platform in 2020–2021. Alternatively, derived type data can be added to TESSy. Both approaches would support multi-country surveys and outbreak investigations by 2021 (using operational model 1, see below section).

### *Bordetella pertussis*

**Method:** WGS

**International molecular/genomic typing schemes and resources:** conventional and cgMLST nomenclature [90] and vaccine antigen phenotype prediction software.

**Rationale:** *Bordetella pertussis* infection (pertussis or whooping cough) constitutes a significant public health problem in Europe. Diverse subunit vaccines are used for infant and adult immunisation programmes in EU/EEA countries. Re-emergence of pertussis in newborns and infants has been related in some European countries to the spread of vaccine antigen escape variants lacking one or more subunit vaccine antigen, such as pertactin [91]. To study vaccine effectiveness and the impact of different vaccination strategies against whooping cough and sustain

pertussis surveillance in infants in the EU/EEA, ECDC established an active sentinel hospital surveillance network for pertussis (PERTINENT) in 2015. This project is funded by the Member States and ECDC. The PERTINENT protocol has been established in seven European study sites comprising 41 hospitals. In addition, ECDC coordinates the EUPert-LabNet, which is a network of EU reference laboratories for pertussis.

Besides antigen characterisation of strains of *B. pertussis*, WGS-based analysis is necessary to determine the impact of current vaccines/immunisation on the emergence and spread of vaccine escape mutants in Europe. Recently, cgMLST was demonstrated to be much more discriminant than PFGE or MLVA for subtyping *B. pertussis* [90]. This analysis will provide actual or predicted effectiveness evidence for guidance on immunisation strategies with current and new vaccines. In addition, this periodic analysis will allow the identification and monitoring of successful clones, the identification of clones, and the identification of virulence factors associated with severe outcome.

**Priorities for preparation and/or implementation in 2019–21:** ECDC, in collaboration with EUPert-LabNet, will coordinate the development of a pilot sentinel for WGS-based typing of *B. pertussis* isolates (complete with protocol) to analyse clonal diversity, identify antigen shifts, and replacement by non-vaccine antigen clones selected by vaccine selective pressure (operational model 3).

## Carbapenem-resistant and/or colistin-resistant Enterobacteriaceae

**Method:** WGS

**International molecular/genomic typing scheme:** phylogenomic analysis of clonality using: cgMLST typing schemes for *K. pneumoniae* and *E. coli*; characterisation of mobilome/resistome/virulome, using international nomenclature for carbapenemase and colistin resistance gene/mutation identification; Inc and pMLST plasmid typing nomenclature.

**Rationale:** According to the EU and WHO action plans on antimicrobial resistance, carbapenem-resistant and/or colistin-resistant Enterobacteriaceae (C/CRE) are in a group of prioritised antimicrobial-resistant pathogens that continue to disseminate in the EU/EEA and have reached an alarmingly high prevalence in several countries. Advanced genomic monitoring of distribution, cross-border spread, and risk factors associated with high-risk clones and plasmid determinants of multiple and extreme resistance is urgently needed to inform prevention and control policies.

**Priorities for preparation and/or implementation in 2019–21:** The WGS-based European carbapenem and/or colistin-resistant Enterobacteriaceae (EURGenCCRE) survey will be conducted in 2019–2020, with EURGen-Net participants from 37 countries. The analysis of the strains will combine standard phenotypic and WGS analysis of clonality based on MLST and cgMLST, plasmid content, resistome and virulome profiling, especially accessory hyper-virulence genes. The EURGenCCRE survey consortium, coordinated by the Swedish Public Health Institute, will conduct the study; a collaborative agreement with the Wellcome Sanger Institute (United Kingdom) was established for joint investment in the WGS analysis of 6 000 isolates of *K. pneumoniae* and *E. coli* with and without resistance to carbapenem and colistin. The project will also provide laboratory manuals and training workshops, hold quality assessment exercises to build capacity, and work on the harmonisation of methods for carbapenem and colistin susceptibility testing in clinical and reference laboratories in Europe.

## Carbapenem-resistant *Acinetobacter baumannii*

**Method:** WGS

**International molecular/genomic typing scheme:** phylogenomic analysis of clonality using: *A. baumannii* cgMLST typing scheme; characterisation of mobilome/resistome/virulome, using international nomenclature for carbapenemase and colistin resistance gene/mutation identification; Inc and pMLST plasmid typing nomenclature.

**Rationale:** Carbapenem-resistant *Acinetobacter baumannii* (CRAB) poses a severe public health threat [92]. *A. baumannii* is the cause of serious infections in healthcare settings, and carbapenem resistance limits treatment options and increases the risk for adverse outcomes. The epidemiological situation in Europe has worsened in the past years, with a higher number of countries reporting interregional spread or endemicity of CRAB. Increased efforts are needed to prevent CRAB from becoming endemic in more European regions [92]. WGS has been used for *A. baumannii* outbreak studies and shown to be superior to other typing techniques [93]. WGS-based core genome typing schemes for *A. baumannii* have been developed, validated and found to be suitable for inter-laboratory comparison [94].

**Priorities for preparation and/or implementation in 2019–21:** The first WGS-based European CRAB survey is proposed to be conducted by the EURGen-Net partners in 2020–21. The sampling strategy as well as the epidemiological and microbiological data collection will be discussed with surveillance partners based on the 'ECDC study protocol for genomic-based surveillance of carbapenem-resistant and/or colistin-resistant Enterobacteriaceae at the EU level' (C/CRE-survey) [95]. The analysis of the strains will likewise combine standard phenotypic and WGS analysis of clonality, plasmid content, resistome and virulome. A coordinating consortium will be tendered out in 2020 through a framework service contract. A call for scientific collaboration will be issued on the WGS analysis

of 2 000 isolates of *A. baumannii* with and without resistance to carbapenem and colistin. The project will also provide laboratory manuals and training workshops, hold quality assessment exercises to build capacity, and work on the harmonisation of methods for carbapenem and colistin susceptibility testing in clinical and reference laboratories in Europe.

## Human immunodeficiency virus (HIV)

**Method:** Sanger, next generation or whole genome sequencing

**International molecular/genomic typing scheme:** Protease-reverse transcriptase and/or integrase gene sequences

**Rationale:** According to the WHO Global Action Plan on HIV drug resistance (HIVDR), preventing and managing the emergence of HIVDR is a key component of a comprehensive and effective HIV response and should be integrated into broader efforts to ensure sustainability and greatest impact of HIV prevention [96]. The Global Action Plan has five strategic objectives: 1) prevention and response; 2) monitoring and surveillance; 3) research and innovation; 4) laboratory capacity; and 5) governance and enabling mechanisms. HIVDR surveillance in the EU/EEA is contributing directly to the WHO Global Action Plan on Monitoring and Surveillance of HIVDR. To support the implementation of the WHO Action Plan, a platform to support the interpretation and storage of sequence data was established. The WHO Regional Office for Europe has agreed to work with ECDC to set up a joint European portal for this platform and initiate joint HIVDR surveillance in the EU/EEA, eastern Europe and central Asia. While this is being established, ECDC will collect aggregated data on drug class resistance from countries who are already able to report this data. This follows the 2015 advice of the ECDC Advisory Forum that supported the development of such a system for the EU-wide collection of HIV resistance data from EU/EEA countries that already operate a surveillance system. HIV resistance data would be collated, analysed and reported in the context of overall annual European HIV resistance prevalence, taking into account the increased use of antiretroviral drugs for pre-exposure prophylaxis and expanding international migration.

**Priorities for preparation and/or implementation in 2019–21:** ECDC will launch a call for aggregated data on transmitted HIVDR based on the variables tested in the recent pilot study [46]. Regular surveillance reports will be prepared to describe trends of transmitted HIVDR prevalence by transmission group and drug class. ECDC will enter into discussion with the WHO Regional Office for Europe, the Member States, and HIV surveillance networks to plan for a legally sound solution for continuous HIVDR surveillance alongside the epidemiological data collection based on the platform set up by WHO (leaning towards a model 3 solution, see below).

## *Streptococcus pneumoniae*

**Method:** WGS

**International molecular/genomic typing schemes and resources:** Conventional MLST nomenclature, core SNP phylogenetic analyses, antibiotic resistance mutation nomenclature and capsular phenotype prediction software [97].

**Rationale:** *Streptococcus pneumoniae* infection constitutes a major public health problem worldwide. Invasive pneumococcal disease (IPD), defined as the isolation or detection of *S. pneumoniae* through PCR or antigen testing in a normally sterile fluid, may present as different syndromes, from mild bacteraemia to pyogenic meningitis and septic shock. Starting in 2009, the old 7-valent pneumococcal vaccine has gradually been replaced by conjugate vaccines covering 10 and 13 serotypes in most European countries. However, initial vaccine success in reducing the incidence of IPD in children was followed by failure in several countries due to replacement by non-vaccine strains such as serotype 22F [97]. To sustain IPD surveillance and vaccination impact assessment in the EU/EEA, ECDC established an active sentinel surveillance network for IPD, the SpIDnet (*Streptococcus pneumoniae* Invasive Disease network) project. In 2015, the project was extended to 15 partners from 10 EU Member States.

Besides capsular serotyping of invasive strains of *S. pneumoniae*, WGS-based analysis is necessary to determine the impact of current vaccines/immunisation on vaccine escape mutants, the spread of successful clones, and the spread of antibiotic resistance [97]. This will provide evidence for guidance on immunisation strategies with current and new vaccines. In addition, this periodic analysis will allow the identification and monitoring of successful clones in the post-vaccination era as well as the identification of clones and virulence factors associated with severe outcome.

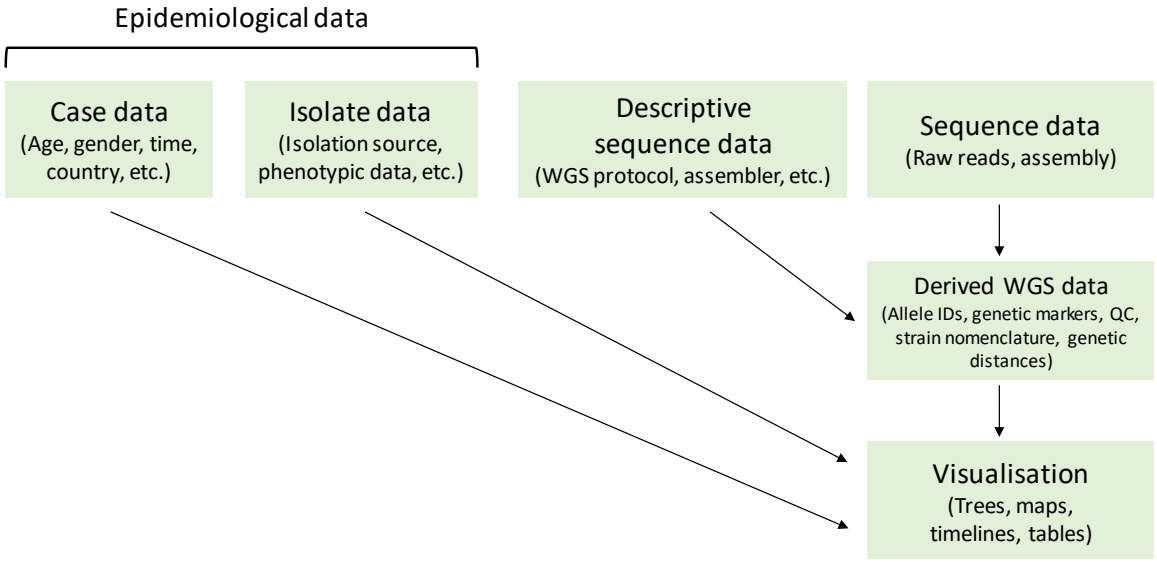
**Priorities for preparation and/or implementation in 2019–21:** ECDC will coordinate the development of a protocol for sentinel WGS-based typing of *S. pneumoniae* isolates from IPD cases. The Centre will also conduct a pilot study on this topic. The objective is to analyse the clonal diversity and identify capsular antigen shifts and replacement by non-vaccine antigen clones selected by vaccine selective pressure (operational model 3).

# Implementation of ECDC/TESSy platform for sequence data sharing, integrative analysis and reporting

This chapter briefly describes envisioned technical solutions for sharing, storage, analysis, and visualisation of WGS typing data. The overall long-term vision is to use a combination of solutions, some delivered by ECDC, while others are provided by external providers:

- Data providers submit sequences and descriptive data about sequences and epidemiological data (Figure 2) in a timely fashion, using an easy-to-use solution for machine-to-machine communication.
- In accordance with the Member States' policies for public WGS data release, WGS data will be uploaded to a protected, access-controlled, reliable and long-term storage solution. WGS data are made public after an initial embargo period if the data provider so desires.
- The WGS data submission process should ensure that every isolate submitted to the WGS storage solution comes with corresponding epidemiological TESSy data.
- Data are analysed, signals detected and visualisations produced with a high level of automation.

**Figure 2. Definitions of the various data types used throughout this chapter**




*Note: Arrows indicate which data are derived based on other data*

Meaningful visualisations that can be used for monitoring, outbreak response, presentations, and reports. Visualisations can be made available to relevant stakeholders through a portal provided by ECDC.

The current system at ECDC is built on the following components:

- Sharing: direct submission of assemblies or the European Nucleotide Archive (ENA) and the Short Read Archive (SRA) accession numbers, as well as epidemiological data and descriptive data about sequences to TESSy, SFTP for raw data, e-mail for assemblies
- Storage: ENA/SRA is used for WGS data generated by an ECDC sequencing provider; it is also used for data published in scientific articles and data already in ENA/SRA. Short-term local storage is used for other WGS data
- Analysis: BioNumerics cgMLST for cluster detection
- Visualisation: BioNumerics trees, MicroReact.

The main bottleneck of the current system is the amount of manual handling involved in data sharing, analysis and visualisation by data providers and ECDC. The reasons for this are mainly the lack of automation at the data providers' side and the lack of reporting of epidemiological data in TESSy. There are several ongoing pilot projects and studies based on different models that could potentially address this issue. There is also a need to create more automated tools for data handling and sharing, for use by both ECDC and data providers.

The main guiding principle for all future systems is that ECDC should not be responsible for the long-term storage of complete genomic raw read data. ECDC therefore pilot-tested the use of protected workspace models with storage and analysis capacity located outside ECDC. This model would allow the Member States to submit data for centralised analysis under defined conditions and with flexibility in terms of public data release. Subsequently, derived genomic data would be integrated with epidemiological data collected in TESSy. The complete dataset would then be graphically visualised and sent back to the Member States. Following the principle of an external

protected workspace, several models were tested for the diseases covered in ECDC genomic roadmap 2.1 [3]. For C/CRE and *N. gonorrhoeae*, a workspace solution hosted by the Wellcome Sanger Institute (United Kingdom) was used. For *N. meningitidis*, EMERT, a protected workspace within the PubMLST.org/neisseria database hosted by the University of Oxford (United Kingdom) is being set up. For *Salmonella*, a workspace located within EBI is being piloted, dubbed the Horizon 2020 COMPARE–ECDC joint project. During 2018, an evaluation of the external protected workspace models was performed, and a general implementation proposal was discussed with the NMFPs and NSFPs.

## Models for EU WGS data sharing, storage, integrated epidemiological analysis and data visualisation

A total of three models were proposed (Figure 3).

**Model 1, in-house solution:** WGS data are submitted to TESSy through an ECDC FTP server or a WGS data repository that is either public or can be accessed by both the submitting institution and ECDC, with epidemiological data submitted to TESSy. ECDC performs analysis and graphical visualisation, and possibly submits WGS data to long-term storage (e.g. externally provided WGS storage component such as ENA). TESSy is capable of receiving assemblies directly and can import locally stored raw reads, but further automation is required to make this method feasible for large data volumes. TESSy is also capable of importing raw reads that are publicly available in SRA/ENA; there is, however, no functionality for directly receiving data from databases restricted to a set of users.

**Model 2, hybrid solution:** WGS raw read data are submitted to an externally provided complete storage and analysis system, epidemiological data are submitted to TESSy. The WGS data are analysed in the external analysis system, and derived data are added to TESSy by ECDC. Graphic visualisations, including the generation of phylogenetic trees from allele identifiers, is performed by ECDC.

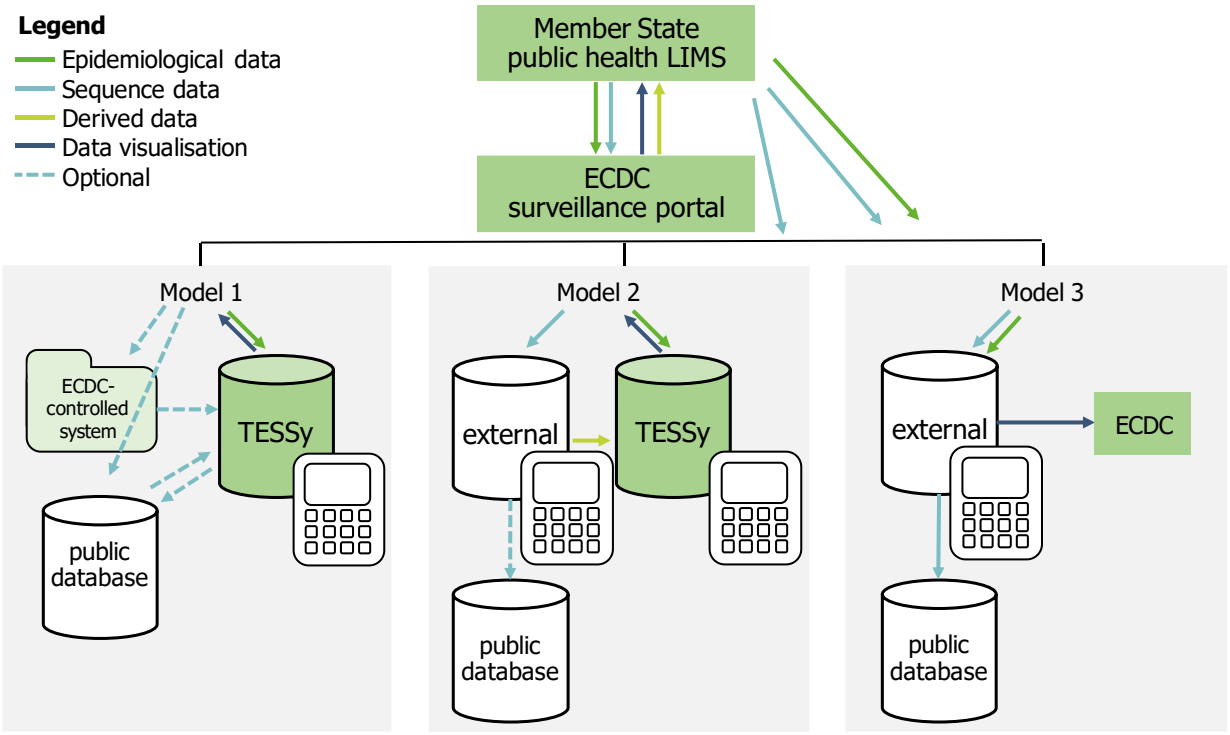
**Model 3, external solution:** Both WGS data and epidemiological data are submitted to an external storage and analysis system; epidemiological data are either provided by ECDC via TESSy or transmitted directly to the external storage solution. Analysis and graphic visualisation is performed in the external system; visualisations are provided through a portal controlled by ECDC.

Models 1 and 2 require a well-working infrastructure for data transfer and temporary storage, automated data submission to the analysis solution used, and the subsequent handling of results. Manual work at all levels of data processing (up to the confirmation of clusters and outbreak response in a routine situation) should be as little as possible. Development, testing, error handling, support and maintenance cannot be automated.

For a number of pathogens, different analytical approaches are possible. Regardless of the chosen approach, all models should conform to the data model used in TESSy in order to ensure a consistent method for data access and visualisation. The exception to this is the external solution (model 3) because WGS-derived data are not added to TESSy, which reduces the value of model 3 because separate external visualisation solutions need to be maintained. The model could still be worth considering, e.g. if many pathogens use the same external system or if there is additional value in using a specific solution for a specific pathogen.

Graphic visualisations of WGS data mainly comprise phylogenetic trees combined with epidemiological data. Visualisations provided by ECDC are meant for internal use in cluster detection and outbreak response as well as for external use by the Member States (e.g. when related to urgent inquiries). The requirements can be different for different applications, which makes it important to ensure that all visualisation needs can be met.

**Figure 3. Schematic models 1 (in-house solution), 2 (hybrid solution) and 3 (external solution)**




*Note: Dashed lines denote optional steps.*

Output visualisation needs are:

- Different types of phylogenetic trees for different needs, e.g. single-linkage-, neighbour joining-, minimum spanning-, and maximum-likelihood trees
- Powerful interactive views with convenient plots and tables for operational work such as outbreak investigations
- Easy-to-understand educational interactive views for presentations
- High resolution technical views/images for reports and scientific articles.

To fulfil these needs, it is likely that several visualisation solutions need to exist in parallel though it is possible that a single solution can meet all needs. Off-the-shelf solutions should be used whenever possible and integrated with ECDC systems. If no available system can fulfil the needs, additional development may be required. If visualisations are provided by external services, they should be integrated into the ECDC web portal or bear an ECDC logotype to indicate that the presented data are provided by TESSy.

It is also critical that an integrated upload solution (data providers upload data to both the WGS storage solution and to TESSy through a single interface) is developed. This solution should work independently of the choice of model for specific pathogens.

The SSR project at ECDC will impact the platform and solutions used for data upload and visualisation. The project includes development of a data warehouse where all surveillance data, including derived WGS data, will be stored in a structured format; it also includes a surveillance web portal through which data and visualisations will be made available to TESSy users.

Factors that could affect the pace of implementation for existing and additional diseases include the following:

- Outcome of ongoing pilots and implementations for WGS-data sharing and analysis
- The implementation of comprehensive typing of *L. monocytogenes* (model 1). If this model turn out to be effective it can be applied to other pathogens as well
- Final results of a pilot project that used protected workspaces at EBI provided by the COMPARE project for *S. enterica* (model 1). These workspaces are an upgrade to the standard ENA which allows for the sharing of sequence data within a restricted set of ENA users
- Changes in the ECDC Disease Programmes and core unit work plans and budgeting.

There is a framework contract between ECDC and the University of Oxford (United Kingdom) for the customisation of the EMERT (II) platform for *N. meningitidis* sequence-based typing data. EMERT use entails is a hybrid solution where typing data are collected and analysed in the external system and derived data are imported into TESSy (model 2).

An external platform (Pathogen Watch, formerly WGSA) is used (model 3) for the ongoing 2018–2020 C/CRE WGS survey with the Public Health Agency of Sweden and the Wellcome Sanger Institute.

Resources for implementation of the strategic framework are needed to support WGS data production, automatisation of data management, data analysis, data interpretation, and communication. The resource needs in the Member States and at ECDC also depend on the amount of microbiological clusters that prompt outbreak response and follow-up actions at EU level. The resources needed at ECDC also depend on the involvement of the surveillance networks and the level of outsourcing for analysis, interpretation and communication.

Table 4 summarises the proposed possible data flow models to consider and test for gradual implementation of EU public health sequence-based molecular data operations in 2019–2021.

**Table 4. Suggested operational models for managing molecular and genomic typing data proposed for priority pathogens for strategic framework implementation activities, 2019–2020**

| Pathogen | Outbreak | Continuous surveillance | Sentinel surveillance or periodic surveys |
|---|---|---|---|
| ECDC programme: Antimicrobial Resistance and Healthcare-Associated Infections (ARHAI) | | | |
| Carbapenem-resistant *Acinetobacter baumannii* | WGS (model 1) | NA | WGS (model 3) |
| Carbapenem-/colistin-resistant Enterobacteriaceae | WGS (model 1) | NA | WGS (model 3) |
| *Clostridium difficile* | WGS (model 1; TBC) | Postponed due to low Member State WGS capacity | PCR-ribotyping (per agreed EU/EEA surveillance scheme) |
| Outbreaks of emerging MDR or XDR pathogens including MRSA | WGS (model 1) | NA | NA |
| Outbreaks of healthcare-associated pathogens with new modes of transmission | WGS (model 1; TBC) | NA | NA |
| ECDC programme: Emerging and Vector-Borne Diseases (EVD) | | | |
| West Nile virus | WGS (model 3) | NA | NA |
| ECDC programme: Food- and Waterborne Diseases and Zoonoses (FWD) | | | |
| *Campylobacter jejuni/C. coli* | Ad hoc WGS support in case of a suspected multi-country event | NA | NA |
| Hepatitis A virus | Gene-sequencing or WGS (model 3) | NA | NA |
| *Legionella spp.* | SBT, WGS (WGS (possibly model 2) | SBT (per agreed EU/EEA surveillance scheme; WGS currently not applicable) | NA |
| *Listeria monocytogenes* | WGS (model 1) | WGS (model 1) | NA |
| *Salmonella* enterica | MLVA, WGS (model 1) | WGS (model 1) | NA |
| Shiga toxin-producing *E. coli* (STEC) | WGS (model1) | WGS (model 1) | NA |
| ECDC programme: HIV, AIDS, STIs and Viral Hepatitis (HSH) | | | |
| Antibiotic-resistant *Neisseria gonorrhoeae* | NA | NA | WGS (model 3) |
| HIV | NA | NA | Gene sequencing or WGS (model 1, model 3)<br>Interim solution: aggregated interpreted data collection |
| ECDC programme: Influenza and other Respiratory Viruses (IRV) | | | |
| Influenza virus | NA | Gene-sequencing or WGS (model 1) | Gene-sequencing or WGS (model 1) |
| ECDC programme: Tuberculosis | | | |
| MDR/XDR TB | WGS (model 3; transition to model 2 aimed for by 2020) | WGS (model 3; transition to model 2 aimed for by 2020) | NA |
| ECDC programme: Vaccine-Preventable Diseases (VPD) | | | |
| *Bordetella pertussis* | NA | NA | WGS (Model 3) |
| *Neisseria meningitidis* | WGS (model 2) | WGS (model 2) | NA |
| *Streptococcus pneumoniae* | NA | NA | WGS (Model 3) |

*Note: NA, not applicable; TBC, to be confirmed*

## WGS data access and use

While the new technical solutions for sharing, storage, analysis, and graphic visualisation of WGS typing data are gradually implemented in TESSy, ECDC is working with each disease network to develop pathogen- or disease-group-specific policies for data access and use. A template for these policies was created in collaboration with the ECDC Legal Section to ensure that all key issues are adequately addressed. Once the new technical solutions for WGS data collection and management are fully implemented, ECDC will create a common access/use policy for WGS data in TESSy that will apply to all pathogens.

## Quality assurance for submitted WGS data

For each disease with WGS implemented as the primary method for molecular typing, a validation study/inter-laboratory ring trial will be conducted before data collection officially begins. The trial will determine a) what quality controls ECDC will perform on raw sequencing reads and, where applicable, assemblies, and b) which genome characterisations (wgMLST, resistome prediction, virulence gene detection, etc.) will be performed (and with which standards). Furthermore, the trial will c) assess whether laboratories are able to generate data of sufficient quality for raw sequencing reads, assemblies, and genome characterisations to ensure full comparability. For the generation of raw sequencing reads, a ring trial where all participating laboratories receive a set of isolates to sequence may be performed, or the results of existing ring trials accepted. For assembly and genome characterisation, a ring trial where all participating laboratories receive a set of raw sequencing reads will be performed. The purpose of this exercise is to ensure the robustness of the technology and validate that each laboratory has the capacity to generate comparable and valid analytical results.

Laboratories with no current WGS capacity, but planning to use the technology at a later stage, will be asked to participate in the trial before officially submitting surveillance data to ECDC in order to verify their qualifications in accordance with mutually agreed minimum quality requirements. The pass-/fail criteria for the qualification testing will be defined – in collaboration with the networks – ahead of the ring trials.

Regular EQAs may no longer be needed to ensure the quality of WGS data for diseases where WGS is implemented as the sole molecular typing tool once the initial ring trial generated satisfactory results for each and every reporting laboratory. The sequence data and associated sequencing and assembly pipeline quality metric metadata ensure a high degree of quality. Laboratories generating low-quality data in routine collection will be offered troubleshooting support.

# ECDC genomic epidemiology support

The governance of the disease-specific genomic epidemiology operations outlined above will follow existing EU mechanisms for surveillance, threat detection and threat alerts in accordance with the EU legal framework, operational agreements, and data protection obligations. In addition to the above-mentioned disease-specific activities, relevant ECDC Disease Programmes and core sections will continue the following generic support actions in order to strengthen genomic epidemiology methods and applications in 2019–2021:

- Map WGS-based public health initiatives and sustain partnerships. This includes advising on the objectives and design of EU-funded research projects and working with EFSA and global partners on WGS-based genotype and inferred phenotype nomenclature, joint databases and surveillance systems in a One Health approach to food-borne pathogens.
- Lead on, and assist with, the integrated analysis of epidemiological and WGS data. This includes working with the Member States to formulate the need for data and analyses that will meet surveillance objectives and ensure that case-based linkage of the epidemiological and microbial information is established at the national reporting level. ECDC will support the testing of WGS analytical platform options to share sequence information and related epidemiological data.
- Gather expert opinion and provide guidance on the appropriate use and validation of WGS-based bioinformatics methods for surveillance. ECDC will contribute to the development of international and cross-sector agreements on WGS quality standards, analytical schemes and genomic type nomenclature for the disease agent/resistance determinants under monitoring, in collaboration with the scientific community, EU and international health agencies, and national reference laboratories.
- Support the professional development of staff in Member States and at ECDC in applied bioinformatics and genomic epidemiology by organising multidisciplinary training workshops for public health microbiologists, epidemiologists and risk managers about analysis, reporting, interpretation and use of integrated genomic epidemiology data for risk assessment and risk communication.
- Develop, run and evaluate selected pilot implementation studies. ECDC will contribute to the evaluation of operational performance, epidemiological validity and effectiveness of the disease-specific WGS-based surveillance selected in the updated roadmap. This includes the design and monitoring of surveillance system performance indicators, including timeliness and statistical robustness of signal detection and attributable disease prevention metrics for the evaluation of public health benefits of system implementation.

Furthermore, to ensure a coordinated, harmonious and timely development of surveillance methods and tools in a global context and to keep up with technological developments in the area of WGS, ECDC will:

- continue to organise disease network meetings and joint annual meetings between NMFPs and NSFPs where key WGS developments and their impact will be appraised and decisions taken on how the EU surveillance system should be adapted; ensure regular reviews of progress towards WGS strategy implementation in the disease networks during annual meetings and ad hoc workshops;
- develop and pilot test surveillance processes and ICT tool functionalities based on a range of assumptions concerning type of data sources, envisaged data flows, ECDC strategies and surveillance objectives. This will be done within the framework of the ECDC SSR project;
- liaise with the European Commission Directorate General for Health and Food Safety (DG SANTE) to ensure prioritisation of notifiable diseases and timely integration of new surveillance case definitions in the legal text; and
- liaise and collaborate with EFSA, EU research and development projects on applied pathogen genomics, WHO and national public health partners at EU and international levels. These collaborations aim at developing international surveillance standards and a sequence-based strain type nomenclature to ensure strategic coherence, coordination and interoperability of data standards and systems, and use of optimal analytical methods and robust interpretation criteria for transmission source identification.

# Implementation and follow-up of the ECDC strategic framework

Issues of optimised epidemiological and microbiological data linkage and timely reporting for control-oriented surveillance and outbreak investigations as well as re-definitions of outbreak and outbreak cases will be resolved through pragmatic problem solving and multidisciplinary discussions in a disease-by-disease manner. These revised risk assessment criteria will be based on demonstrable benefits for infection control. They may lead to modernisation of current surveillance programmes and health protection or food safety regulations.

It is important to underline that the thresholds of sequence relatedness within cluster signals from genomic surveillance analysis that are considered adequate for undertaking further epidemiological cluster investigations have to be agreed upon by the Member States at the operational level. This will be resolved on a disease-by-disease basis by the participants of dedicated surveillance and response networks and laboratory consortia with the goal to optimally fulfil their statutory obligations to protect public health.

Implementation of the proposed actions will be subject to the available resources (technical equipment, staff and operational personnel) at the ECDC and Member State levels. The decision on whether and when to participate in any of the proposed molecular or genomic surveillance activity on a voluntary basis is up to the Member States. Likewise, the amount of sequence data shared through the ECDC platform for European surveillance is decided by the Member States, based on expected benefits from cross-country comparisons weighed against the burden of uploading substantial amounts of data. The level of resources required for molecular typing data management support to data providers depends on the amount of microbiological clusters that prompt outbreak investigation and response; on the level of automation achieved for data sharing, analysis and reporting; and also on the distribution of analysis, interpretation, and communication workload between ECDC and the participating networks.

Evaluation will be monitored by reporting on the relevant process, outcome and impact indicators for molecular surveillance as included in the Agency's annual work plans. These indicators are defined in accordance with the ECDC public health microbiology strategy 2018–2022 [98] and the ECDC surveillance strategy 2014–2020 [99]. In addition, joint implementation research, including longitudinal time series modelling analyses, will be desirable to evaluate the long-term effectiveness and cost-effectiveness of WGS-enhanced surveillance and control programmes at national and EU-wide levels.

The next strategic framework revision is scheduled for 2021.

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# Annex 1

## Risk and outbreak assessments of multi- country food- and waterborne outbreaks reported, 2016–2018

| Pathogen (number of assessments) | Title, publishing date and reference |
|---|---|
| *Salmonella* enterica (11) | Rapid risk assessment: Multi-country outbreak of *Salmonella* Enteritidis phagetype 8, MLVA type 2-9-7-3-2 infections. 5 September 2016. [1] |
| | Multi-country outbreak of *Salmonella* Enteritidis phage type 8, MLVA type 2-9-7-3-2 and 2-9-6-3-2 infections. 27 October 2016. [2] |
| | Rapid risk assessment: Multi-country outbreak of *Salmonella* Enteritidis PT8 infection, MLVA type 2-10-8-5-2, associated with handling of feeder mice. 5 December 2016. [3] |
| | Rapid risk assessment: Increase in *Salmonella* Stourbridge infections in Germany during 2016 - 1st update. 27 January 2017. [4] |
| | Re-emerging multi-country WGS-defined outbreak of *Salmonella* Enteritidis, MLVA type 2-12-7-3-2 and 2-14-7-3-2. 3 February 2017. [5] |
| | Rapid outbreak assessment: multi-country outbreak of *Salmonella* Enteritidis phage type 8, MLVA profile 2-9-7-3-2 and 2-9-6-3-2 infections. 7 March 2017. [6] |
| | Rapid risk assessment: Cluster of new *Salmonella* serotype cases in four EU Member States. 20 March 2017. [7] |
| | Rapid outbreak assessment: Multi-country outbreak of new *Salmonella* enterica 11:z41:e,n,z15 infections associated with sesame seeds. 14 June 2017. [8] |
| | Rapid risk assessment: Multi-country outbreak of *Salmonella* Enteritidis phage types 56 and 62, MLVA profile 2-11-3-3-2 and 2-12-3-3-2 infections. 20 July 2017. [9] |
| | Multi-country outbreak of *Salmonella* Enteritidis infections linked to Polish eggs. 12 December 2017. [10] |
| | Joint rapid outbreak assessment: Multi-country outbreak of *Salmonella* Agona infections linked to infant formula. 16 January 2018. [11] |
| *Listeria monocytogenes* (2) | Multi-country outbreak of *Listeria monocytogenes* PCR serogroup IVb, MLST ST6. 6 December 2017. [12] |
| | Multi-country outbreak of Listeria monocytogenes serogroup IVb, multi-locus sequence type 6, infections probably linked to frozen corn. 22 March 2018. [13] |
| *Legionella* (2) | Rapid risk assessment: Increase of Legionnaires' disease in EU travellers returning from Dubai since October 2016. 21 September 2017. [14] |
| | Outbreak of travel-associated Legionnaires' disease – Palmanova, Mallorca (Spain), September–October 2017. 23 October 2017. [15] |
| Hepatitis A (2) | Rapid risk assessment: Hepatitis A outbreak in the EU/EEA mostly affecting men who have sex with men, 3rd update. 28 June 2017. [16] |
| | Rapid risk assessment: Multi-country outbreak of hepatitis A virus genotype IA infections affecting EU countries in 2018. 21 May 2018. [17] |
| STEC (1) | Rapid outbreak assessment: multi-country foodborne outbreak of Shiga toxin-producing *Escherichia coli* infections associated with haemolytic uraemic syndrome. 6 April 2016. [18] |

# Annex 2

## Pilot studies and collaborative initiatives on molecular typing for pathogens and diseases not prioritised in current ECDC implementation framework

A number of organisations and collaborative projects have established molecular typing schemes for different or larger groups of pathogens. A list of earlier projects was presented in an expert opinion on whole genome sequencing for public health surveillance [19]. Additional projects are described below.

## Hepatitis E virus

Hepatitis E virus (HEV) is one of the leading causes of acute viral hepatitis worldwide. Hepatitis E is an underdiagnosed disease and shows locally high endemicity, with increased case numbers in some EU/EEA countries [20,21]. In 2015, ECDC initiated activities on hepatitis E to better understand the epidemiological situation in the EU/EEA Member States. An ECDC HEV expert group [22] was established with national experts and representatives from WHO and EFSA. A semi-structured survey was circulated to the ECDC national focal points for food- and waterborne diseases and zoonoses in January 2016 to assess the hepatitis E surveillance activities in EU/EEA Member States. Twenty of 30 responding countries have a specific national hepatitis E surveillance system, with case definitions and guidelines in place [21,23]. Of the 26 EU/EEA Member States that test for HEV, 17 reported that they also conduct HEV sequencing [23].

The ECDC HEV expert group recommended the establishment of a sequence database for the support of molecular epidemiological investigations across countries. ECDC supported RIVM in their initiative to establish the HEV sequence database, HEVnet [24]. This voluntary initiative has been available for interested experts working in the field of HEV since 2017. In 2017, the first HEVnet meeting was held at RIVM. It was supported by ECDC to review the overall objectives and conduct a first data analysis. In 2017, EFSA published a scientific opinion on the public health risk associated with HEV as food-borne pathogen [25]. An increasing number of EU countries invested in studies on HEV from a public health point of view but also with regard to veterinary or food safety. Several EU/EEA Member States initiated blood donation screening programmes because of hepatitis E risk assessments over the last few years. ECDC conducted an expert consultation to review the data and assess the risk and prevention of hepatitis E virus transmission through substances of human origin in Lisbon in 2016 [26]. Hepatitis E is also included in WHO's action plan for the health sector response to viral hepatitis [27]. To support the EU/EEA Member States' response to WHO's action plan, ECDC and the ECDC HEV expert group are currently developing a technical guidance document entitled 'Suggestions for national testing and surveillance for hepatitis E in the EU/EEA', which will be published in 2019. ECDC continues its activities on hepatitis E but is currently not prioritising HEV for routine molecular surveillance at the European level.

## Non-polio enteroviruses

Non-polio enteroviruses (NPEVs) cause a high burden with regard to a wide range of diseases, ranging from mild respiratory symptoms to severe neurological outcomes and even death. NPEVs circulate year-round, with higher circulation in summer and autumn in the USA [28] and Europe [29,30]. Enteroviruses (EVs) are RNA viruses and include polioviruses (PV), coxsackie A viruses (CAV), coxsackie B viruses (CBV), echoviruses (E) and numbered EVs. EVs cause a wide spectrum of infections in humans, including non-specific febrile illness and viral exanthema, respiratory infections, hand-foot-and-mouth disease (HFMD), myocarditis, meningitis, encephalitis, and, rarely, acute flaccid paralysis (AFP) or acute flaccid myelitis (AFM) or even death.

The main public health needs for NPEV surveillance include outbreak detection and response, and monitoring of EV types associated with severe disease. In 2014, severe respiratory outbreaks of EV-D68 occurred in the USA [31], Canada, and Europe [32,33]; 59% of all EV-D68 cases (data from three US states) had to be submitted to intensive care units [31]. A total of 120 AFM cases were reported from 34 states [34]. In Europe, three AFP cases and one death were associated with these outbreaks in 2014 [32]. In 2016, several European countries reported severe infections with NPEVs [35], following an EV-A71 outbreak with severe neurological symptoms among 87 children in Spain [36].

To better respond to NPEV outbreaks, the European surveillance and laboratory capacity for detection of NPEVs was mapped [37]: 26 of 29 EU/EEA Member States conduct laboratory-based NPEV surveillance and 24 included neurological infections in their surveillance. Only five Member States reported using AFP surveillance. Nineteen countries reported using sequence-based EV typing. As the enterovirus diagnostics and characterisation showed to be quite variable, recommendations for specimens to be collected as well as laboratory methods to be used were

prepared [38]. At the same time, the European Non-Polio Enterovirus Network (ENPEN) was established [38]. Because of the voluntary and passive nature of most enterovirus surveillance systems in Europe, NPEV infections are not well recognised and underreported. Prospective studies evaluating the NPEV disease burden in Europe are needed to determine the public health needs for establishment of continuous surveillance of NPEVs.

## Respiratory syncytial virus

Respiratory syncytial virus (RSV) is the major pathogen causing severe lower respiratory tract infections among infants and young children. RSV is the most common cause of hospitalisation for acute lower respiratory tract infection in children younger than five years and is estimated to cause between 66 000 and 199 000 deaths worldwide every year [39]. The year-round circulation of RSV causes annual global epidemics that show a seasonal pattern. RSV seasons peak later and last longer with increasing latitude in Europe [40]. Immunoprophylaxis to prevent RSV infection with a neutralising monoclonal antibody, palivizumab, is available for certain target groups and given on a monthly basis during the RSV season, and a vaccine is expected to enter the market within 5–10 years, presumably by 2025 [41].

RSV infection is not notifiable in most of the EU/EEA countries, however, weekly detection data are collected in TESSy on a voluntary basis through the influenza-like illness or acute respiratory infection surveillance systems [40]. RSV disease burden and clinical impact studies are ongoing in Europe [42], and RSV surveillance systems were recently mapped [43]. The majority of EU/EEA countries have active surveillance and sufficient laboratory capacity for RSV, but case definitions and laboratory methods should be standardised. Typing is performed in 20 Member States, and the majority of countries use the N gene for typing and the G gene for genotyping. Sequencing is performed in eight Member States, and 12 Member States plan to introduce next generation sequencing in the near future. Seventeen Member States reported participating in external quality assessments for RSV detection every year [43]. WHO has developed a strategy for a global pilot project on RSV surveillance and appointed three RSV reference laboratories, one of which is the one run by Public Health England in the United Kingdom [44].

There is no RSV-specific surveillance in the EU/EEA. Surveillance, however, is a priority due to the impending launch of vaccine. This was discussed both in ECDC expert consultations [45] and in the ECDC Advisory Forum. Several surveillance options were discussed in the European Influenza Surveillance Network annual meeting 2017, where all Member States supported the establishment of RSV surveillance, mainly through enhanced acute respiratory infection surveillance (GP based) as well as enhanced sentinel paediatric hospital surveillance. For the monitoring of circulating viruses and comparisons to the vaccine strains, it will be crucial to introduce sequence-based monitoring of RSV in the EU/EEA [27]. However, at this stage, ECDC is not prioritising RSV for routine molecular surveillance at the European level.

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