D2.1 List of other pathogens

Work Package 2: Implementation of Whole Genome Sequencing Protocols

Author(s): Ákos Tóth, Bernadett Pályi, Judit Henczkó

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Other authors	Ákos Tóth, Bernadett Pályi		
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1.0	21.08.2023	Final version after integration of suggestions of consortium partners		NNK
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TABLE OF CONTENTS

1. EXECUTIVE SUMMARY

HERA 2 is the continuation of the HERA project, which aimed to establish RT-PCR and WGS for SARS-CoV-2. In the scope of the HERA 2, the consortium aims to ensure the sustainable use and integration of enhanced infrastructure into routine surveillance and outbreak investigation activities, in synergy with relevant on-going work at (inter)national level. The focus lies on the joint continuation of automation, optimisation, and validation of WGS/RT-PCR capacities (laboratory, bioinformatics, IT) and the establishment and validation of new genome sequencing methods (laboratory and bioinformatics). Another central aspect is the distribution of knowledge among the consortium members and other relevant stakeholders.

As each consortium country has a different background in terms of already implemented protocols, a survey was conducted to analyse and understand the current status and needs.

The consortium partners (Austria, Croatia, Greece, and Hungary) are parts or leaders of their national public health laboratory system, and they work with different pathogens. However, each country has a specific focus on the most targeted pathogens due to geographical differences and the implementation situation within the country. To facilitate focused work in the frame of the survey, the prioritised pathogens were collected from each country.

The pathogen list was drawn up according to the ECDC roadmap, this roadmap (Version 2.1, 2016–19) presents the recommended priority list of pathogens/diseases and technical implementation options for the medium-term integration of molecular/genomic typing into EU-level surveillance and epidemic preparedness. Additional inputs from the consortium partners were integrated, according to the public health priority and potential added value offered by WGS implementation. Prioritisation of the different pathogens was determined according to the agenda of the national agencies.

2. LIST OF ABBREVIATIONS

EU	European Union
NGS	Next Generation Sequencing
mNGS	Metagenomic Next Generation Sequencing
RT-PCR	Reverse Transcription Polymerase Chain Reaction
WGS	Whole Genome Sequencing
WHO	World Health Organization
ECDC	European Centre for Disease Prevention and Control
AMR	Antimicrobial resistance

3. BACKGROUND OF THE PROJECT

The HERA2 project has a duration of 42 months and is founded by the EU. It aims to ensure the sustainable use and integration of enhanced infrastructure into routine surveillance and outbreak investigation activities. The goal of work package 2 is to set up and implement the appropriate WGS methods and protocols at all consortium partners institutions.

The consortium conducted a survey to identify the main pathogens of public health relevance at the national level, assessing currently available and already implemented methods, as well as current needs. Once the pathogen list is available, the consortium will start to implement the necessary WGS methods to reduce the risk of cross-border outbreaks. All working groups will develop SOPs and guidelines on requirements the generation of whole genome sequencing (WGS) data and the use of the results of the WGS. This implementation will include short-read and long-read sequencing methods, including mNGS methods and bioinformatics. To facilitate cross-border searching, linking and analysis of genomic and health data, this WP will identify gaps and best practices.

4. OBJECTIVES AND CONTENTS

The objectives of the HERA2 Deliverable 2.1 support the specific objectives of the project, in order to provide solutions for consortium partners to implement and validate different WGS methodologies.

The diversity of communicable diseases is a major challenge for prioritisation of pathogens. Different infections, caused by emerging and re-emerging pathogens, or antimicrobial resistant bacteria, are considered as one of the most complex global health challenges today. Although differences in the distribution of the different pathogens, risk factors between countries are evident, gaps in surveillance, and a lack of standards for NGS methodology and data sharing particularly affect the public health response and the possible risk of cross- border threats as well. Infection prevention and control are the major options at our hands to reduce the burden of different infections. Nowadays, we are at the dawn of the age of genomics and we are witnessing a revolution. We can also be shapers of this change, as many areas of public health genomics are unfortunately still untapped. In this project, our task is to identify the gaps in our system at national level and to define the necessary method implementations, validations, or standardizations.

Considering that the entire NGS diagnostic process is complex, we will focus on the generation of validated output, rather than trying to standardize all steps along the way. The output should reach an agreed level of quality and only data that reaches this level will be entered into the system.

The main objectives and related works are:

1. Review and revise the project-related, relevant priority pathogens with epidemic and pandemic potential.
    - For this purpose, a survey was conducted on PCR/WGS capacities, enhancement potential and important pathogens
    - The survey was generated and shared between the project partners and national institutes from the countries. The original documentation was anonymised by indicating the country (but not required).
2. Develop and review the Pathogen List, which will be the basis for the implementation of the different NGS methods
    - The completed surveys were evaluated using Microsoft Office program.
    - The extracted draft pathogen list was checked and revised according to the WHO and ECDC priority list guidelines and for legal compliance purposes and unique national needs (different geolocations, public health prioritizations, communicable disease list, etc).
3. Provide and evaluate a final pathogen list by the partners for implementation of different whole genomic sequencing methods, including:
    - Short -read sequencing methods,
    - Long- read sequencing methods,
    - Metagenomics (short-read and long- read as well)

    and the relevant bioinformatics pipelines

5. FRAMEWORK

The purpose of the project delivery framework is to set the expectations and standards for how the project cycle should play out, from initiation to closure and post-implementation review. The framework should take into account the skills and training of the project partners, as well as the reporting requirements within the project.

Table 1. Framework of D2.1

Report on the survey was sent out	11.05.23	AGES
General report and individual country-specific reports were sent out/uploaded in confluence (WP2)	15.05.23	AGES, CIPH, EODY, NNK
Prioritisation of the pathogen list by every partner until 19.05. and input to NNK	19.05 2023	AGES, CIPH, EODY, NNK
End of May first draft of the Deliverable 2.1 was prepared by NNK	30.05.2023	NNK
Monitoring and proposed amendments	July 2023	AGES, CIPH, EODY, NNK
Create Final report of the Deliverable 2.1 by NNK	July 2023	NNK

6. TOOLS

The survey was generated using the software application Askallo. The results of the survey were analyzed among partners and used in the preparation of this deliverable.

7. RESULTS

All consortium partners defined the list of pathogens within their own competence considering national needs. An initial design of a first draft of the lists of pathogens was provided by AGES. The proposal was developed with the involvement of all partners and in line with ECDC and WHO recommendations (including priority setting).

Austria
8 surveys were filled out by Austria. One survey was from an organisation with 21-50 members, another one from an organisation with 51-100 members, one from an organisation with 101-200 members. The other 5 were returned by members of an organisation with more than 500 members.

Croatia
6 surveys were filled out by Croatia. One survey was from an organisation with 21-50 members, another one from an organisation with 101-200 members, 3 from an organisation with 201-500 members. The last survey was returned by a member of an organisation with more than 500 members.

Greece
4 surveys were filled out by Greece. 2 surveys were from an organisation with 1 to 10 members, 1 from an organisation with 21-50 members, The last survey was returned by a member of an organisation with 51-100 members.

Hungary
7 surveys were filled out by Hungary. 2 surveys were from an organisation with 1 to 10 members, 3 were from an organisation with 11-20 members, 1 from an organisation with 21-50 members, The last survey was returned by a member of an organisation with 101-200 members.

	Austria	**Croatia**	**Greece**	**Hungary**
PCR	West Nile virus	Shiga Toxin-producing *Escherichia coli* (STEC)	Norovirus-adenovirus-enterovirus, STEC, Shigella sp.,Campylobacter sp.	No plan to implement PCR method during the project
	Campylobacter sp.	Varicella zoster virus		
	Salmonella sp.	*Candida auris*		
	Listeria sp.	Disease X		
NGS	*Salmonella* sp.	*Campylobacter* typing + AMR	*Legionella* sp.	*Legionella* sp.
	Campylobacter sp.	*Legionella pneumophila* typing +AMR	Influenza virus	Disease X
	Carbapenem resistant *Pseudomonas aeruginosa*	*Listeria monocytogenes* typing + AMR	Norovirus	*Bordetella* sp.
	West Nile virus	*Salmonella* typing + AMR	RSV	Carbapenem-resistant *Enterobacterales*
	Norovirus	STEC + AMR		Enterovirus
	Influenza virus	Carbapenem resistant *Enterobacterales*		Respiratory Syncytial virus (RSV)
		Disease X		Influenza
		Adenoviruses		
		Enteroviruses		
		Influenza A + AVIAN influenza		
		Influenza B		
		Respiratory Syncytial virus		
		West Nile virus		

Table 2. Priority pathogen list of molecular detection and genotyping of pathogens of interest by country.

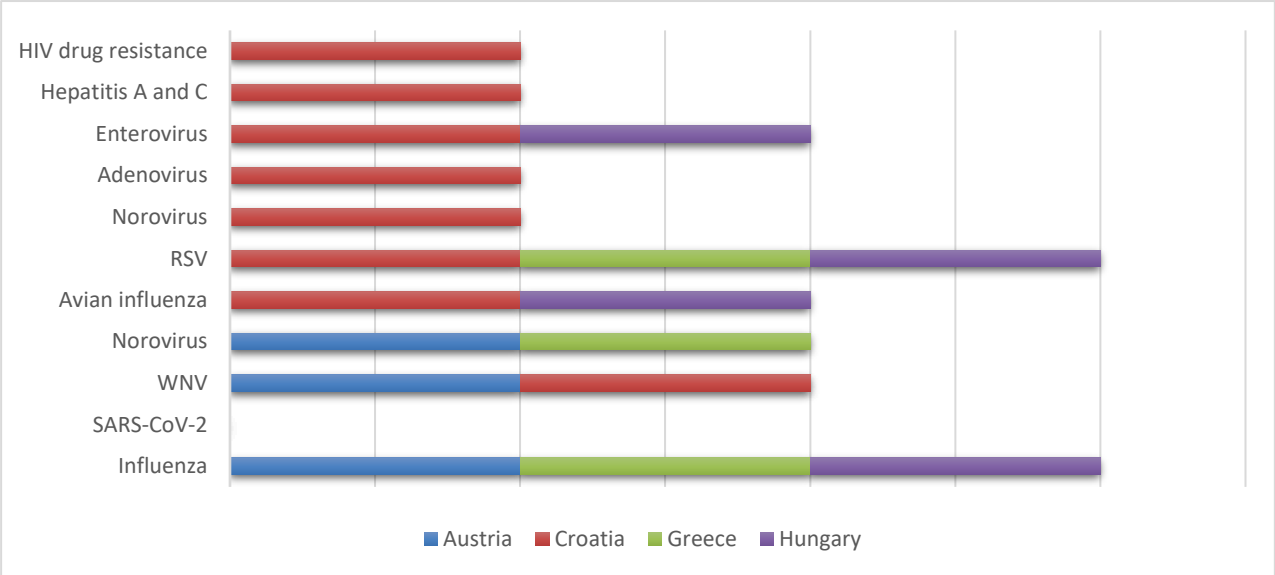

Figure 1. Priority list for Viral NGS

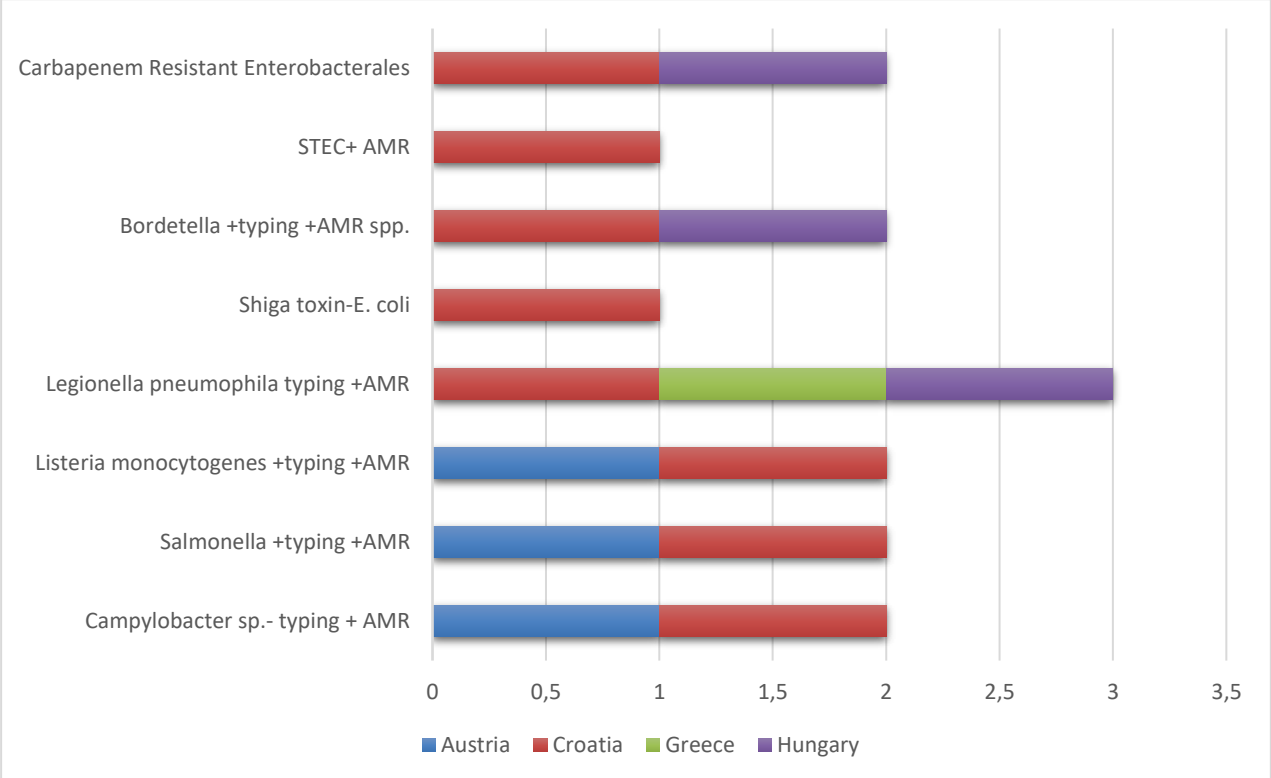

Figure 2. Priority list for Bacterial NGS

Outcomes

According to the conducted survey on PCR and WGS priority pathogens, there are differences in the ratio for bacteriological and virological agents. Figure 3 below visualizes the differences between PCR and NGS pathogens type:

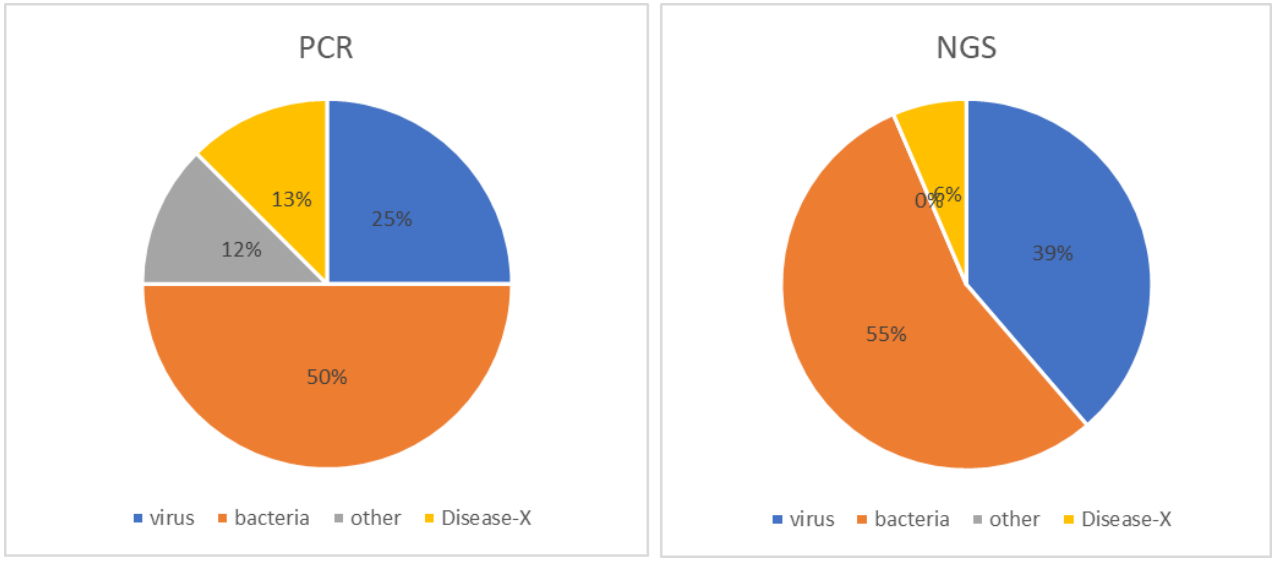

Figure 3. Comparison of priority list for PCR and NGS by type of pathogens.

For PCR one out four countries, Hungary, does not plan the implementation of any PCR based method. Austria, Croatia, and Greece have different priorities: Austria and Greece focus on food-borne related pathogens, while Croatia has no specific focus.

The differences between countries in terms of NGS are even more nuanced. Austria, Croatia, Greece, and Hungary focus on respiratory (all countries have highlighted influenza and RSV as a priority) and enteric viral pathogens. The following bacteriological pathogens are of high interest in all the consortium member countries: *Legionella pneumophyla*, *Bordetella* spp., carbapenem-resistant *Enterobacterales*, *Listeria monocytogenes*, *Salmonella enterica* and *Campylobacter* spp. are among the top priorities. Disease-X, which could be the next pandemic, is a focus in Croatia and Hungary.

According to the objectives reviewed and revised on the "survey on PCR/WGS capacities, enhancement potential and important pathogens", the HERA 2 survey was evaluated by the partners. Based on this survey, a summarized Pathogen List was developed according to the national needs and legal compliance. The pathogens listed can cause serious and often fatal infections, such as bloodstream infections, pneumonia, or diarrhoea. The listed bacteria have become resistant to a number of antibiotics, including carbapenems and third-generation cephalosporins or, depending on their specific virulence characteristics, can cause large and severe outbreaks.

These outcomes may include appropriate method selection for implementation.

8. CONCLUSIONS AND NEXT STEPS

The outcomes of the survey show that the consortium partners have diverse implementation plans. These variations are due to variations in technical capabilities, protocols implemented so far, and a different epidemiological focus. The next steps include the individual determination of needs and procurement of the appropriate reagents and consumables, after which the implementation will start. The final goal is to develop reliable and highly accurate sequencing protocols and bioinformatics pipelines.

9. APPENDIX

Survey on PCR/WGS capacities, enhancement potential and important pathogens: project HERA 2 Original empty Survey

ECDC tool for the prioritisation of infectious disease threats:

https://www.ecdc.europa.eu/sites/default/files/documents/Tool-for-disease-priority-ranking_handbook_0_0.pdf

ECDC roadmap for integration of molecular typing and genomic typing into European-level surveillance and epidemic preparedness – Version 2.1, 2016-19:

https://www.ecdc.europa.eu/sites/default/files/media/en/publications/Publications/molecular-typing-EU-surveillance-epidemic-preparedness-2016-19-roadmap.pdf

Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis:

https://www.who.int/publications/i/item/WHO-EMP-IAU-2017.12