TECHNICAL REPORT

Monitoring the use of whole-genome sequencing in infectious disease surveillance in Europe 2015–2017

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Monitoring the use of whole-genome sequencing in infectious disease surveillance in Europe

2015–2017

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Abbreviations

cgMLST	Core genome multilocus sequence typing
MDR	Multidrug-resistant
MLVA	Multiple-locus variable number tandem repeat analysis
NGS	Next-generation sequencing
NMFP	National Microbiology Focal Points
NRL	National reference laboratory
PFGE	Pulsed-field gel electrophoresis
RT-PCR	Reverse transcription polymerase chain reaction
SNP	Single nucleotide polymorphism
STEC	Shiga toxin-producing *Escherichia coli*
WGS	Whole-genome sequencing

Executive summary

Whole-genome sequencing (WGS) coupled with epidemiological and environmental investigations delivers ultimate resolution for detecting and analysing transmission routes and tracing sources of epidemic infections as well as assessing microbial virulence and antimicrobial drug resistance determinants. WGS-based typing and phenotype prediction has therefore become an essential tool for public health surveillance and molecular epidemiology of infectious diseases and antimicrobial drug resistance.

Since 2015, the ECDC National Microbiology Focal Points have been monitoring the level of implementation of WGS-based typing for national public health surveillance and investigation of prioritised diseases across the European Union (EU) and European Economic Area (EEA). The 2017 survey aims to take stock of EU/EEA national plans and technical capacity for use of WGS in public health surveillance operations. This information update will be essential for the revision of ECDC's roadmap on disease priorities for molecular typing integration into surveillance and epidemic preparedness. It will also guide ECDC action to support equitable public health access to genomic-based typing methods across the EU/EEA.

All 30 EU/EEA countries participated in the 2017 survey. The vast majority of national public health reference laboratories had access to WGS-based typing for public health applications. Illumina was the most frequently used technology, followed by Ion Torrent technology. The access to bioinformatics expertise for routine WGS data analysis was reported as a common limiting factor. By mid-2017, two-thirds of the EU/EEA countries were using WGS analysis, either as first- or second-line typing method for surveillance of the pathogens and antibiotic resistance issues identified as EU priorities. The sampling frame used and the bioinformatics analysis varied according to the pathogen/resistance issue and the country. Core genome multi-locus allelic profiling was the most frequently used analytical approach to genotyping bacterial genomes, suggesting that there is potential bioinformatics pipeline and nomenclature compatibility. Further capacity development for WGS-based typing is ongoing, with 29 countries either using or planning to start genomic surveillance operations by 2019.

Building upon this tremendous opportunity, ECDC will help to consolidate and harmonise WGS-based typing methods to enable pan-EU genomic surveillance and multi-country outbreak investigations by developing secure and flexible sequence and epidemiological data exchange, management and analysis systems.

1 Introduction

Whole-genome sequencing (WGS) provides higher resolution and accuracy than classical molecular typing methods, such as PFGE or MLVA, contributing to a better understanding of infectious disease and drug resistance transmission patterns and thereby improving the effectiveness of interventions for their control. As the technology progresses, it becomes increasingly efficient and cost-competitive for diagnostic and surveillance purposes. Despite these advantages, challenges with the costs of implementing the technologies, lack of expertise and the need to adapt epidemiological investigation methods may limit its use by public health laboratories. Moreover, further harmonisation for bioinformatic analysis, smart and secure information technology solutions for WGS data storage and sharing, and trained staff with new skills mixes will ensure that genomic epidemiology translates into real-life infection control and prevention [1].

The state of the art is evolving towards WGS as a replacement of other molecular methods for surveillance and outbreak investigations. Taking stock of the latest advances, ECDC has outlined a priority list of diseases for which WGS data will be gradually integrated into EU-level surveillance systems and multi-country investigations of cross-border outbreaks [2]. This ambitious European cooperative process builds upon the operational capacity to implement WGS-based typing for public health applications among Member States of the European Union and European Economic Area (EEA) [2,3]. To assess the EU/EEA Member States' national capacities to implement WGS-based typing, ECDC and the National Microbiology Focal Points (NMFPs) have jointly conducted annual web-based surveys (2015–2016). These surveys map access of national public health reference laboratories (NRL) to NGS technologies and bioinformatics expertise and the use by these laboratories of WGS-based typing for national surveillance and outbreak investigations of the eight highest-priority foodborne, antimicrobial resistant and vaccine-preventable pathogens selected for European genomic surveillance [1]. The 2017 survey is presented for comparison with the findings of previous annual surveys. The main aim of this monitoring activity is to inform the revision of ECDC's roadmap for integration of molecular and genomic typing into European surveillance and epidemic preparedness so that it can be aligned with national surveillance programmes to maximise EU public health added value.

2 Methods

ECDC used the online survey software (https://ec.europa.eu/eusurvey/) for the collection of relevant information by the NMFPs from the 30 EU/EEA countries. The surveys collected information on WGS practice and development plans as of mid-2015 to 2017 by the competent NRL. The detailed methodology used for the 2015–2016 surveys is described in the previous publication [1], whereas the technical report here describes the 2017 survey. The invitation to respond to the survey for data as of July 2017 was sent to the NMFPs on 16 October 2017 and the survey was open until 8 December 2017.

The 2017 survey contained ten questions for each of the eight pathogens prioritised for European genomic surveillance, including foodborne pathogens [*Listeria monocytogenes*, *Salmonella enterica* and Shiga toxin-producing *Escherichia coli* (STEC)], antimicrobial-resistant pathogens (carbapenemase-producing *Enterobacteriaceae*, antibiotic-resistant *Neisseria gonorrhoeae* and multidrug-resistant *M. tuberculosis*) and vaccine-preventable diseases (*Neisseria meningitidis* and human influenza virus). These questions covered public health NRL access to WGS, bioinformatics expertise and WGS-based operational typing capacity and practice for outbreak investigations or national surveillance. NMFPs could choose whether to collect all the information and respond in a single survey or to forward individual pathogen-specific links to the respective national public reference laboratories. All NMFPs received their respective national surveys and a brief summary upon completion. A total of two reminders were sent for the data collection.

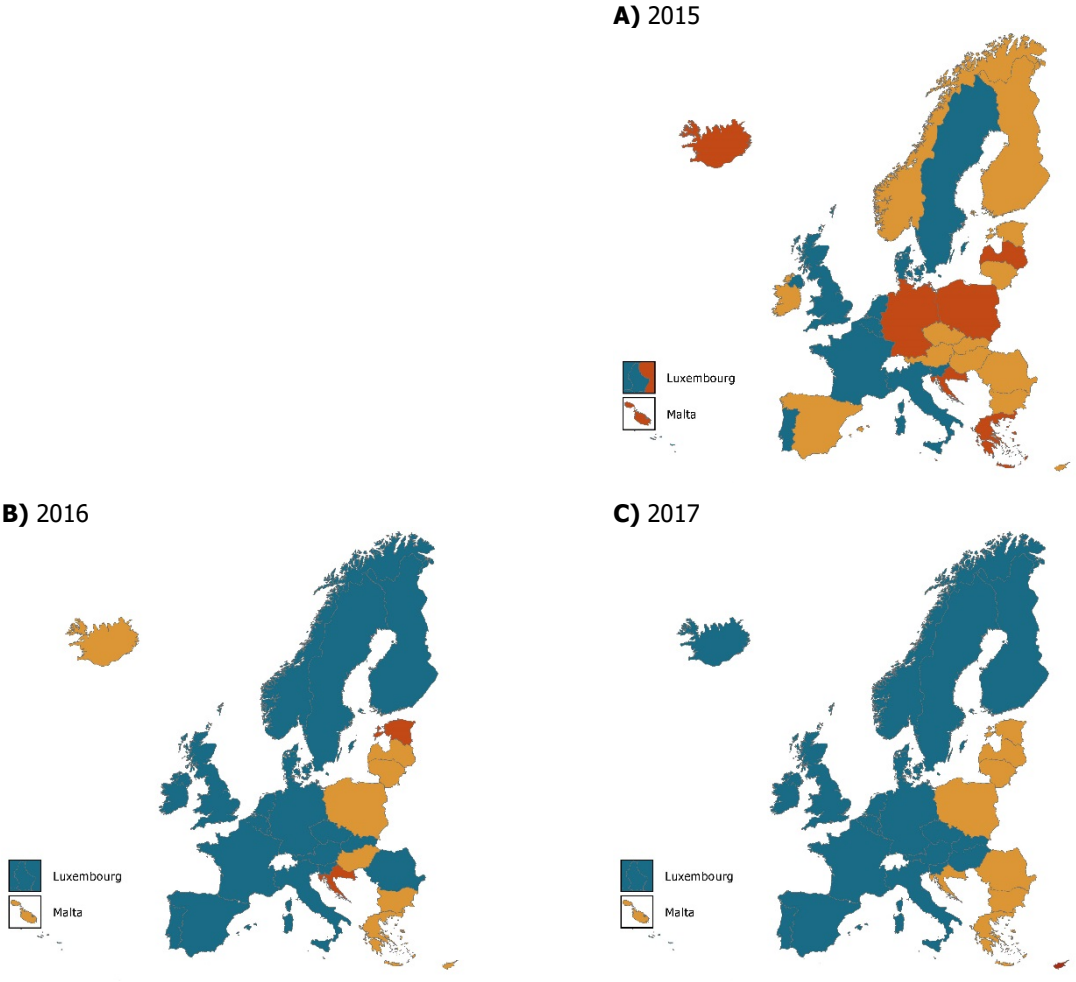
The analysis included a mapping of the countries using, or planning to use WGS-based typing for either outbreak investigation, surveillance or both, and related temporal trends by year of survey. To estimate the maximum fraction of EU/EEA population and EU/EEA total notified diseases potentially covered by genomic-based surveillance at national level in 2017, the data on user countries was extrapolated to calculate their country population divided by the total EU/EEA population (source: Eurostat 2016), and the user country notified cases divided by total number of EU/EEA notified cases of disease, if available (source: ECDC Atlas, 2016 data). The bioinformatics approaches used and information outputs produced were described and compared by user country and target pathogen or resistance phenotype.

3 Results and discussion

The total number of respondents for the surveys 2015-2017 was 28, 29 [1] and 30, respectively. For both 2015 and 2016 data collection, data was further validated with the EULabCap surveys [5-7]. Regarding the 2017 survey, data was collected incompletely for carbapenemase-producing *Enterobacteriaceae* (n=28 countries responded) and antibiotic-resistant *Neisseria gonorrhoeae* (n=29 countries responded). In addition, one Member State did not complete the survey in its entirety, having only replied completely to three (invasive *N. meningitidis*, human influenza virus and MDR-TB) out of eight diseases and partially to three diseases (*L. monocytogenes, S. enterica* and STEC).

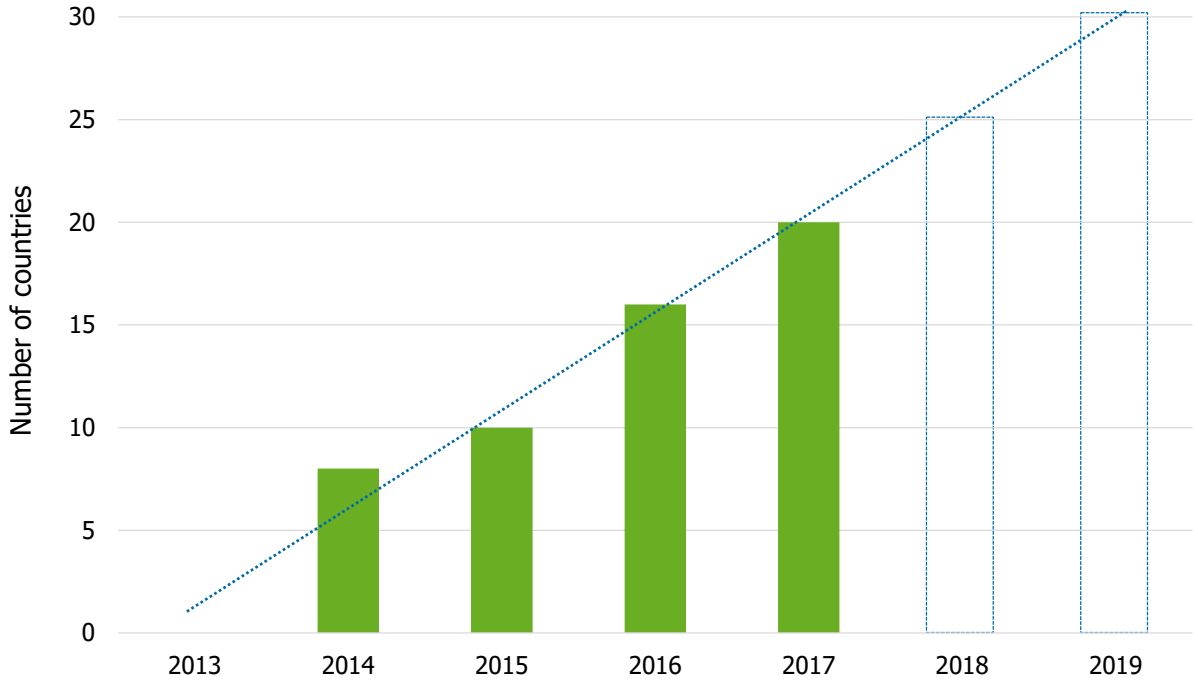
The number of EU/EEA countries reporting WGS-based typing by the National Public Health Reference Laboratories for routine surveillance and outbreak investigations of at least one human pathogen markedly has increased since 2013: no EU/EEA country in 2013 [4], eight EU/EEA countries in 2014 [5], ten EU/EEA countries in 2015 [1,6] and sixteen countries in 2016 [1]. In 2017, a total of 20 EU/EEA countries used WGS-based typing routinely for national surveillance of at least one human pathogen (Figure 1 and 2). In addition, more non-user countries reported that they had started planning to implement WGS-based typing by 2019 for surveillance applications (either for outbreak investigations or for routine surveillance, with only one country not using and not planning for at least one human pathogen (Figure 1 and 2).

Figure 1. National public health reference laboratories use of WGS-based typing for national surveillance of at least one human pathogen



Legend: blue: in use; light orange: a plan in place/in progress within three years; dark orange: no use or national plan in place within three years. EU/EEA countries, 2015–2017.

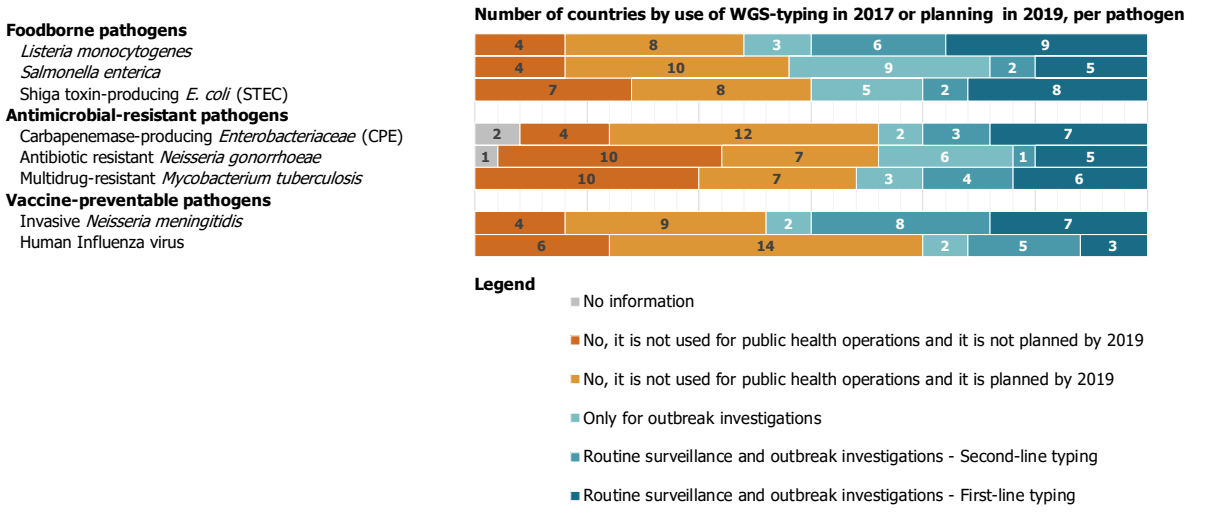
Figure 2. WGS-based typing trend in the EU/EEA, WGS used routinely for national surveillance for at least one human pathogen



Based on the EULabCap survey for 2013–2014 [5-6], surveys 2015-2016 and prevision for 2018 [1] and the 2017 status and prevision for 2019 (present survey).

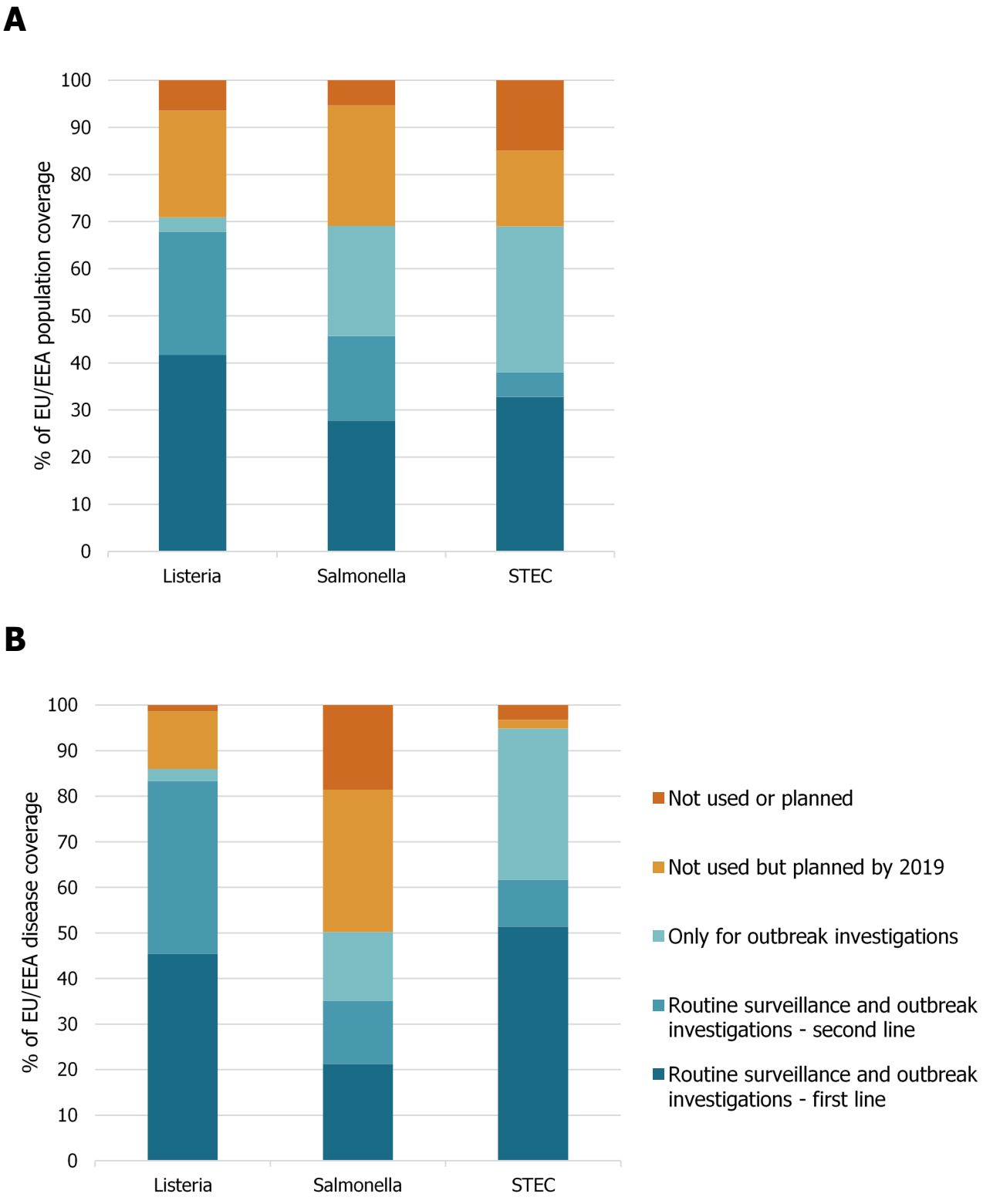
The target pathogens for which countries most frequently used WGS-based typing in 2017 for both outbreak and surveillance applications were, in order of decreasing frequency, *N. meningitidis*, followed by *L. monocytogenes* and STEC (Figure 3), a trend that has endured since 2015 [1].

Figure 3. Number of EU/EEA countries using WGS-based typing as first or second-line method for routine surveillance and outbreak investigations in National Public Health Reference Laboratories by disease group and pathogen, 2017



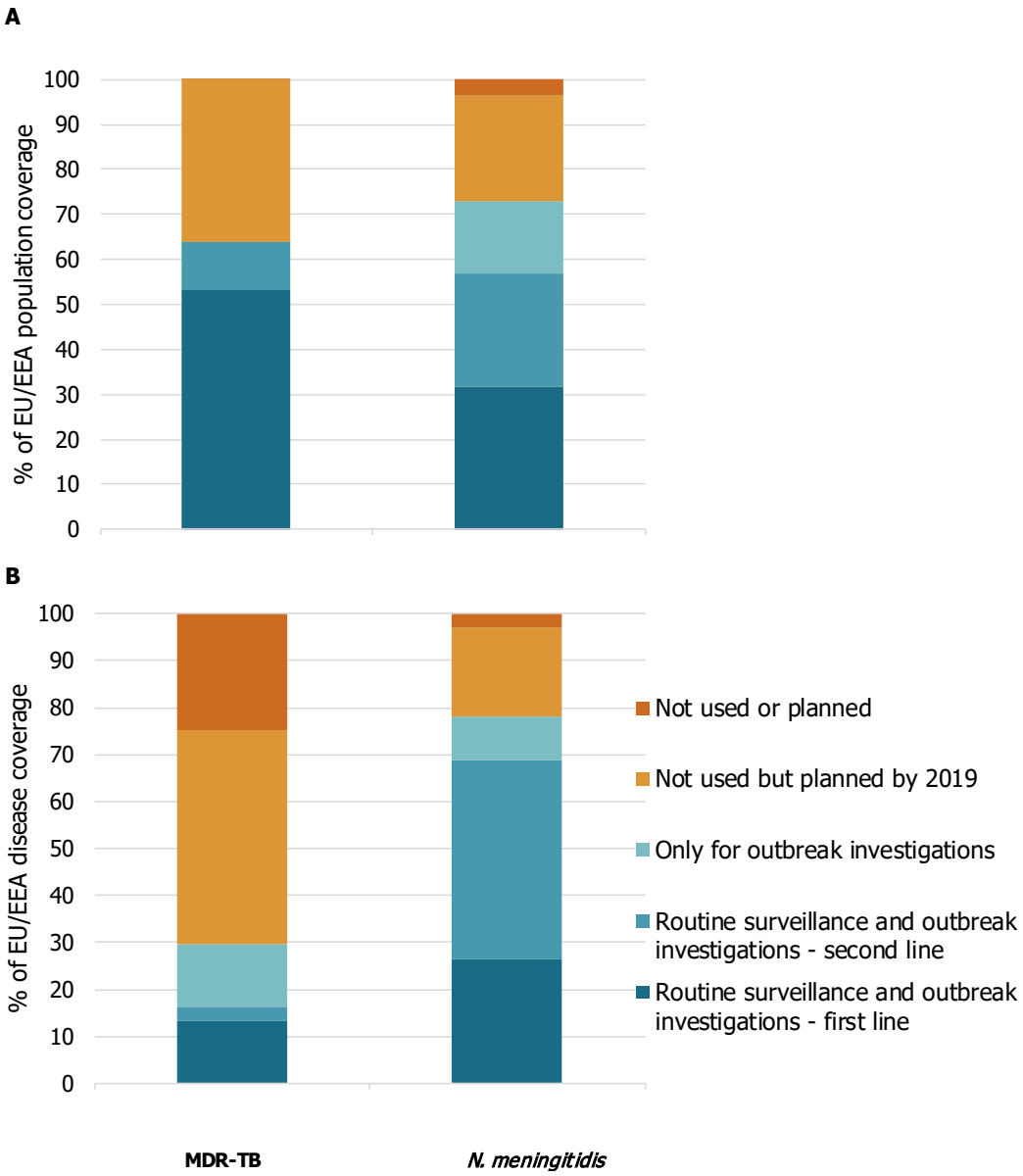
L. monocytogenes was the food-borne pathogen most frequently typed using WGS for surveillance and outbreak investigations, followed by STEC and *S. enterica* (Figure 3). When weighted in terms of proportion of population and disease burden, WGS-based surveillance of food-borne infections ranged from 38–68% of the EU/EEA population and 35–83% of all notified cases in the EU/EEA (Figures 4A and 4B respectively). For *L. monocytogenes* and STEC, population coverage was lower than disease coverage, but for *S. enterica,* population coverage was higher (Figure 4).

Figure 4. WGS-based typing surveillance for food-borne diseases, 2017 coverage by exposed population (A) and total notified cases (B), EU/EEA, 2016



In terms of proportion of population and disease burden, WGS-based surveillance of MDR-TB infections was estimated to be 64% of EU/EEA population coverage in contrast with only 16% of notified cases in the EU/EEA (Figure 5A and 5B, respectively). On the other hand, coverage for invasive *N. meningitidis* infections was estimated to be 57% of the EU/EEA population and even higher in terms of disease burden, with a total of 69% of total notified cases in the EU/EEA (Figure 5A and 5B, respectively).

Figure 5. WGS-based typing surveillance for MDR-TB and invasive N. meningitidis diseases, 2017 coverage by exposed population (A) and total notified cases (B), EU/EEA, 2016

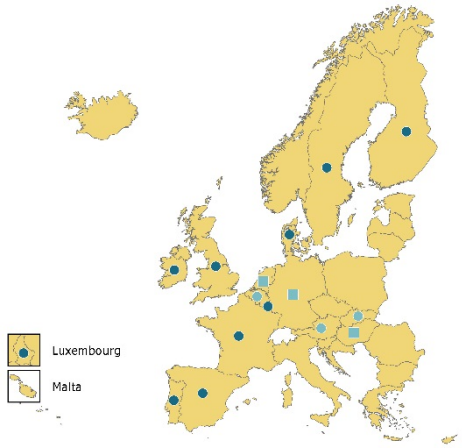


The use of WGS-based typing in 2017 in the EU/EEA countries for routine surveillance and its respective surveillance sample frame and pathogen typing scheme are presented in Figures 6 and 7.

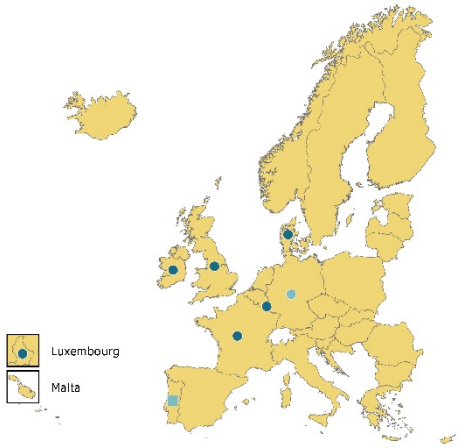
With regard to the use of WGS-based typing for food-borne pathogens in 2017, the pathogen that was most comprehensively characterised by WGS as first line typing method was *Listeria monocytogenes* (nine countries), followed by STEC (eight countries) and *S. enterica* (five countries) (Figure 6 and Table 3).

Figure 6. EU/EEA national public health reference laboratories' use of NGS technologies for typing (routine surveillance and outbreak investigation), as of July 2017

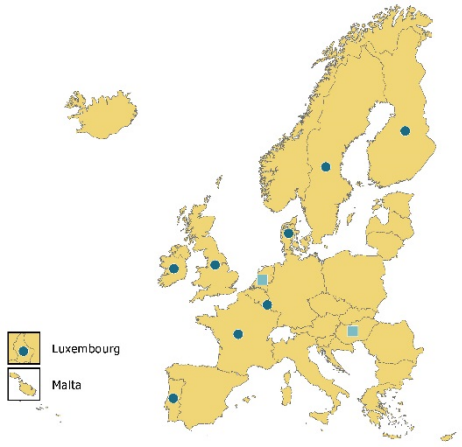
A) *Listeria monocytogenes*



B) *Salmonella enterica*



C) STEC



Yellow: participation in the survey; sampling frame (comprehensive – circle and sentinel/subset of samples – square) and typing scheme (first line – dark blue and second line – light blue) for A) Listeria monocytogenes, B) Salmonella enterica, C) STEC

In 2017, countries using next generation sequencing (NGS) technologies for either outbreak investigations or routine surveillance most frequently used Illumina, followed by Ion Torrent technology (Table 1). The technology distribution found in the 2017 survey is similar to that for previous surveys [1].

Table 1. Number of EU/EEA countries with one or more national public health reference laboratories having access to next-generation sequencing (NGS) technologies for routine public health operations, by technology and instrument used, 2017

		Food-borne pathogens			Antimicrobial-resistant pathogens			Vaccine-preventable diseases	
NGS technology	**Instrument**	*L. monocytogenes*	*S. enterica*	STEC	CPE	AR-N. *gonorrhoeae*	MDR-TB	*N. meningitidis*	Human Influenza virus
Illumina	HiSeq series	3	3	3	2	1	2	3	1
	HiSeq X series						1		
	MiniSeq	1	2	3	2		4		1
	MiSeq series	12	10	7	7	10	7	13	7
	NextSeq	2	2	2	3	1	3	2	2
Ion Torrent	S4								1
	S5	1	1	1	1		1		
	S5 XL								1
	PGM		1	1		1		1	1
	Proton							1	1
Oxford Nanopore Technologies	MinION		1	1	2		1	1	1
Pacific Biosciences-PacBio	PacBio RS II		1		2				
	Sequel		1	1					
Other not specified	-	3	2	2	1	1	1	1	

Among diverse bioinformatic pipelines used by the national public health reference laboratories for WGS data analysis, the core genome multilocus sequence typing (cgMLST), often used in combination with single-nucleotide polymorphisms (SNP) analysis, was the most commonly used approach across pathogens (Table 3). Other commonly produced outputs included the seven housekeeping genes MLST and the phenotype prediction (resistome/virulome/mobilome and serogroup), which were often used in combination (Table 3). The type of software used by the EU/EEA countries for the bioinformatic analysis of food-borne pathogens in 2017 included a mixture of either commercial or open source software (Table 2).

Table 2. Bioinformatics tools used by the National Public Health Reference Laboratories using WGS-based typing for surveillance and outbreak investigations of food-borne pathogens, July 2017*

	Number of EU/EEA countries		
Tools used for sequence analysis	*L. monocytogenes* (n=14)	*S. enterica* (n=7)	STEC (n=9)
Commercial software	9	4	4
Open source software	4	3	5
In-house suite of customised tools	4	2	2

* Not mutually exclusive

Among the antimicrobial-resistant pathogens surveyed, multidrug-resistant (MDR) *M. tuberculosis* was the most intensively typed by WGS as first-line typing method in the largest number of countries for a continuous, comprehensive sample of cases. This was followed by CPE or carbapenem-resistant *Enterobacteriaceae* (Table 3). In contrast, first-line WGS-based typing was typically restricted to a sentinel subset of samples for surveillance of antibiotic-resistant *N. gonorrhoeae* (Table 3).

The sequential typing scheme definition differs in interpretation for bacterial pathogens and for viral pathogens. For bacterial pathogens first-line typing means that WGS is used as the primary typing method while second-line typing means it is used as a secondary method to complement results from another typing method. With regard to viral pathogens, particularly human influenza virus, sequencing is used for further characterisation of a sample subset. Indeed, in laboratories from the eight countries that reported routinely using WGS for influenza typing, the first-line (sub)typing is performed by RT-PCR, followed by genotyping using either Sanger or NGS technology as second-line typing.

Figure 7. EU/EEA national public health reference laboratories use of NGS technologies for typing (routine surveillance and outbreak investigation), as of July 2017

Yellow: participation in the survey; grey: no participation in the survey; sampling frame (comprehensive – circle and sentinel/subset of samples – square) and typing scheme (first line – dark blue and second line – light blue) for A) MDR TB, B) invasive N. meningitidis, C) antibiotic-resistant N. gonorrhoeae and D) carbapenemase-producing Enterobactereaceae and E) human influenza virus.

For WGS data storage, the NRL in the vast majority of EU/EEA countries deposited their raw sequence (fastq files) data in dedicated closed databases (either national or international) rather than public repositories such as ENA (Table 3). The most frequently reported reason for this practice was the priority given to this information for national reporting and risk assessment, followed by the need for permission for scientific publication of original data. The third most cited reason was the need for personal data protection.

Table 3. Number of EU/EEA countries using WGS-based typing for surveillance and outbreak investigations in the national public health reference laboratories and respective typing scheme, sampling frame, bioinformatics analysis, and raw data storage practice by pathogen, 2017

	2017	Food-borne pathogens			Antimicrobial resistant pathogens			Vaccine preventable pathogens	
		L. monocytogenes	*S. enterica*	STEC	CPE	AR-*N. gonorrhoeae*	MDRTB	Human influenza virus	*N. meningitidis*
Number of countries using WGS for routine surveillance and outbreak investigations		14	7	9	10	6	10	8	15
Typing scheme	First-line WGS	9	5	8	7	5	6	3	7
	Second-line WGS	5	2	1	3	1	4	5	8
Sampling frame	Continuous comprehensive	12	6	8	7	2	9	-	15
	Sentinel/ subset of case samples	2	1	1	3	4	1	8	-
Bioinformatic analysis *	cgMLST	12	6	5	6	4	5	-	12
	SNP	7	5	5	5	2	7	-	5
	Resistome prediction	4	5	7	8	4	6	-	3
	wgMLST	5	3	3	2	2	2	-	2
	Virulome/ mobilome prediction	4	2	9	5	1	-	-	1
	MLST prediction	12	6	8	3	-	-	-	2
	Serogroup prediction	7	6	9	1	-	-	-	2
	NG-MAST	-	-	-	-	3	-	-	-
	Speciation	-	1	1	-	-	3	-	1
	Hemagglutinin and neuraminidase sequence prediction	-	-	-	-	-	-	4	-
	Phylogenetic relationship	-	1	1	1	-	-	7	1
	Identification of specific point mutations	-	1	1	-	-	-	6	1
	rMLST	-	-	-	-	-	-	-	5
	MLST+*porA* VR1 and VR2+*fetA*	-	-	-	-	-	-	-	12
	Vaccine antigen prediction	-	-	-	-	-	-	-	9
	Other not specified	-	-	-	1	-	3	3	1
Raw sequence data storage *	Dedicated closed database(s)	13	5	7	10	6	10	6	12
	Publicly available database(s)	1	2	2	-	1	1	2	3

**: not mutually exclusive.*

4 Conclusion

The results of the third pan-EU/EEA survey presented herein showed that by mid-2017 the vast majority of national public health reference laboratories in EU/EEA countries had access to WGS-based typing of diverse microbial pathogens for investigations of infection and drug resistance transmission. Two-thirds of the countries were routinely using WGS in 2017 for national surveillance of at least one human pathogen. Moreover, WGS use was planned to be implemented within three years in the non-user countries. This observation confirms and extends the previously reported rapid public health implementation of the technology across the EU/EEA [1].

This emerging mainstream practice should enable pan-European WGS-derived data exchange in the medium-term, subject to harmonisation of sequence analysis pipelines for output compatibility, agreement on international WGS-derived type nomenclature and development of secure and efficient international data sharing and management platforms. The findings of the latest survey also confirm that current bottlenecks mainly relate to development of expertise in epidemiological-WGS data integrative analysis and access to user-friendly international nomenclature. Together with its partners, ECDC will contribute to identifying solutions and broadening capacities in these areas, with the primary aim of facilitating inter-operability with EU and national surveillance and outbreak response programmes. To this end, ECDC will consider the projected WGS-data outputs across the EU/EEA when updating its disease priority roadmap for the integration of epidemiological and WGS-based typing data into European surveillance systems in line with national practice.

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Annex 1.

NMFP questionnaire on 2017 national capacity for Whole Genome Sequencing (WGS) use for Public Health applications in EU/EEA countries

Fields marked with * are mandatory.

Dear NMFP,

This survey aims at taking stock of EU/EEA National public health plans and current technical capacity for use of Whole Genome Sequencing (NGS/WGS) for public health surveillance operations. This information update will be taken into consideration for the revision in 2018 of the ECDC roadmap for molecular typing integration into surveillance and epidemic preparedness and to identify the needs for supporting the transition to genomic-based typing methods.

In case of any question or if assistance is required do not hesitate to contact us at ECDC. Microbiology@ecdc.europa.eu or by phone +46(0)858601216.

Thank you for your inputs!

Kind regards,

Joana Revez, survey coordinator and HelpDesk,
Microbiology Coordination Section, ECDC

Country information

* Please specify the country you represent

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- United Kingdom

* Please enter your name

* Please enter your email

Current national NGS/WGS practices, as of July 2017

General Questions

* **1.** Do Public Health reference laboratories in your country have access to Next Generation Sequencing NGS technology for public health operations?

(tick more than one if applicable; **if you only select the option "No", then please select Not applicable from questions 3-5)**

- [ ] Yes, internal access to NGS
- [ ] Yes, external access to NGS (isolates or genetic material is sent elsewhere)
- [ ] No
- [ ] Do not know

If external access to NGS, please specify where [e.g. other national laboratories, commercial provider(s)].

* **2.** If limited or no access, what is(are) the reason(s)?

(tick more than one if applicable)

- [ ] Current plan to implement NGS/WGS by 2019
- [ ] No plan to implement NGS/WGS by 2019
- [ ] Delayed implementation until further development of the application
- [ ] Gaps/lack of expertise
- [ ] Lack of funding
- [ ] Lack of staff to implement
- [ ] Not applicable
- [ ] Other

If other**,** please provide further details.

* **3.** Do public health reference laboratories have access to sufficient bioinformatics expertise and competence for routine WGS data analysis?

- ( ) Yes, sufficient bioinformatics expertise and competence within public health reference laboratories
- ( ) Yes, some degree of expertise and competence supplemented with external expertise

- Yes, fully outsourced to external service
- Other
- Not applicable

If other, please specify.

* **4.** In your public health reference laboratories, what are the training needs for public health operations?
(tick more than one if applicable)

- Bioinformatic analysis
- Genome-based typing nomenclature for international comparison
- Integration/interpretation of WGS data into public health risk assessment methods
- Other

If other, please specify.

Typing practices

ECDC roadmap V2.1 categorises pathogens/diseases by priority for EU surveillance integration:
1. **Operationalisation of EU wide WGS-based surveillance systems in the near term:** *Listeria monocytogenes*, *Neisseria meningitidis*, Carbapenemase-producing *Enterobacteriaceae* (CPE) and antibiotic-resistant (AR) *Neisseria gonorrhoeae*.
2. **Operationalisation of WGS-based surveillance systems deferred until the required technical capacity across the EU/EEA is met:** human influenza virus, *Salmonella enterica*, Shiga-Toxin producing *E. coli* (STEC) and multidrug-resistant (MDR) *Mycobacterium tuberculosis*.
3. **Further required evidence of the opportunities and challenges:** PCR-ribotyping for *Clostridium difficile* surveillance, and sequence-based surveillance of anti-viral drug resistance in human immunodeficiency virus (HIV) and Hepatitis C virus (HCV).
4. **Postpone until next roadmap revision in 2018:** West Nile virus (WNV) and meticillin-resistant *Staphylococcus aureus* (MRSA).

For the first two above mentioned prioritization categories of pathogens (a total of 8 human pathogens), please reply to the respective tailored questions.

5. Do public health reference laboratories use NGS technologies for typing, as of July 2017?

	No, it is not used for public health operations and it is not planned to implement it by 2019	No, it is not used for public health operations and it is planned to implement it by 2019	Only for outbreak investigations	For routine surveillance and outbreak investigations

* *L. monocytogenes*				
* *N. meningitidis*				
* CPE				
* AR-*N. gonorrhoeae*				
* Influenza				
* *S. enterica*				
* STEC				
* MDR-TB				

6. Which NGS/WGS technology platform(s) is(are) used?

	Illumina MiniSeq	Illumina MiSeq series	Illumina NextSeq	Illumina HiSeq series	Illumina HiSeq X series	Pacific Bioscience PacBio RS II	Pacific Bioscience Sequel	Ion Torrent PGM	Ion Torrent Proton	Ion S5	Ion S5 XL	Oxford Nanopore MinION	Other	Not applicable
* *L. monocytogenes*	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
* *N. meningitidis*	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
* CPE	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
AR-*N. gonorrhoeae*	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Influenza	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
S. enterica	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
STEC	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
MDR-TB	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

If other, please specify which NGS/WGS technology platform(s) is used.

7. If WGS used by reference laboratories for public health operations, which typing scheme is used, as of July 2017?

	Not applicable, as these technologies are not used for public health operations	Second-line typing method, as complement to other methods (If WGS used as second line of typing, please specify to which typing method(s) it is complementary)	First-line typing method (defined as the primary method of choice for fine-typing)
* *L. monocytogenes*	○	○	○
* *N. meningitidis*	○	○	○
* CPE	○	○	○
* AR-*N. gonorrhoeae*	○	○	○
* Influenza	○	○	○
* *S. enterica*	○	○	○
* STEC	○	○	○
* MDR-TB	○	○	○

For *L. monocytogenes,* please specify to which typing method(s) it is complementary

For invasive *N. meningitidis,* please specify to which typing method(s) it is complementary

For CPE, please specify to which typing method(s) it is complementary

For AR-*N. gonorrhoeae,* please specify to which typing method(s) it is complementary

For Influenza*,* please specify to which typing method(s) it is complementary

For *S. enterica,* please specify to which typing method(s) it is complementary

For STEC*,* please specify to which typing method(s) it is complementary

For MDR-TB*,* please specify to which typing method(s) it is complementary

8. Please describe the sampling frame applied for typing with NGS technologies, as of July 2017.

	Not applicable, as these technologies are not used for public health operations	Repeat surveys samples	Sampling a subset of specimens /sentinel cases	Continuous comprehensive sampling	Other
* *L. monocytogenes*	○	○	○	○	○
* *N. meningitidis*	○	○	○	○	○
* CPE	○	○	○	○	○
* AR-*N. gonorrhroeae*	○	○	○	○	○
* Influenza	○	○	○	○	○
* *S. enterica*	○	○	○	○	○
* STEC	○	○	○	○	○
* MDR-TB	○	○	○	○	○

Please specify the sampling frame

9. Which tool(s) are used for the sequence data analysis?

	Not applicable, as these technologies are not used for public health operations	commercial software (e.g. BioNumerics)	open sourced software	in-house suit of customized tools	Other
* *L. monocytogenes*	○	○	○	○	○
* *N. meningitidis*	○	○	○	○	○
* CPE	○	○	○	○	○
* AR-*N. gonorrhroeae*	○	○	○	○	○
* Influenza	○	○	○	○	○
* *S. enterica*	○	○	○	○	○
* STEC	○	○	○	○	○
* MDR-TB	○	○	○	○	○

Please specify which kind of software

10. Please describe what kind of bioinformatic analysis is(are) done for the above public health applications

	Not applicable, as these technologies are not used for public health operations	cgMLST (Core genome MLST) scheme	SNP mapping	Resistome prediction	wgMLST (Whole genome MLST) scheme	Virulome/ mobilome prediction	Housekeeping gene MLST scheme	Serogroup /serotype prediction	NG-MAST sequence based scheme	Speciation	Haemagglutinin and neuraminidase sequences prediction	Phylogenetic relationships	Identification of specific point mutations	rMLST (ribosomal MLST) scheme	MLST+ *porA* VR1+ *porA* VR2+ *fetA* EMGM scheme	Vaccine antigens prediction	Other
* *L. monocytogenes*	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
* *N. meningitidis*	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
* CPE	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
* AR-*N. gonorrhoeae*	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
* Influenza	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
* *S. enterica*	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
* STEC	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
* MDR-TB	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Please specify which kind of other bioinformatic analysis are performed

11. Where are the raw sequence data (fastaq files) stored?

	Not applicable, as these technologies are not used for public health operations	Dedicated closed databases (national or international)	Publicly available databases (e.g. ENA etc) deposited along with epidemiological data (or so called "WGS metadata")	Publicly available databases (e.g. ENA etc) deposited without metadata
* *L. monocytogenes*	○	○	○	○
* *N. meningitidis*	○	○	○	○
* CPE	○	○	○	○
* AR-*N. gonorrhoeae*	○	○	○	○
* Influenza	○	○	○	○
* *S. enterica*	○	○	○	○
* STEC	○	○	○	○
* MDR-TB	○	○	○	○

General comments

General comments to ECDC

Thank you for taking the time to answer and for your support to ECDC and the Microbiology Coordination Section

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