Provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in priority healthcare-associated infections

EURGen-RefLabCap

Final Report

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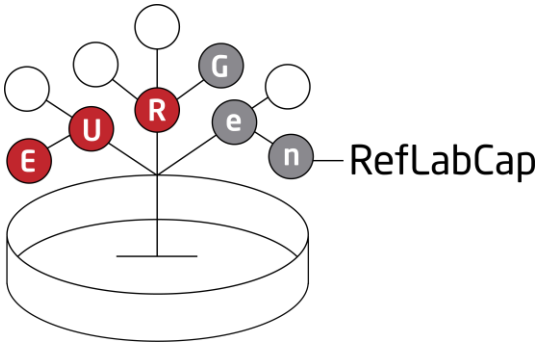

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Acronyms and abbreviations

AC	Additional country
AST	Antimicrobial susceptibility testing
CL	Clinical laboratory
CRE	Carbapenem-resistant Enterobacterales
CCRE	Carbapenem- and/or colistin-resistant Enterobacterales
CRAb	Carbapenem- and/or colistin-resistant *Acinetobacter baumannii* complex
CRPa	Carbapenem- and/or colistin-resistant *Pseudomonas aeruginosa*
DTU	Technical University of Denmark
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EFSA	European Food Safety Authority
EQA	External quality assessment
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EURGen-Net	European Antimicrobial Resistance Genes Surveillance Network
FAO	Food and Agriculture Organization
HaDEA	European Health and Digital Executive Agency
HAI	Healthcare-associated infections
IQC	Internal quality control
NEL	National expert laboratory
NRL	National reference laboratory
ONT	Oxford Nanopore Technologies
PC	Priority country
QC	Quality control
SSI	Statens Serum Institut
WGS	Whole-genome sequencing
WS1	Workstream 1 of the EURGen-RefLabCap project
WS2	Workstream 2 of the EURGen-RefLabCap project

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Disclaimer

Avertissement

Country abbreviations

Albania	AL	Latvia	LV
Austria	AT	Lithuania	LT
Belgium	BE	Luxembourg	LU
Bosnia and Herzegovina	BA	Malta	MT
Bulgaria	BG	Moldova	MD
Croatia	HR	Montenegro	ME
Cyprus	CY	Netherlands	NL
Czechia	CZ	North Macedonia	MK
Denmark	DK	Norway	NO
Estonia	EE	Poland	PL
Finland	FI	Portugal	PT
France	FR	Romania	RO
Germany	DE	Serbia	RS
Greece	EL	Slovakia	SK
Hungary	HU	Slovenia	SI
Ireland	IE	Spain	ES
Iceland	IS	Sweden	SE
Italy	IT	Türkiye	TR
Kosovo*	XK		

Source: Eurostat - Glossary: Country codes

*This designation is without prejudice to positions on status and is in line with United Nations Security Council Resolution 1244/99 and the International Court of Justice Opinion on the Kosovo declaration of independence

Abstract

The EURGen-RefLabCap project provided networking and technical support activities to the public health national reference laboratories (NRLs) in 37 European countries, with the goal of controlling the threat to human health caused by antimicrobial-resistant pathogens, in those countries and across borders.

The priority pathogens targeted by the project were healthcare-associated carbapenem- and/or colistin-resistant Enterobacterales (CCRE), *Pseudomonas aeruginosa* (CRPa) and *Acinetobacter baumannii* (CRAb).

The support aimed at strengthening the quality of antimicrobial resistance (AMR) surveillance systems by taking advantage of whole-genome sequencing (WGS) technology, leading to improved capacities and capabilities for testing and typing priority pathogens, detecting AMR and performing surveillance and outbreak investigations by the participating NRLs and associated local networks of clinical laboratories (CLs).

Support was provided through activities such as network meetings, technical training courses, external quality assessment exercises and virtual simulated exercises for outbreak investigation. Targeted support was provided to a subset of countries defined as priority countries (PCs) including country visits and the opportunity to conduct genomic pilot studies and receive sequencing equipment.

All activities carried out during the project had very positive outcomes and led to beneficial developments in the NRLs. Capacities and capabilities were developed in all main areas of the project, which has improved AMR surveillance in Europe.

Résumé

Le projet EURGen-RefLabCap a fourni des activités de mise en réseau et de soutien technique aux laboratoires nationaux de référence (LNR) de santé publique de 37 pays européens dans le but de contrôler la menace pour la santé humaine causée par les pathogènes résistants aux antimicrobiens, dans ces pays et au-delà des frontières.

Les agents pathogènes prioritaires ciblés par le projet étaient les entérobactéries résistantes aux carbapénèmes et/ou à la colistine (CCRE), *Pseudomonas aeruginosa* (CRPa) et *Acinetobacter baumannii* (CRAb).

Ce soutien vise à renforcer la qualité des systèmes de surveillance de la résistance aux antimicrobiens (RAM) en tirant parti de la technologie du séquençage du génome entier (whole-genome sequencing -WGS), ce qui permettra d'améliorer les capacités de test et de typage des agents pathogènes prioritaires, de détection de la RAM et de réalisation d'enquêtes de surveillance et d'épidémies/outbreak par les laboratoires nationaux de référence participants et les réseaux locaux de laboratoires cliniques qui y sont associés.

Le soutien a été fourni par le biais d'activités telles que des réunions de réseautage des cours de formation technique, des exercices d'évaluation externe de la qualité et des exercices virtuels de simulation d'enquête sur les épidémies. Un soutien ciblé a été apporté à un sous-ensemble de pays définis comme prioritaires (PCs), notamment par des visites dans les pays et la possibilité de mener des études pilotes en génomique et de recevoir du matériel de séquençage.

Toutes les activités menées au cours du projet ont eu des résultats très positifs et ont conduit à des développements bénéfiques dans les LNR. Des capacités ont été développées dans tous les principaux domaines du projet, ce qui a amélioré la surveillance de la RAM en Europe.

Executive summary

Context

Antimicrobial resistance (AMR) poses a major threat to human health in terms of deaths, burden of disease, hospital length of stay, healthcare costs and socio-economic impact.

The purpose of the European Antimicrobial Resistance Genes – Reference Laboratory Capacity project (EURGen-RefLabCap), funded by the European Commission (EC), was to provide services to strengthen coordination, support and capacity in national public health reference laboratory functions for AMR. The EURGen-RefLabCap project specifically addressed the objectives of the "European One Health action plan on AMR (2017)", which aims at making the European Union (EU) a 'best practice' region in the area of prevention and control of AMR.

The EURGen-RefLabCap project provided networking and support activities to the NRLs in EU and other European countries to support the declared EU goal of controlling the threat to human health caused by antimicrobial-resistant pathogens, in all countries and across borders.

Objectives and scope

The general objective of EURGen-RefLabCap was to support European countries to enhance the validity and accuracy of surveillance data of selected priority antimicrobial-resistant infections from health systems, in order to enable better detection and control of AMR. The specific objective was to support the coordination and operation of public health reference laboratories by providing networking and capacity-building activities including training, quality assurance, country visits, and in particular contribute to the implementation of whole-genome sequencing (WGS) for accurate testing and typing of priority pathogens in the NRLs.

The support delivered to the participating national reference laboratories (NRLs) aimed at strengthening the coverage, timeliness and usability of AMR surveillance systems at local, regional and national levels, by taking advantage of WGS technology and the improved capabilities of the NRLs for testing and typing priority pathogens and sharing alerts on incidents and outbreaks.

Methodology

All members of the existing EURGen-Net network were invited to participate in the EURGen-RefLabCap project. NRLs in 37 European countries participated in the project. The antimicrobial-resistant priority pathogens of the project included carbapenem- and/or colistin-resistant Enterobacterales (CCRE), which were targeted in Workstream 1 (WS1) during 2021-2024. Two additional pathogens were targeted in Workstream 2 (WS2) during 2022-2024, specifically carbapenem- and/or colistin-resistant *Pseudomonas aeruginosa* (CRPa) and *Acinetobacter baumannii* (CRAb).

All countries were enrolled in WS1 while 32 countries were enrolled in WS2 The network for the project was established at a virtual kick-off meeting in May 2021.

Thirteen priority countries (PCs) were selected through a mapping exercise based on their relatively higher need to receive targeted and bespoke support to develop NRL capacity for the priority pathogens (BA, BG, HR, CY, CZ, EE, EL, IT, LV, LT, RO, RS and SI). Four additional countries (ACs) were also selected based on their relatively higher need to receive targeted support (MD, PL, PT and ES).

The gaps identified in NRL capacity for the priority pathogens in a number of countries are considered a weakness in the European surveillance for AMR (and overall preparedness

for AMR) and cause further concerns as the occurrence of AMR is relatively high in most of the PCs. Consequently, a significant part of the support activities and resources of the EURGen-RefLabCap project were dedicated to the PCs.

The support provided applied a range of methods and approaches to promote the development of capacities and capabilities in the ECDC-defined core functions of NRLs.

Support provided to the participants of the EURGen-RefLabCap project

Activities	Target countries	Attendance
Kick-off meeting	All	32 countries
Three network meetings	All	35, 32, 29 countries
Nineteen webinars	All	Median of 35 individuals
Survey mapping NRL capacity for CCRE	All	37 countries
Survey mapping NRL capacity for CRAb/CRPa	All in WS2 (32 countries)	23 countries
Train-the-trainer workshop on capacity building	All	33 countries
Training workshop on Illumina sequencing	All	27 countries
Training workshop Data for Action	All	27 countries
Three genomic external quality assessment exercises	All	27, 29, 31 laboratories
Five virtual simulated outbreak exercises	All	26, 28, 19, 21, 20 countries
National mapping exercise on the methods used at the local clinical laboratories including financial support	All	27 countries
Two protocols with guidance for WGS-based surveillance and outbreak detection	All	Shared with all
Two guidance documents with methods for internal quality control	All	Shared with all
Implementation of national action plan to building capacity for NRL core functions	PCs	13 countries
Regular bespoke consultancy sessions	PCs	12 countries
Genomic surveillance pilot study including financial support	PCs and ACs	15 countries
Guidance and financial support to acquire WGS equipment	PCs and ACs	16 laboratories
Train-the-trainer workshop on pedagogical strategies	PCs and ACs	13 countries
Two virtual project management workshops	PCs	13, 8 countries
Training workshop on Nanopore sequencing	PCs and ACs	16 countries
Two country visits by the EURGen-RefLabCap team and ECDC representatives	PCs	12, 11 countries

Outcomes

Increased awareness of best practice, hands-on skills and technical knowledge trough training, external quality assessment exercises and step-by-step guidance

The participation in all activities was consistently very high throughout the project and the participant feedback was constantly positive. The NRLs in 12 PCs highlighted in structured interviews that the learning from the training workshops contributed to the implementation, development or modernisation of their WGS protocols and setup of bioinformatics analysis. The genomic external quality assessment (EQA) exercises were, for many participants, their first opportunity to perform bioinformatics analysis. The learning from the EQAs contributed to development of new bioinformatics pipelines, quality assurance and adjustment of existing WGS and bioinformatics approaches. The two step-by-step protocols for WGS-based national surveillance and outbreak investigation, developed in the project, provided a technical framework of great utility to the implementers of the WGS technology. The five simulated exercises were important activities that increased the technical capacity for cluster analysis for surveillance and for outbreak detection purposes, and also clarified the biological and epidemiological relevance of the results obtained through cluster analysis.

These activities increased the NRL's hands-on skills and technical knowledge and thereby enhanced the capability for best practice in the NRLs throughout Europe.

Increased awareness of NRL role and functions and capacity building in priority countries through bespoke support

Creation of individual NRL action plans in the PCs raised awareness about the NRL roles and core functions and helped the PCs to identify current gaps in capacity, and to plan and implement actions for improvement. The NRLs in 13 PCs (BA, BG, HR, CY, CZ, EE, EL, IT, LV, LT, RO, RS, SI) prepared and implemented an action plan to strengthen their capabilities and capacities to detect and control the spread of CCRE. The action plans were used as management tools for new developments, and in some countries, they were also used to engage with stakeholders to argue for resources and positioning in their health systems. The process of creating the action plans resulted in heightened awareness of the NRL core functions as a technical framework for providing laboratory reference services.

Comparison of indicator ratings of provision of NRL roles and core functions obtained by the PCs at the beginning of the project to those obtained at the end of the project highlights that the greatest progress has been made in the core function of 'reference diagnostics', including the transition to using WGS in all PCs. Progress in capacity for 'monitoring, alert and response', including WGS-based surveillance, has also been obtained but to a lesser degree. Capacity gaps persist in digital infrastructure, coverage of surveillance systems and reporting of data and alerts nationally and to ECDC monitoring systems.

Modernisation of reference testing in priority countries aided by bespoke operational support

The individual NRL action plans showed that the bespoke operational support should have a strong focus on the implementation of WGS and bioinformatics analysis, and also guided the design of project activities to bridge the gaps in NRLs roles and core functions. The country visits in the PCs were instrumental for successfully delivering technical and operational support to the PCs. Regular bespoke consultancy sessions have kept the momentum of improvement and ensured steady progress. In some PCs, organisational barriers to implementation of certain items of the action plans were also discussed during the country visits. In addition, some NRLs used the opportunity of these visits to engage

with key stakeholders in their own or external organisations (such as ministries of health, public health organisations, hospitals and other NRLs/CLs) by including them in the visits.

Importantly, the pilot genomic surveillance studies allowed the PCs and ACs to apply newly acquired knowledge and technical skills in WGS and bioinformatics analysis and generate for some the first genomic data on AMR in the priority pathogens in their country. The NRLs in 12 PCs (BA, BG, HR, CY, CZ, EE, EL, LV, LT, RO, RS, SI) and 2 ACs (PT, ES) completed pilot genomic surveillance studies and 2 additional NRLs (BA, MD) initiated pilot studies but did not complete the data analysis within the timeline. Moreover, the NRLs experienced first-hand the potential of genomic epidemiology for AMR surveillance and outbreak detection and inspired them to establish closer national and international collaborations to continue building capacity in this area. Finally, Purchase of ONT equipment and reagents, and training in its use, has provided NRLs with state-of-the-art methods for WGS.

Improved capacity for WGS-based routine analysis

The development of expertise for performing and implementing WGS into routine reference services were of high priority for all PCs. The NRLs in BA, HR, CY, EE, EL, LT, RO and RS developed expertise and capacity in performing WGS and bioinformatic analysis, and initiated implementation of quality assured WGS testing for surveillance and outbreak detection purposes. The NRLs in BG, CZ, LV and SI, who already implemented WGS, further developed and improved bioinformatics pipelines for analysing Illumina and/or ONT data.

Improved capacity for WGS-based national and international AMR surveillance

WGS-based surveillance systems were developed or improved in CY, CZ, EE, EL, LV, LT, RS and SI covering selected regions/health systems or entire countries. The newly developed capacity for genomic surveillance of AMR pathogens will from now on enable countries, for which epidemiology of priority pathogens was largely unknown prior to this project, to detect and investigate outbreaks enhancing the effectiveness of national and international surveillance. The NRLs in BG, EE, LV and LT further reported key findings on emerging resistance to the European surveillance portal for infectious diseases (EpiPulse) and the NRLs in LT and EL published preliminary national genomic data in scientific journals. Reporting of alerts on outbreaks to EpiPulse has heightened the contribution to the AMR surveillance in Europe. Moreover, the NRL in LT presented findings on a hybrid plasmid sustaining a multi-hospital outbreak of OXA-48 producing *K. pneumoniae* in Lithuania, while the NRL in LV presented finding on genomic clusters of carbapenemase-producing *K. pneumoniae* circulating in healthcare facilities in Latvia at the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE). However, there remain gaps in the sharing of genomic AMR surveillance data between institutions at national/regional levels and the uploading of data into European alert and monitoring systems. In addition, concerns of insufficient policy and financial support during and beyond the EURGen-RefLabCap project were expressed by most of the countries.

Enhanced NRL advisory role and collaboration with national networks of clinical laboratories

The coordination and completion of the mapping exercise on capacity for detection of the priority pathogens among networks of CLs by the NRLs resulted in strengthened engagement of the NRLs with their respective networks. Especially among the PCs, this facilitated building a new or stronger advisory role of the NRLs towards the CLs that persisted after the mapping exercise. Some NRLs experienced improved or more formalised collaboration with their networks of CLs, more regular communication, and increased referral of samples to the NRL. The two guidance documents for internal quality

control were also a useful tool both for the NRLs but also for further distribution within their national networks.

The NRLs in CY, HR, CZ, EE, EL, LV, RO and RS highlighted that key outcomes of their participation in the project were obtained in association with the development of their advisory and coordinating role towards the local networks of CLs. The NRLs obtained the following specific outcomes regarding networks of CLs: formation of national network of CLs and enhanced collaboration (CY, RO), consolidation of existing regional networks or more formalised collaboration with national networks of CLs (HR, LV, RS), providing laboratory training to personnel from the national CLs (CZ), establishment of framework for provision of technical support and scientific advice to national network of CLs (EE), and improved and more frequent communication with the network of CLs (EL).

Strengthened cross-border collaborations

The multiple networking and training activities provided in the project have enabled the NRL representatives to meet and establish collaborations with NRLs in other countries. Participation in all networking activities was high and the ratings of the utility and execution were also consistently high. From participant feedback surveys, it is concluded that the PCs and ACs greatly benefitted from all types of networking and support activities, and that they had needs for further support activities in the future. In a few instances, collaborations were initiated between groups of countries with connected epidemiological situations. The most prominent collaboration that has been established to date include the Baltic States (EE, LV, LT) that formed a three-nations NRL network with the aim of strengthening AMR surveillance in the geographical region. Multinational networks like this are greatly supporting the development of cross-border preparedness in line with the aim of the EU regulation 2022/2371 on the Serious cross-Border threat to health.

Other collaborations between neighbouring European countries have been explored but not yet formalised. In the long term, strong international networks are essential to maintaining NRL capacities and preparedness of all countries.

Options for future actions

At European level, continued efforts to sustain and support quality controlled WGS-based systems for characterization of priority pathogens and for national and international surveillance should be provided to all countries. It is recommended that the majority of the support activities delivered in the EURGen-RefLabCap project are continued in other EU-funded projects, including the European Reference Laboratory for public health on AMR (EURL-PH-AMR).

In particular, at European level, this should include the continued delivery of training exercises in laboratory techniques, WGS and bioinformatics analysis, and regular EQA/proficiency testing exercises for benchmarking the methods and quality of the WGS-data analysis. Moreover, evidence-based protocols on WGS and bioinformatics developed in the project should be updated as more scientific knowledge and bioinformatics tools and approaches will become available. Due to gaps in the monitoring, alert and response functions, training of the NRLs should involve reporting phenotypic and genomic data into national surveillance systems and the issuing of alerts on relevant pathogens and outbreaks to the European surveillance portal for infectious diseases, EpiPulse. Provision of scientific and technical support to the EU disease networks concerning AMR, including EURGen-Net and EARS-Net, should also be continued. Moreover, participation of the European NRLs in other regionally or internationally relevant research and development projects and initiatives should be continued to maintain knowledge and expertise.

At individual country level, gaps remain in infrastructures essential to laboratory surveillance and data storage, handling, sharing and reporting. Countries should continue to build, upgrade and maintain scalable infrastructure for WGS data handling and storage, and consolidate and secure reporting of NRL testing results to the national networks of CLs and public health authorities. In some countries, digitalisation of laboratory data in the NRL and/or CLs is still lacking, and improvement of local, regional and national integrated digital systems are required. Finally, strengthening the engagement with key stakeholders and decision-makers to obtain funding and structural support for NRL services, national surveillance and preparedness for cross-border threats to health is essential to the provision of all NRL roles and functions.

Sommaire

Contexte

La résistance aux antimicrobiens (RAM) constitue une menace majeure pour la santé humaine en termes de décès, de charge de morbidité, de durée d'hospitalisation, de coûts des soins de santé et d'impact socio-économique.

L'objectif du projet européen Antimicrobial Resistance Genes - Reference Laboratory Capacity (EURGen-RefLabCap), financé par la Commission européenne (CE), était de fournir des services pour renforcer la coordination, le soutien et la capacité des fonctions des laboratoires nationaux de référence en santé publique pour la résistance aux antimicrobiens. Le projet EURGen-RefLabCap répondait spécifiquement aux objectifs du "Plan d'action européen One Health sur la RAM (2017)", qui vise à faire de l'Union européenne (UE) une région de "meilleures pratiques" dans le domaine de la prévention et du contrôle de la RAM.

Le projet EURGen-RefLabCap a fourni des activités de mise en réseau et de soutien aux LNR de l'UE et d'autres pays européens afin de soutenir l'objectif déclaré de l'UE de contrôler la menace pour la santé humaine causée par les agents pathogènes résistants aux antimicrobiens, dans tous les pays et par-delà les frontières.

Objectifs et champ d'application

L'objectif général d'EURGen-RefLabCap était d'aider les pays européens à améliorer la validité et la précision des données de surveillance des infections prioritaires résistantes aux antimicrobiens provenant des systèmes de santé, afin de permettre une meilleure détection et un meilleur contrôle de la résistance aux antimicrobiens. L'objectif spécifique était de soutenir la coordination et le fonctionnement des laboratoires de référence de santé publique en proposant des activités de mise en réseau et de renforcement des capacités, notamment des formations, l'assurance qualité, des visites dans les pays et, en particulier, de contribuer à la mise en œuvre du WGS pour des tests précis et le typage des agents pathogènes prioritaires dans les laboratoires nationaux de référence.

Le soutien apporté aux LNR participants visait à renforcer la couverture, la rapidité et la facilité d'utilisation des systèmes de surveillance de la RAM aux niveaux local, régional et national, en tirant parti de la technologie WGS et des capacités améliorées des LNR pour tester et typer les agents pathogènes prioritaires et partager les alertes sur les incidents et les épidémies.

Méthodologie

Tous les membres du réseau EURGen-Net existant ont été invités à participer au projet EURGen-RefLabCap. Des LNR de 37 pays européens ont participé au projet. Les pathogènes prioritaires résistants aux antimicrobiens du projet comprenaient les entérobactéries résistantes aux carbapénèmes et/ou à la colistine (CCRE), qui ont été ciblées dans le cadre du Workstream 1 (WS1) au cours de la période 2021- 2024. Deux autres pathogènes ont été ciblés dans le cadre du Workstream 2 (WS2) pendant la période 2022-2024, à savoir *Pseudomonas aeruginosa* (CRPa) et *Acinetobacter baumannii* (CRAb) résistants aux carbapénèmes et/ou à la colistine.

Tous les pays ont été inscrits à WS1, tandis que 32 pays ont été inscrits à WS2. Le réseau du projet a été établi lors d'une réunion virtuelle de lancement en mai 2021.

Treize pays prioritaires (PCs) ont été sélectionnés par un exercice de cartographie sur la base de leur besoin relativement élevé de recevoir un soutien ciblé et sur mesure pour développer la capacité des LNR pour les agents pathogènes prioritaires (BA, BG, HR, CY,

CZ, EE, EL, IT, LV, LT, RO, RS et SI). Quatre pays supplémentaires (ACs) ont également été sélectionnés sur la base de leur besoin relativement plus important de recevoir un soutien ciblé (MD, PL, PT et ES).

Les lacunes identifiées dans la capacité des LNR pour les agents pathogènes prioritaires dans un certain nombre de pays sont considérées comme une faiblesse dans la surveillance européenne de la RAM (et dans la préparation générale à la RAM), et suscitent d'autant plus d'inquiétudes que l'occurrence de la RAM est relativement élevée dans la plupart des PCs. Par conséquent, une part importante des activités de soutien et des ressources du projet EURGen-RefLabCap a été consacrée aux PCs.

Le soutien apporté a appliqué une série de méthodes et d'approches visant à promouvoir le développement des capacités et des compétences dans les fonctions essentielles des LNR définies par l'ECDC.

Soutien aux participants du projet EURGen-RefLabCap

Activités	Présence	Présence
Réunion de lancement	Tous	32 pays
Trois réunions de réseau	Tous	35, 32, 29 pays
Dix-neuf webinaires	Tous	Médiane de 35 personnes
Enquête sur la capacité du LRN pour le CCRE	Tous	37 pays
Enquête sur les capacités du LRN pour le CRAb/CRPa	Tous dans WS2 (32 pays)	23 pays
Atelier de formation des formateurs sur le renforcement des capacités	Tous	33 pays
Atelier de formation sur le séquençage Illumina	Tous	27 pays
Atelier de formation "Data for action”	Tous	27 pays
Trois exercices d'évaluation de la qualité externe de la génomique	Tous	27, 29, 31 laboratoires
Cinq exercices virtuels de simulation d'épidémie	Tous	26, 28, 19, 21, 20 pays
Exercice de cartographie nationale des méthodes utilisées dans les laboratoires cliniques locaux, y compris le soutien financier	Tous	27 pays
Deux protocoles d'orientation pour la surveillance et la détection des épidémies par WGS	Tous	Partagé avec tous
Deux documents d'orientation présentant des méthodes de contrôle interne de la qualité	Tous	Partagé avec tous
Mise en œuvre du plan d'action national visant à renforcer les capacités pour les fonctions essentielles du LNR	PCs	13 pays
Séances régulières de conseil sur mesure	PCs	12 pays
Étude pilote de surveillance génomique, y compris le soutien financier	PCs et ACs	15 pays

Conseils et soutien financier pour l'acquisition d'équipements WGS	PCs et ACs	16 laboratoires
Atelier de formation des formateurs sur les stratégies pédagogiques	PCs et ACs	13 pays
Deux ateliers virtuels de gestion de projet	PCs	13, 8 pays
Atelier de formation sur le séquençage par nanopore	PCs et ACs	16 pays
Deux visites de pays par l'équipe EURGen-RefLabCap et des représentants de l'ECDC	PCs	12, 11 pays

Résultats

Sensibilisation accrue aux meilleures pratiques, aux compétences pratiques et aux connaissances techniques par le biais de formations, d'exercices d'évaluation externe de la qualité et d'orientations étape par étape

La participation à toutes les activités a été constamment très élevée tout au long du projet et le retour d'information des participants a été constamment positif. Les LNR de 12 PCs ont souligné, lors d'entretiens structurés, que les enseignements tirés des ateliers de formation avaient contribué à la mise en œuvre, au développement ou à la modernisation de leurs protocoles WGS et à la mise en place d'analyses bioinformatiques. Les exercices d'évaluation externe de la qualité (EQA) génomique ont constitué, pour de nombreux participants, leur première occasion d'effectuer une analyse bioinformatique. Les enseignements tirés des EQA ont contribué au développement de nouveaux pipelines bioinformatiques, à l'assurance qualité et à l'ajustement des approches WGS et bioinformatiques existantes. Les deux protocoles étape par étape pour la surveillance nationale basée sur le WGS et l'investigation des épidémies, élaborés dans le cadre du projet, ont fourni un cadre technique d'une grande utilité pour les personnes chargées de la mise en œuvre de la technologie WGS. Les cinq exercices de simulation ont été des activités importantes qui ont renforcé la capacité technique d'analyse des clusters à des fins de surveillance et de détection des épidémies, et ont également clarifié la pertinence biologique et épidémiologique des résultats obtenus par l'analyse des clusters.

Ces activités ont permis d'améliorer les compétences pratiques et les connaissances techniques des LNR et, partant, de renforcer la capacité des LNR européens à mettre en œuvre les meilleures pratiques.

Sensibilisation accrue au rôle et aux fonctions des LNR et renforcement des capacités dans les pays prioritaires grâce à un soutien sur mesure

La création de plans d'action individuels pour les LNR dans les PCs a permis de sensibiliser aux rôles et aux fonctions essentielles des LNR et a aidé les PCs à identifier les lacunes actuelles en matière de capacité, ainsi qu'à planifier et à mettre en œuvre des actions d'amélioration. Les LNR de 13 PCs (BA, BG, HR, CY, CZ, EE, EL, IT, LV, LT, RO, RS, SI) ont préparé et mis en œuvre un plan d'action pour renforcer leurs capacités à détecter et à contrôler la propagation du CCRE. Les plans d'action ont servi d'outils de gestion pour les nouveaux développements et, dans certains pays, ils ont également été utilisés pour dialoguer avec les parties prenantes afin d'obtenir des ressources et un positionnement dans leurs systèmes de santé. Le processus d'élaboration des plans d'action a permis de mieux faire connaître les fonctions essentielles du LNR en tant que cadre technique pour la fourniture de services de référence en laboratoire.

La comparaison des évaluations des indicateurs relatifs à la fourniture des rôles et des fonctions essentielles des LNR obtenues par les PCs au début du projet et celles obtenues

à la fin du projet montre que les progrès les plus importants ont été réalisés dans la fonction essentielle de "diagnostic de référence", y compris la transition vers l'utilisation du WGS dans tous les PCs. Des progrès ont également été réalisés, mais dans une moindre mesure, en ce qui concerne la capacité de "surveillance, d'alerte et de réaction", y compris la surveillance basée sur le WGS. Des lacunes persistent au niveau de l'infrastructure numérique, de la couverture des systèmes de surveillance et de la communication des données et des alertes au niveau national et aux systèmes de surveillance de l'ECDC.

Modernisation des tests de référence dans les pays prioritaires grâce à un soutien opérationnel sur mesure

Les plans d'action individuels des LNR ont montré que le soutien opérationnel sur mesure devait être fortement axé sur la mise en œuvre de la WGS et de l'analyse bioinformatique, et ont également guidé la conception des activités du projet afin de combler les lacunes dans les rôles et les fonctions essentielles des LNR. Les visites de pays dans les PCs ont joué un rôle déterminant dans la fourniture d'un soutien technique et opérationnel aux PCs. Des sessions régulières de conseil sur mesure ont permis de maintenir la dynamique d'amélioration et de garantir des progrès constants. Dans certains PCs, les obstacles organisationnels à la mise en œuvre de certains points des plans d'action ont également été examinés lors des visites dans les pays. En outre, certains LNR ont profité de ces visites pour nouer le dialogue avec des acteurs clés au sein de leur propre organisation ou d'organisations externes (comme les ministères de la santé, les organisations de santé publique, les hôpitaux et d'autres LNR/LC) en les associant aux visites.

Il est important de noter que les études pilotes de surveillance génomique ont permis aux PC et aux CA d'appliquer les connaissances et les compétences techniques nouvellement acquises en matière de WGS et d'analyse bioinformatique et de produire, pour certains, les premières données génomiques sur la résistance aux antimicrobiens des agents pathogènes prioritaires dans leur pays. Les LNR de 12 PC (BA, BG, HR, CY, CZ, EE, EL, LV, LT, RO, RS, SI) et de 2 AC (PT, ES) ont achevé des études pilotes de surveillance génomique et 2 autres LNR (BA, MD) ont lancé des études pilotes mais n'ont pas achevé l'analyse des données dans les délais impartis. En outre, les LNR ont pu constater par eux-mêmes le potentiel de l'épidémiologie génomique pour la surveillance de la RAM et la détection des épidémies, ce qui les a incités à établir des collaborations nationales et internationales plus étroites afin de continuer à renforcer les capacités dans ce domaine. Enfin, l'achat d'équipements et de réactifs ONT, ainsi que la formation à leur utilisation, ont permis aux LNR de disposer de méthodes de pointe pour le WGS.

Amélioration de la capacité d'analyse de routine basée sur le WGS

Le développement de l'expertise pour la réalisation et la mise en œuvre de WGS dans les services de référence de routine était une priorité élevée pour tous les PCs. Les LNR de BA, HR, CY, EE, EL, LT, RO et RS ont développé leur expertise et leur capacité à réaliser des WGS et des analyses bioinformatiques, et ont commencé à mettre en œuvre des tests WGS de qualité assurée à des fins de surveillance et de détection des épidémies. Les LNR de BG, CZ, LV et SI, qui réalisaient déjà des WGS, ont continué à développer et à améliorer les pipelines bioinformatiques pour l'analyse des données Illumina et/ou ONT.

Amélioration de la capacité de surveillance nationale et internationale de la résistance aux antimicrobiens fondée sur la technologie WGS

Des systèmes de surveillance basés sur le WGS ont été développés ou améliorés à CY, CZ, EE, EL, LV, LT, RS et SI, couvrant des régions/systèmes de santé sélectionnés ou des pays entiers. La nouvelle capacité de surveillance génomique des agents pathogènes de la RAM permettra désormais aux pays, pour lesquels l'épidémiologie des agents

pathogènes prioritaires était largement inconnue avant ce projet, de détecter et d'étudier les épidémies, ce qui renforcera l'efficacité de la surveillance nationale et internationale. Les LNR de BG, EE, LV et LT ont en outre communiqué au portail européen de surveillance des maladies infectieuses (EpiPulse) des résultats clés sur la résistance émergente, et les LNR de LT et EL ont publié des données génomiques nationales préliminaires dans des revues scientifiques. La transmission d'alertes sur les épidémies à EpiPulse a renforcé la contribution à la surveillance de la résistance aux antimicrobiens en Europe. En outre, le LNR de LT a présenté les résultats d'un plasmide hybride à l'origine d'une épidémie multihospitalière de *K. pneumoniae* producteur d'OXA-48 en Lituanie, tandis que le LNR de LV a présenté les résultats de clusters génomiques de *K. pneumoniae* producteur de carbapénémase circulant dans les établissements de santé en Lettonie lors de la conférence scientifique européenne sur l'épidémiologie appliquée aux maladies infectieuses (ESCAIDE). Toutefois, des lacunes subsistent dans le partage des données génomiques de surveillance de la RAM entre les institutions aux niveaux national et régional et dans le téléchargement des données dans les systèmes européens d'alerte et de surveillance. En outre, la plupart des pays se sont inquiétés de l'insuffisance du soutien politique et financier pendant et après le projet EURGen-RefLabCap.

Renforcement du rôle consultatif du LNR et de la collaboration avec les réseaux nationaux de laboratoires cliniques

La coordination et l'achèvement de l'exercice de cartographie des capacités de détection des pathogènes prioritaires au sein des réseaux de CL par les LNR ont permis de renforcer l'engagement des LNR auprès de leurs réseaux respectifs. En particulier au sein des PCs, cela a facilité la mise en place d'un rôle de conseil nouveau ou plus fort des LNR vis-à-vis des CL, qui a perduré après l'exercice de cartographie. Certains LNR ont vu leur collaboration avec leurs réseaux de CL s'améliorer ou se formaliser, la communication devenir plus régulière et le nombre d'échantillons renvoyés au LNR augmenter. Les deux documents d'orientation pour le contrôle interne de la qualité ont également constitué un outil utile à la fois pour les LNR et pour une distribution ultérieure au sein de leurs réseaux nationaux.

Les LNR de CY, HR, CZ, EE, EL, LV, RO et RS ont souligné que les principaux résultats de leur participation au projet ont été obtenus en lien avec le développement de leur rôle de conseil et de coordination auprès des réseaux locaux de CL. Les LNR ont obtenu les résultats spécifiques suivants concernant les réseaux de CL: formation d'un réseau national de CL et renforcement de la collaboration (CY, RO), consolidation des réseaux régionaux existants ou collaboration plus formelle avec les réseaux nationaux de CL (HR, LV, RS), formation en laboratoire du personnel des CL nationaux (CZ), établissement d'un cadre pour la fourniture d'un soutien technique et de conseils scientifiques au réseau national de CL (EE), et communication améliorée et plus fréquente avec le réseau de CL (EL).

Renforcement des collaborations transfrontalières

Les multiples activités de mise en réseau et de formation prévues dans le cadre du projet ont permis aux représentants des LNR de rencontrer des LNR d'autres pays et d'établir des collaborations avec eux. La participation à toutes les activités de mise en réseau a été élevée et les évaluations de l'utilité et de l'exécution ont également été élevées. Les enquêtes de retour d'information des participants ont permis de conclure que les PCs et les CAs ont grandement bénéficié de tous les types d'activités de mise en réseau et de soutien, et qu'ils avaient besoin d'autres activités de soutien à l'avenir. Dans quelques cas, des collaborations ont été initiées entre des groupes de pays présentant des situations épidémiologiques similaires. La collaboration la plus importante à ce jour concerne les États baltes (EE, LV, LT) qui ont formé un réseau LNR à trois nations dans le but de renforcer la surveillance de la RAM dans la région géographique. Les réseaux multinationaux de ce type contribuent grandement au développement de la préparation transfrontalière,

conformément à l'objectif du règlement (CE) n° 2022/2371 relatif à la menace transfrontalière grave pour la santé.

D'autres collaborations entre pays européens voisins ont été envisagées mais n'ont pas encore été formalisées. À long terme, des réseaux internationaux solides sont essentiels pour maintenir les capacités des LNR et l'état de préparation de tous les pays.

Options pour les actions futures

Au niveau européen, tous les pays devraient poursuivre leurs efforts pour maintenir et soutenir les systèmes basés sur le WGS à qualité contrôlée pour la caractérisation des agents pathogènes prioritaires et pour la surveillance nationale et internationale. Il est recommandé que la majorité des activités de soutien menées dans le cadre du projet EURGen-RefLabCap soient poursuivies dans le cadre d'autres projets financés par l'UE, notamment le laboratoire européen de référence pour la santé publique en matière de résistance aux antimicrobiens (EURL-PH-AMR).

En particulier, au niveau européen, cela devrait inclure la poursuite des exercices de formation aux techniques de laboratoire, à l'analyse WGS et à la bioinformatique, ainsi que des exercices réguliers d'EQA/de vérification des compétences pour comparer les méthodes et la qualité de l'analyse des données WGS. En outre, les protocoles fondés sur des données probantes en matière de WGS et de bioinformatique élaborés dans le cadre du projet devraient être mis à jour à mesure que de nouvelles connaissances scientifiques et de nouveaux outils et approches bioinformatiques deviendront disponibles. En raison des lacunes dans les fonctions de surveillance, d'alerte et de réaction, la formation des LNR devrait porter sur la communication des données phénotypiques et génomiques aux systèmes de surveillance nationaux et sur l'émission d'alertes sur les agents pathogènes et les épidémies au portail européen de surveillance des maladies infectieuses, EpiPulse. Il convient également de continuer à apporter un soutien scientifique et technique aux réseaux de lutte contre les maladies de l'UE concernant la RAM, notamment EURGen-Net et EARS-Net. En outre, la participation des LNR européens à d'autres projets et initiatives de recherche et de développement pertinents au niveau régional ou international devrait être poursuivie afin de maintenir les connaissances et l'expertise.

Au niveau national, des lacunes subsistent dans les infrastructures essentielles à la surveillance des laboratoires et au stockage, au traitement, au partage et à la communication des données. Les pays devraient continuer à construire, mettre à niveau et entretenir des infrastructures évolutives pour le traitement et le stockage des données WGS, et consolider et sécuriser la communication des résultats des tests des LNR aux réseaux nationaux de CL et aux autorités de santé publique. Dans certains pays, la numérisation des données de laboratoire dans les LNR et/ou les CL fait encore défaut, et il est nécessaire d'améliorer les systèmes numériques intégrés aux niveaux local, régional et national. Enfin, il est essentiel de renforcer l'engagement auprès des acteurs clés et des décideurs afin d'obtenir un financement et un soutien structurel pour les services des LNR, la surveillance nationale et la préparation aux menaces transfrontalières pour la santé, afin d'assurer tous les rôles et toutes les fonctions des LNR.

1. Introduction

1.1. Overview of the report

This document constitutes the final report of the EURGen-RefLabCap project delivered under the *European Health and Digital Executive Agency (*HaDEA*)* Service Contract (SC 2019 74 01) with the title: 'Provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in priority healthcare-associated infections.'

The purpose of the European Antimicrobial Resistance Genes – Reference Laboratory Capacity project (EURGen-RefLabCap) is to provide services to strengthen coordination, support and capacity in national microbiology reference laboratory functions for antimicrobial resistance (AMR). This report aims at fulfilling the requirements for reporting at the end of the 4-year project period and includes a summary of work carried out; all activities and the main outcomes produced, and any lessons learned and proposals for further work based on feedback from the participating national reference laboratories (NRLs)/national expert laboratories (NELs) (in the remainder of this report NRLs and NELs are referred to as NRLs for brevity). The content of the report is structured as described in Table 1.

Table 1: Structure of the report

Section	Section title	Content and purpose
1.1	Overview of the report	Introduction of the project and the aim of the report
1.2	Context of the project	Key points of effective action against AMR relating to this project
1.3	Action at European level	European Union (EU) policy in the area of detection and monitoring of AMR
2	Methodology	Summary of the methodological approach applied in individual tasks and activities
3	Support activities and outcomes	Summary of support activities and outcomes of all completed tasks and activities
4	Evaluation	Evaluation approach and outcome of participant feedback and any lessons learned
5	Conclusions	Main conclusions from the project and next steps
6	Annexes	Annexes including lists of completed activities, participation in activities, guidance documents, training materials, weblinks and bibliography

1.2. Context of the project

AMR poses a major global threat to human health in terms of deaths, burden of disease, hospital length of stay, healthcare costs and socio-economic impact. AMR is considered one of the highest global public health and development threats. Alone in Europe, it has been estimated that annually up to over 800 000 infections are caused by bacteria resistant to antimicrobials, which result in up to over 38 000 deaths, while more than 1 million disability life years (DALYs) are attributable to infections with antibiotic-resistant bacteria.[1] Similarly, it has been estimated that over 70% of cases of infections with antibiotic-resistant bacteria are healthcare-associated infections (HAI). It is accepted that the misuse and overuse of antimicrobials in humans, animals and plants are the main drivers of the development of AMR, and that AMR makes infections harder to treat and a variety of medical procedures much riskier to perform.[2]

Well-functioning reference laboratory services are essential to support the action to control antimicrobial resistance (AMR) as set out in the 'European One Health action plan against AMR (2017)' and 'Decision (EU) 1082/2013 on serious cross-border health threats'(now superseded by the Regulation (EU) 2022/2371 on serious cross-border threat to health).[3, 4]

The purpose of the European Commission (EC) funded EURGen-RefLabCap project is to provide services to strengthen coordination, support and capacity in national microbiology reference laboratory functions for AMR. The EURGen-RefLabCap project specifically addresses objectives of the European One Health action plan on AMR (2017), which aims at making the EU a 'best practice' region.[3] The activities of the EURGen-RefLabCap project are aimed at complementing existing activities of the European Centre for Disease Prevention and Control (ECDC) who is tasked with detection, surveillance and risk assessment of threats to human health from communicable diseases. As ECDC does not operate their own laboratories they rely on laboratory services provided by national laboratories and associated public health structures. However, from 2025, the EURL-PH-AMR will provide laboratory services for ECDC.

1.3. Action at European level

At the turn of the millennium, the EU recognised the urgency of tackling AMR in all health sectors. Since then, the EC has continuously supported efforts against AMR, making Europe a leader in AMR research and development of initiatives to strengthen the monitoring and prevention of AMR and the development of new medicines to treat or prevent resistant bacterial infections. The European efforts on AMR are aligned with global efforts on AMR set out in the WHO Global Action Plan on AMR (2015).[5] More recently, EU action plans have focussed increasingly on One Health approaches to tackling AMR due to the interconnectedness of human and animal disease and environmental spread of AMR. In 2023, the Council of the EU adopted Recommendation on stepping up EU actions to combat antimicrobial resistance (AMR) in a One Health approach (2023/C 220/01), which includes reduction targets for the occurrence of antimicrobial-resistant bacteria to be achieved in the EU by 2030.[6]

During the last decade there has been a focus on improving epidemiological surveillance, monitoring, 'early warning' systems, preparedness and response planning and coordination between European countries. However, the SARS-CoV-2 pandemic further demonstrated and reinforced the need in Europe for harmonisation and expansion of the capacity for surveillance and detection of outbreaks of emerging infectious threats. The new EU Regulation (2022/2371) on 'serious cross-border health threats' aims at strengthening epidemiological surveillance and monitoring, networks of epidemiological surveillance, the Early Warning and Response System (EWRS), coordination of response to public health

emergencies at EU-level and establishes a network of EU reference laboratories for public health.[7]

2. Methodology

2.1. Objectives and scope

The general objective of EURGen-RefLabCap is to support EU Member States and other countries participating in the Third EU Health Programme to enhance the validity and accuracy of surveillance data of selected priority resistant bacteria (mainly causative agents of HAIs) from health systems, in order to enable better detection and control of cross-border threats to human health from AMR.

The specific objective is to support the coordination and operation of public health reference laboratories by providing networking and capacity-building activities including training, quality assurance, country visits, and in particular supporting the implementation of whole genome sequencing (WGS) for accurate testing and typing of specified pathogens in the NRLs.

The overall expected outcome is to further strengthen the coverage, timeliness and usability of AMR surveillance systems at local, regional and national levels, by taking advantage of the applied WGS technology, validated analysis tools and the improved capabilities of the public health reference laboratories for diagnosing and typing priority pathogens and sharing alert information on AMR events, including outbreaks and incidents. This is intended to improve effectiveness for early warning and targeted control of AMR at the point of transmission and enhance preparedness.

The support delivered in the project falls within three areas: 1) building capacity in the public health reference laboratories for diagnosing, typing and reporting on the priority pathogens, 2) strengthening the role of the public health reference laboratories in building capacity in the networks of clinical laboratories (CLs) in their respective countries, and 3) modernising diagnostic testing and molecular typing in the health systems for the priority pathogens using WGS (Table 2).

The priority pathogens of the project include carbapenem- and/or colistin-resistant Enterobacterales (CCRE), which are targeted in Workstream 1 (WS1) from year 1 to year 4 (2021-2024). Two additional pathogens are targeted in Workstream 2 (WS2) from year 2 to year 4 (2022-2024), specifically carbapenem- and/or colistin-resistant *Pseudomonas aeruginosa* (CRPa) and *Acinetobacter baumannii* (CRAb).

Participating countries include the 27 EU Member States, 2 EEA-countries and 8 non-EU/EEA countries.

Of these, 13 ‘priority countries’ (PCs) were selected based on their relatively higher need to receive targeted and bespoke support to develop national public health reference laboratory capacity for the priority pathogens (Table 2). Four ‘additional countries’ (AC) were selected (among the remaining 24 countries without PC status) based on their relatively higher need to receive targeted support in relation to the implementation of WGS in the NRLs for public health purposes. All 37 countries were enrolled in WS1, while only 32/37 countries were enrolled in WS2.

Table 2: Scope of the EURGen-RefLabCap project

Scope	Description
Areas of support	Area 1: Activities aimed at building capacity in the NRLs for the specified priority pathogens to improve their core functions for AMR in their countries. Area 2: Activities aimed at strengthening the role of the NRLs to build capacities in regional and local networks of CL in health systems of their countries. Area 3: Activities aimed at modernising diagnostic and molecular typing tests in health systems for the specified pathogens using whole genome sequencing (WGS).
Geographical region	37 participating countries in total including 27 EU Member States, two EEA countries: NO and IS; and eight non-EU/EEA countries: AL, BA, XK, MD, ME, MK, RS and TR.
Priority pathogens	The project includes antimicrobial-resistant pathogens that primarily are grouped as causative agents of HAI. The priority pathogens are: CCRE: WS1 of the project, engaging 37 countries. CRPa and CRAb: WS2 of the project, engaging 32 countries (AL, AT, BA, BG, HR, CY, CZ, DK, EE, FI, FR, DE, EL, HU, IS, IE, IT, XK, LT, LU, MT, MD, NL, MK, NO, PL, PT, SK, SI, ES, SE and TR).
Priority countries	Thirteen countries that accepted to receive additional and targeted support to develop national public health reference laboratory capacity for the priority pathogens. These include: BA, BG, HR, CY, CZ, EE, EL, IT, LV, LT, RO, RS and SI.
Additional countries	Four countries that accepted to receive targeted support in relation to implementation of WGS in the NRLs for public health purposes. These include: MD, PL, PT and ES.
Period	January 2021 - December 2024.

2.2. Methodology

The support provided to the participating countries in this project applied a range of methods and approaches to support the development of capacities and capabilities in the core functions of NRLs.

All countries were offered to participate in the following activities:

- Surveys of capacity for core functions of public health reference laboratories
- Networking activities (including network meetings and workshops)
- Activities to enhance the advisory and coordinating role of the reference laboratories in their respective countries
- External quality assessments (EQAs) of WGS and bioinformatics analyses
- Multidisciplinary training using virtual simulated outbreak exercises
- Webinars on clinical, technical and practice-related issues

The PCs were offered to participate in additional activities:

- Introduction to creating and implementing ‘NRL action plans’ for building capacity for public health reference laboratory core functions
- NRL visits by the country teams and ECDC scientific advisors to identify areas for improvement and support the implementation of required actions

- Regular bespoke consultancy sessions for each country (by phone, video-call, email, etc.), including provision of advice on technical and practice-related issues and motivational dialogue
- Implementation of surveys on capacity for detection and reporting priority pathogens in networks of CLs aimed at enhancing the advisory role, support, and reference functions of the NRLs towards the CLs
- Conducting a WGS pilot study on selected strains of priority pathogens collected in their respective countries
- Laboratory-based training courses and workshops aimed at implementation of WGS and bioinformatics analysis

The ACs were offered to participate in additional activities:

- Conducting a WGS pilot study on selected strains of priority pathogens collected in their respective countries
- Laboratory-based training courses and workshops aimed at implementation of WGS and bioinformatics analysis

Financial support, paid out as fixed-amounts reimbursements, was offered to PCs and ACs after their participation in certain capacity building activities. Specifically, reimbursement could be obtained after completing a mapping survey on laboratory capacity among networks of CLs, after completion of a pilot genomic surveillance study report, and for the acquisition of WGS equipment from Oxford Nanopore Technologies (ONT) (Oxford Nanopore Technologies, Inc., Oxford, UK).

To assess progress in the PCs, indicators of capacity for NRL core functions were used to gauge the capacity for provision of NRL core functions on: reference diagnostics (1), scientific advice (3), and monitoring, alert and response (3) at the end of the project (2024) compared with that of the capacity at the beginning of the project (2021) assessed via a detailed mapping survey (Section 3.1.2). Evaluation of support activities was carried out through participant feedback surveys after each activity, two interim evaluation surveys (Section 4.1). Structured interviews were also conducted with NRLs in the PCs to collect detailed feedback.

Details of the approaches used to deliver the support activities are described in the following section (Section 3).

3. Support activities and outcomes

The networking and support activities were developed based on the outcomes of initial mapping and scoping exercises (Section 3.1). This section also describes the establishment of the network of NRLs for the EURGen-RefLabCap project. The initial mapping of reference laboratory capacity led to the identification of PCs having NRLs that needed enhanced and targeted technical support, and selection of pathogens for the two dedicated workstreams (WS1 and WS2). Support activities dedicated to the PCs are described in Section 3.2. Development of guidance on methods for WGS based AMR surveillance and outbreak detection are described in Section 3.3 and general training activities in Section 3.4. Overviews of the activities offered to all countries or PC and AC and the participation during the project are presented in Annex 6.1 and Annex 6.2.

3.1. Establishment of the network and scoping the project

3.1.1. Establishment of network for the purpose of the contract

Key outcomes:

A network of 38 national reference laboratories (NRLs) from 37 countries in Europe was established for the EURGen-RefLabCap project, promoting collaboration between NRLs nominated as contacts for WS1 pathogens (CCRE). A network of NRLs was also established for the WS2 pathogens (CRAb, CRPa) and consisted of 34 laboratories from 32 countries.

The network for the EURGen-RefLabCap project was established as follows:

- the 29 EU/EEA countries (27 EU Member States, IS and NO) were invited through the National Focal Points for Antimicrobial Resistance (and their alternates), National Coordinators (Coordinating Competent Bodies), and EURGen-Net National Technical Coordinators;
- the seven non-EU Member States (BA and RS, as participants in the Third EU Health Programme; ME, MK and TR, as EU candidate countries; and AL and XK, as potential candidate countries) were invited via the National ECDC Correspondents in the Western Balkans and Türkiye; and
- MD (participating in the Third EU Health Programme*)* was invited through the National Focal Point.

Invitations to join the EURGen-RefLabCap project were emailed in March/April 2021, and all 37 countries accepted, each assigning a laboratory to participate. CZ and SI assigned two laboratories to share participation. The kick-off meeting, introducing the EURGen-RefLabCap initiative and its objectives to the established network, was held in May 2021.

Establishment of the network to include contacts for WS2 pathogens (CRAb and CRPa) was initiated in May 2022. All countries were invited by ECDC to assign a contact. In December 2022 there was a final list with contacts from 28 countries (AL, AT, BG, HR, CY, CZ, DK, EE, FI, FR, DE, EL, HU, IS, IE, XK, LT, LU, MT, NL, MK, NO, PL, PO, SI, ES, SE, TR). In addition, three countries (BA, IT, MD) joined in June 2023 and one country (SK) joined in January 2024. All countries assigned the same laboratory as for WS1 except for BA that included two new laboratories as contacts for both WS1 and WS2. Five countries (BE, LV, MN, RO, RS) never assigned a contact for WS2 and therefore were not invited to participate in WS2 activities.

AL, ME, MK, XK, and TR were not participants in the Third EU Health Programme in 2021 and were invited to join the project as observers. Their involvement in the EURGen-RefLabCap project was unfunded. Consequently, an annual contract was negotiated with WHO Europe to provide financial support to these five countries. The contracts covered expenses for one representative per country to attend in-person meetings, workshops, EQAs, and simulated exercises. All online activities, such as the kick-off meeting, network meeting, virtual workshops and webinars were offered free of charge.

The project website domain (www.eurgen-reflabcap.eu) was launched in May 2021. Throughout the project, the website has been continuously updated with documents related to the activities, including protocols, anonymised survey outcomes, internal quality control (QC) documents, EQA results, presentations, and materials from in-person meetings. Additionally, it provides links to supporting online resources.

3.1.2. Identification of gaps and needs for capacity building

Key outcomes:

Individual NRLs had diverse capacities and capabilities for the provision of the five 'NRL core functions'. Important differences were observed in implementation of genomic surveillance, provision of scientific advice to clinical laboratories and public health authorities and availability of national integrated digital systems for collecting and reporting AMR data. This information was key to identify and prioritise capacity building needs.

Public health reference laboratories play a pivotal role in prevention and control of AMR in countries across Europe. Having sufficient capability and capacity to detect, assess, monitor and report infectious threats in each country strengthens the European public health microbiology system and contributes to effective prevention and control of infectious diseases and AMR.

The five ECDC-defined NRL core functions that reference laboratories are expected to undertake include: 1) reference diagnostics, 2) reference material resources, 3) scientific advice, 4) collaboration and research, and 5) monitoring, alert and response.[8] Indicators of capacity for these core functions were defined to allow for analysis of the capacity of the NRLs included in the project.

In July 2021, a review was prepared focusing on the publicly available information on the capability and capacity of the NRLs to perform the five core functions. However, most of these documents were grey literature issued by public health organisations and lacked detailed information about the capacities and capabilities of the individual NRLs and countries.

To obtain more granular information at country level, a detailed survey on laboratory capacity was developed and distributed as an online questionnaire to the laboratories of the 37 participating countries in July 2021.

The NRLs in most countries performed phenotypic antimicrobial susceptibility testing (AST) and genotypic characterisation of CCRE but important gaps were observed in the indicators regarding commitment to quality assurance (e.g. measured by 'participation in EQAs for phenotypic AST') (Table 3). WGS was implemented for reference purposes for CCRE at the NRL in 17 countries (AT, BE, CZ, DK, FI, FR, DE, IE, HU, IT, LU, NL, NO, PT, SI, ES and SE) and further 17 NRLs (BA, BG, HR, CY, EE, EL, IS, XK, LV, MT, MD, ME, MK, PL, RO, SK and TR) were planning the implementation of WGS for these purposes within 1-4 years. There was also variation in how the 'scientific advisory role' was carried out by the respective NRLs, and gaps were identified in relation to the provision of scientific and technical advice to CLs and public health authorities. Moreover, involvement in the development of national infection prevention and control guidance for CCRE was lacking in some NRLs. There were also capacity gaps in NRLs for several aspects of the core function for 'monitoring, alert and response', which included the setup of surveillance systems for CCRE, data collection and reporting, outbreak monitoring, alerts and early warning functions and defined outbreak support functions for the NRL staff. The results of the capacity survey were presented at the first network meeting (December 2021) and reported in the 'Results of the questionnaire: Public Health Microbiology Laboratory capacity for CRE/CCRE' (June 2022) (Annex 6.2). Additionally, in-depth analysis of the phenotypic and molecular methods available at the NRLs for AST and strain typing of CCRE, with particular focus on genomic approaches, was conducted. The results were provided in 'Report on the gaps in WGS capacity and molecular testing equipment, software and analytical skills at national laboratory level' (Section 3.3.1).

The findings of the capacity survey were also used to inform i) the development of: 'Work plan for networking and technical support activities for all countries in the EURGen-RefLabCap project' (December 2021), and ii) the selection of PCs and ACs, that is

documented in an addendum to the survey report (May 2022) and explained in Section 3.1.4.

A similar in-depth analysis of laboratory functions and capacity for molecular and genomic AMR prediction and strain typing methods was carried out for WS2 pathogens (CRAb and CRPa) in December 2022. Compared to the WS1 survey, the WS2 survey focussed more narrowly on laboratory methodologies applied for CRAb and CRPa as many of the other NRL core functions were more general public health laboratory functions not specific to certain pathogens, and that information was available through the first capacity survey.

When WS2 was initiated in 2022, addressing additional pathogens, a similar in-depth analysis of laboratory functions and capacity for molecular and genomic AMR prediction and strain typing methods was carried out for CRAb and CRPa. Compared to the WS1 survey, The WS2 survey focussed more narrowly on laboratory methodologies applied for CRArab and CRPa as many of the other NRL core functions were more general public health laboratory functions not specific to certain pathogens, and information was available through the first capacity survey.

The results were reported for 23 countries in WS2 in the 'Survey of the current molecular or genomic AMR prediction and strain typing methods used in NRLs for public health with competence for carbapenem- and/or colistin-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*' (August 2023) and presented to the NRLs in a webinar. This was accompanied by an ECDC presentation on the current epidemiological situation of CRAb and CRPa in the EU/EEA (Annex 6.2).

In general, fewer countries had implemented phenotypic and genotypic testing and typing methodologies for CRAb and CRPa than reported for CCRE (Table 3). Although, it is uncertain what the NRL capacity was in the countries that did not yet join WS2 (nor replied to questionnaire). There were also large gaps detected in commitment to quality assurance in the NRLs of WS2 and the implementation of WGS was at early stages in many countries.

Table 3: Number of countries with NRL capacity for phenotypic AST and genotypic characterisation of AMR genes (not including WGS), participation in EQAs and implementation of WGS surveyed for CCRE (July 2021) and CRAb and CRPa (December 2022)

NRL capacities	CCRE	CRAb	CRPa
Number of countries (respondents)	**37**	**23**	**23**
Confirmatory phenotypic AST	32	18	18
EUCAST guidelines used for AST and/or molecular characterisation	30	22	22
Participation in EQA for phenotypic AST	27	16	16
Genotypic characterisation of AMR genes	32	19	19
Participation in EQA for genotypic testing	17	6	6
WGS implemented	17	13	14
WGS planned	17	10	9

3.1.3. Implementation of networking and support activities

Key outcomes:

The capability and capacity for national surveillance and outbreak investigation of the priority pathogens (CCRE, CRAb and CRPa) have been strengthened in the European NRLs as a result of their active participation in the networking and technical support activities implemented in the EURGen-RefLabCap project.

The activities outlined in the tender for the EURGen-RefLabCap project addressed the need for improved surveillance of antimicrobial-resistant healthcare-associated pathogens through the WGS in the NRLs. This, in turn, should allow for the generation of more accurate and comparable surveillance data that enable a rapid response to emerging or epidemic infections caused by antimicrobial-resistant pathogens within the European countries and across borders.

During the first seven months of the project, the EURGen-RefLabCap team developed a work plan to achieve the overall aim and objectives of the project: 'Work plan for networking and technical support activities for all countries in the EURGen-RefLabCap project'. The scope, content and schedule of the networking and technical support activities were developed following a review of the publicly available literature and with information on the current capacity NRL core functions provided in a mapping survey completed by the NRLs of the project in mid-2021. Especially the identified gaps and needs for development for NRL capacity informed the support activities available to all countries (Section 3.1.2.). The scope and design of the planned technical support activities were further refined, as more information about the capacity and laboratory practices for WGS-based surveillance and bioinformatics methods of all participating countries became available in later surveys.

The networking and support activities **for all NRLs** in EURGen-RefLabCap were developed in an effort to engage with the network members, as much as possible across borders, provide 'state of the art' technical support and facilitate shared learning and exchange of best practice and collaborative working between the network members. Agendas and presentations can be found here: https://www.eurgen-reflabcap.eu/meetings

All countries were offered to participate in the following activities:

- Network meetings
- On-site training workshops
- Virtual training workshops
- Virtual simulated outbreak exercises
- EQA exercises
- Webinars

The networking and technical support activities were attended by a high number of participants (Annex 6.1). These activities were rated continuously by collecting user feedback and the feedback scores were predominantly high. The impact on the actual NRL capacity in each country is best evidenced in the interviews with the coordinators of the PCs (Section 4.2).

3.1.4. Identification of priority countries and additional countries

Key outcomes:

The EURGen-RefLabCap team, DG SANTE and ECDC identified countries with the greatest need for capacity building in AMR surveillance for priority healthcare-associated infections (PCs) and identified countries for activities to support NRLs in their advisory role towards networks of CLs for AMR surveillance in their countries (ACs). Almost all invited PCs and ACs agreed to participate in the bespoke support activities and improved their capacity for genomic surveillance of AMR pathogens.

To strive towards timely availability of consistent surveillance data on AMR pathogens across European countries, networking and capacity building activities in EURGen-RefLabCap were adapted to the context of each country.

The EURGen-RefLabCap team, DG SANTE and ECDC jointly established criteria to rank NRLs/countries based on their need for capacity building activities for surveillance of AMR in priority healthcare-associated infections in September 2021. A similar exercise was repeated in May 2022, to identify NRLs that would benefit most from activities to support their role in working with and building the capacity of the CL networks in their countries for CCRE, CRAb and CRPa. The guiding principle for establishing the criteria was to use the most recent and comparable data describing the public health microbiology capacity of NRLs, including surveillance and detection of outbreaks caused by CCRE, CRAb and CRPa in their respective countries. Details on the agreed criteria are presented in Table 4.

Table 4: Criteria for identifying PCs and ACs to be offered bespoke support activities in the EURGen-RefLabCap project

Scope	Criteria	Rationale and relevance to scoring
Identification of PCs	Selected questions from the EURGen-RefLabCap questionnaire on Public Health Microbiology Laboratory capacity for CCRE.	Reference diagnostics. Participation in the planned activities is expected to improve the foundation of functions of NRLs.
		WGS. Participation in the planned activities is expected to enable NRLs to use WGS for AMR surveillance.
		Reference material resources. Proficiency in this core function is relatively easy to achieve without the bespoke support of EURGen-RefLabCap.
		Scientific advice. Proficiency in this core function may be good without having fully implemented WGS.
		Collaboration. This core function will become more important once the capacity for WGS is established in the NRLs.
		Monitoring, alert and response. Proficiency in this core function may be good without having fully implemented WGS.
	Correct species identification for isolates sent for CCRE survey	Participation in the planned activities is expected to improve quality of results.
	Score in EURGen-Net CCRE EQA	Participation in the planned activities is expected to improve quality of results.
	Contribution of WGS data for ECDC Rapid Risk Assessments	Participation in the planned activities is expected to enable countries to contribute WGS data to international AMR surveillance.
Identification of ACs	Selected questions indicative of collaboration and coordination between the NRL and the CLs from the EURGen-RefLabCap questionnaire on Public Health	Participation in the planned activities is expected to improve scoring in these questions by strengthening the collaboration between the NRL and the CLs.

Scope	Criteria	Rationale and relevance to scoring
	Microbiology Laboratory capacity for CCRE	
	EARS-Net 2020 data[9] including: • National population coverage • Geographical representativeness • Hospital representativeness • Patient and isolate representativeness • *E. coli* – carbapenem resistance percentage and trend 2016-2020 • *K. pneumoniae* – carbapenem resistance percentage and trend 2016-2020 • *P. aeruginosa* – carbapenem resistance percentage and trend 2016-2020 • *A. baumannii* – carbapenem resistance percentage and trend 2016-2020	EARS-Net 2020 data provide an overview of AMR in Europe. Participation in the planned activities is expected to improve representativeness of EARS-Net data by strengthening the collaboration between the NRL and the CLs. Furthermore, participation in the planned activities, although not directly affecting the percentages of carbapenem-resistant isolates, will result in an improved ascertainment of the priority pathogens, which is expected to benefit countries having high resistance percentages.

Note: colours indicate relevance to the scoring. Dark orange, very high importance; light orange, high importance; yellow, medium importance; green, low importance.

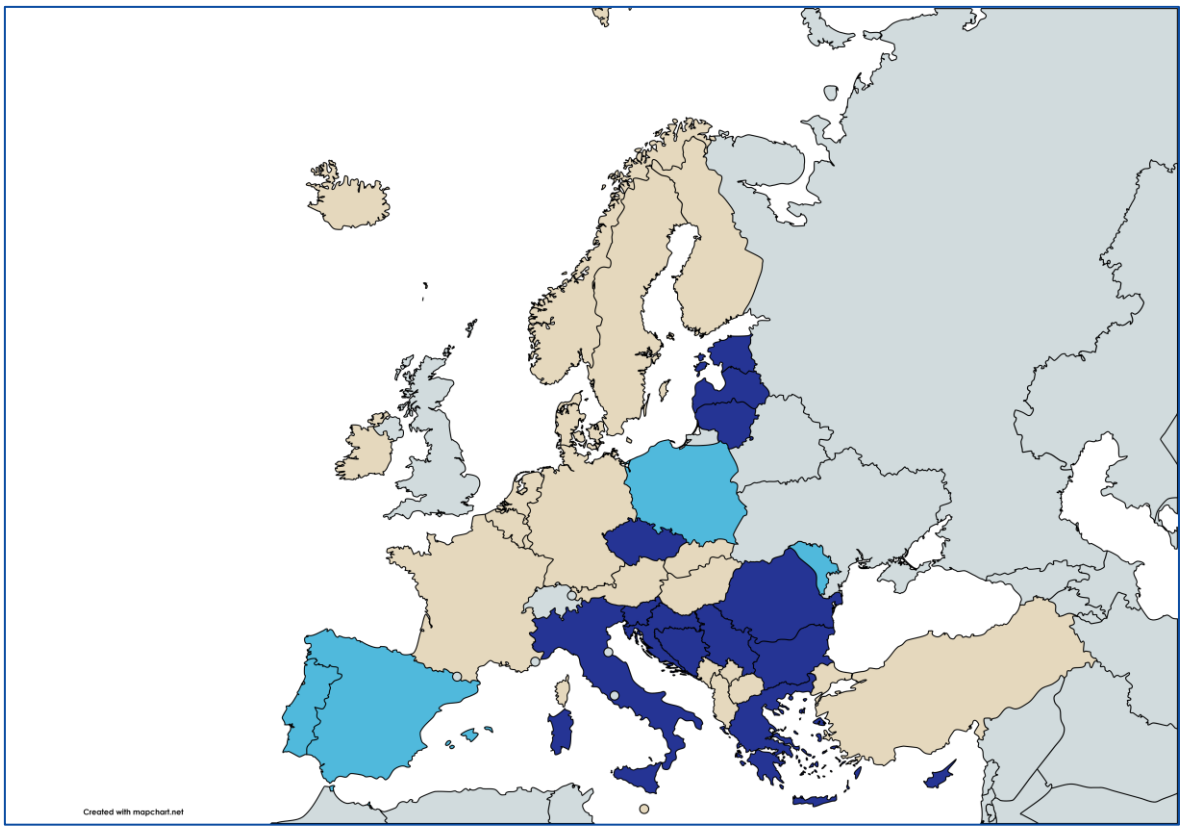
Based on the financial and human resources of the project, the EURGen-RefLabCap team could support 8-12 PCs, which could be extended to a maximum of 16 countries for activities to improve collaboration between NRLs and CLs in national networks. In addition, only countries included in the Third EU Health Programme were eligible for financial support in EURGen-RefLabCap activities.

The top 14 eligible countries in the ranking were contacted individually to explain the activities being offered. Thirteen countries accepted the PC designation, while one country declined the offer, as it did not have the human resources to carry out the activities. Similarly, countries eligible for AC were contacted, and four countries accepted the nomination as an AC.

The 13 countries designated as PC were: BA, BG, HR, CY, CZ, EE, EL, IT, LV, LT, RO, RS, SI (Figure 1). The four countries designated as AC were: MD, PL, PT, ES.

Most PCs and ACs were highly engaged and completed all of the project's bespoke support activities (Annex 6.1)).

Figure 1: Countries participating in the EURGen-RefLabCap project



Dark blue: priority countries (PCs). Light blue: additional countries (ACs). Light brown: other countries.

3.1.5. Selection of additional priority pathogens and expansion of the network

Key outcomes:

CRAb and CRPa were selected as additional priority antimicrobial-resistant pathogens for WS2 of the EURGen-RefLabCap project and molecular surveillance in EURGen-Net, based on the outcome of a questionnaire distributed to ECDC National Focal Points for AMR and ECDC National Correspondents for EU Enlargement countries.

The initial part of the project focused on CCRE. In order to further improve and modernise the diagnostic and reference testing methodologies of the NRLs and molecular surveillance in European countries, two additional healthcare-associated antimicrobial-resistant priority pathogens were identified in 2022. The inclusion of additional pathogens in the EURGen-RefLabCap and EURGen-Net was aimed at accelerating the transition to WGS in the NRLs and enhancing genomic surveillance across Europe. Implementation of the WGS technology streamlines the laboratory workflows by combining a number of investigative steps previously performed using different methods, and allows upscaling of the analysis. Moreover, the data outputs of WGS are more easily exchanged and compared between laboratories and countries due to the digitalisation of the WGS data.

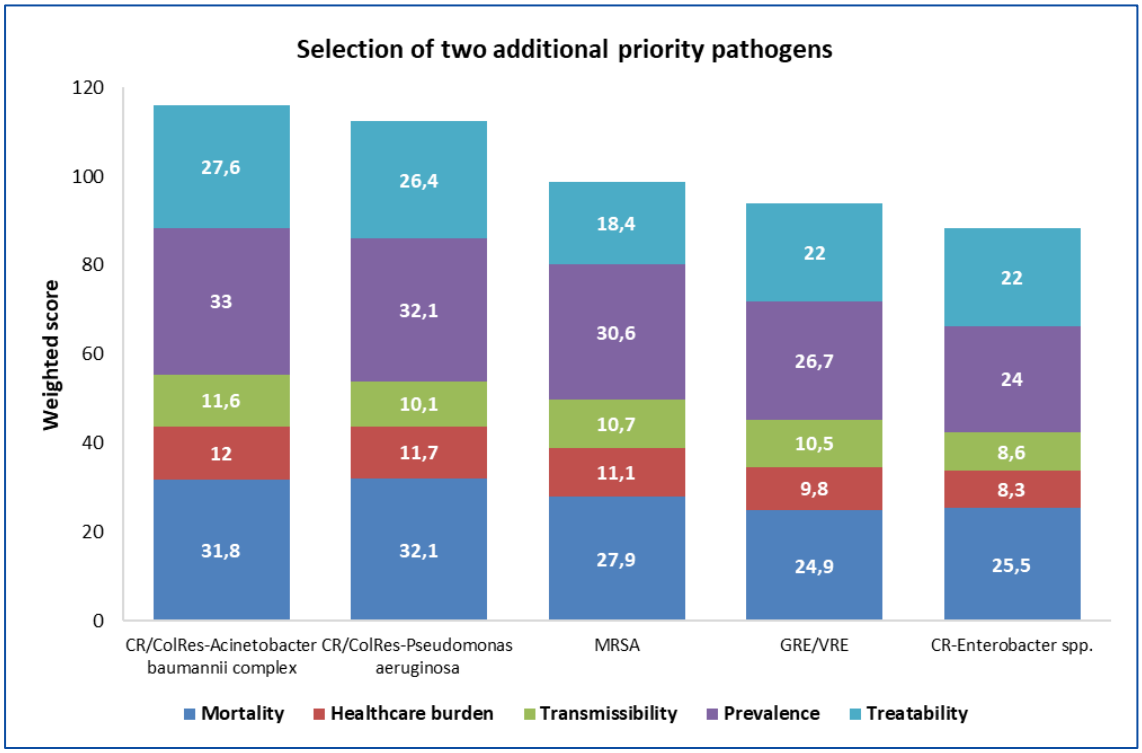
A prioritisation exercise to select additional pathogens was organised by the EURGen-RefLabCap team in collaboration with DG SANTE, HaDEA, ECDC and the National Focal Points for AMR. The exercise was carried out by distributing a questionnaire to the ECDC National Focal Points for AMR and ECDC National Correspondents for EU Enlargement countries (AL, BA, XK, MD, ME, MK and TR). The exercise aimed at selecting two additional priority pathogens from the following pre-defined list:

- Carbapenem- and/or colistin-resistant *Pseudomonas aeruginosa* (CRPa)
- Carbapenem- and/or colistin-resistant *Acinetobacter baumannii* (CRAb)
- Extended-spectrum beta-lactamase-producing *Enterobacter* spp.
- Meticillin-resistant *Staphylococcus aureus* (MRSA)
- Glycopeptide/vancomycin-resistant *Enterococcus* spp. (GRE/VRE)

The respondents completed the questionnaire after being presented to five criteria associated with each of the five pathogens: mortality, healthcare burden, transmissibility, prevalence and treatability, as well as quantitative and qualitative data on these criteria.

Representatives from 30 (AL, BE, BG, HR, CY, CZ, DK, EE, FI, FR, DE, HU, IS, IT, LV, LU, MT, MD, ME, NL, MK, NO, PL, PT, RO, SK, SI, ES, SE, TR) out of 37 European countries replied to the questionnaire. The respondents were asked to rate the pathogens on a scale from 1 to 5 (1= not important, 2= slightly important, 3= important, 4= fairly important and 5= very important, and an option of 'no opinion') against the five criteria. Weighting factors were applied to the scores of each criterium based on the quality of the evidence available, and with quantitative rated over qualitative evidence, with severe rated over mild outcome, with impact on large populations rated over impact on smaller populations affected by disease, and finally by the relevance to the EURGen-RefLabCap project (Figure 2). The results were submitted to HaDEA and ECDC to inform them of the outcome. The results and draft conclusions were further discussed with the ECDC National Focal Points for AMR and ECDC National Correspondents for EU Enlargement Countries before a final decision was made. Ultimately this resulted in the selection of CRAb and CRPa as the additional pathogens.

Figure 2: Assigned total weighted scores and weighted scores by category for each of the five pathogens based on replies from 30 European countries.



*CR/ColRes; carbapenem-resistant and/or colistin-resistant. CR-*Enterobacter *spp.: Extended-spectrum beta-lactamase-producing* Enterobacter *spp. GRE/VRE: Glycopeptide/vancomycin-resistant* Enterococcus *spp. MRSA: Methicillin-resistant* Staphylococcus aureus*.*

The network of laboratories for WS2 was created through a nomination process led by ECDC using the framework of the Stakeholder Relations Management system. Thirty-two countries (AL, AT, BA, BG, HR, CY, CZ, DK, EE, FI, FR, DE, EL, HU, IE, IS, IT, XK, LT, LU, MT, MD, NL, MK, NO, PL, PT, SK, SI, ES, SE and TR) nominated laboratories to participate in WS2.

3.2. Support activities for priority countries

3.2.1. Support for local capacity development through action plans for priority countries

Key outcomes:

The NRLs in the 13 PCs (BA, BG, HR, CY, CZ, EE, EL, IT, LV, LT, RO, RS, SI) prepared and implemented an action plan to strengthen their capabilities and capacities to detect and control the spread of CCRE in healthcare settings at local and national level, across Europe and globally.The key outcomes following on from the action plans are described at country level (Table 5).

Action plans are established project management tools that list specific steps, tasks and resources needed to achieve desired goals. Due to the complexity of the EURGen-RefLabCap project, which involved multiple activities with many stakeholders over a four-year period, the EURGen-RefLabCap team invited the NRLs in PCs to identify and prioritise areas for development to strengthen their capabilities and capacities to detect and control the spread of CCRE in healthcare settings nationally and internationally.

To ensure a harmonised approach across countries, the EURGen-RefLabCap team prepared a Microsoft Excel template for action plans and presented it to each PC in individual online meetings during 2021, and at the first workshop for the PCs in December 2021. The template was then shared by email to be completed by the PCs.

The action plan template included specific aims and objectives for achieving competence in each of the five NRL core functions (Annex 6.4), which the NRLs could further elaborate on, or delete, according to their individual needs. For each relevant objective, the NRLs had to describe their planned actions, the resources required and the timeframe, and define the deliverables and milestones foreseen to achieve the objective, as well as the responsible persons.

The draft action plans produced by the NRLs in BA, BG, HR, CY, CZ, EE, EL, IT, LV, LT, RO, RS and SI were discussed and finalised during the first country visits (Section 3.2.3) and in online bespoke consultancy meetings. By June 2022, all PCs had produced an action plan. As expected, the action plans differed from country to country, reflecting the different capabilities of NRLs in the five NRL core functions (Section 3.1.2). Independent of the country, all action plans included actions to reach the overall aims to perform WGS-based typing of CCRE, to provide scientific advice and technical support to public health authorities and to provide input to national recommendations for public health notification.

The EURGen-RefLabCap team clearly explained that the action plan was intended to be a working document and a supporting tool that would be regularly reviewed and updated as necessary. The NRLs in the PCs used the action plans in different ways, but all PCs reviewed their action plans at least once during the second country visits (Section 3.2.3). An exception was the NRL in IT, that developed an action plan but did not have the resources to prioritise further engagement in the bespoke support activities to implement such plan.

The PCs provided feedback on the action plans in structured interviews at the end of the project (Section 4.2). The initiative of creating action plans was praised as it enabled the NRLs to reflect on their needs and, in some cases, even to identify what they needed to

achieve to fulfil their role as NRLs. Having agreed the action plan also enhanced their commitment to change, and made it easy to assess progress.

The key outcomes obtained as a result of completing and implementing the action plan highlighted by the respective PCs in the structured interviews are listed in Table 5.

The capacity building efforts among the PCs mainly focused on aspects of NRL core function 1 - reference diagnostics, including confirmatory reference testing, phenotypic and genotypic testing and typing, and commitment to quality assurance. All PCs prioritised the development of expertise for performing and subsequently implementing WGS into routine reference services.

At the start of the project, only **four** NRLs in PCs (BG, CZ, LV and SI) had capacity and capability for performing WGS (by Illumina) for reference purposes.

Skills and expertise for performing WGS in the laboratory, including DNA preparation and completion of sequencing runs, were developed in **seven** NRLs (HR, CY, EE, EL, LT, RO, RS). The NRL in BA participated in online and on-site trainings and the EQAs which led to initiation of planning of the implementation of WGS.

Eleven NRLs (BG, HR, CZ, CY, EE, EL, LV, LT, RO, RS, SI) further developed laboratory skills in ONT sequencing and data analysis.

WGS pipelines supporting routine workflows were either implemented and/or improved in **ten** NRLs (BG, HR, CY, CZ, EE, EL, LV, LT, RO and SI).

Fewer PCs implemented improvements related to NRL core function 2 – reference material resources. The NRL in CZ extended their available reference collection of isolates while the NRL in RO sequenced isolates of their existing reference collection for quality assurance purposes.

Many other improvements in PCs were obtained within aspects of NRL core function 3 – Scientific advice, NRL core function 4 – collaboration and NRL core function 5 – monitoring, alert and response. The establishment and/or consolidation of networks of CLs improved the communication and collaboration between the NRLs and CLs in their respective countries. The improvement of the CL networks' setup and activity was highlighted as a key outcome in **11** NRLs (BG, HR, CZ, CY, EE, EL, LT, LV, RO, RS, SI) (see also Section 3.2.3 for details).

With regards to improvements in NRL core function 5 – 'monitoring, alert and response', the NRLs in **six** countries (BG, EE, EL, LV, LT, SI) improved their national surveillance systems and capability to detect outbreaks by implementation of quality assured WGS testing and sub-typing, while NRLs in **five** countries (HR, CY, CZ, RO, RS) progressed actions towards setting up WGS-based surveillance.

Progress in capacity for NRL core functions in all PCs was assessed by using indicators see Section 4.2.

Table 5: Key outcomes obtained by the NRLs in PCs. The statements were obtained from a series of structured interviews held to gather feedback from the PCs in September 2024

Country	Key outcomes in priority countries
BA	Heightened awareness of the NRL core functions including use of WGS for AMR surveillance purposes. Development of a standard operating procedure for confirmatory diagnostic testing for atypical CCRE isolates.

Country	Key outcomes in priority countries
BG	Improvement in bioinformatics pipeline for analysis of ONT data through participation in EQAs. Improvement of national AMR surveillance.
HR	Developed expertise in WGS using Illumina and ONT and in bioinformatics analysis. Implementation of analytical pipelines for WGS data based on open source solutions. Improvement of the framework for collaboration with the local CLs.
CY	Implementation of WGS and bioinformatics analysis with Illumina and ONT technologies. Formation of National Network of Microbiology Laboratories with enhanced collaboration between laboratories. Increased cooperation with Veterinary Services for shared WGS equipment and workflow.
CZ	Implementation of ONT sequencing. Development of short- and long-read pipeline for automatic data assembly and generation of results using online WGS tools. Update of national guidelines for detection of CRE/CCRE isolates in clinical practice including outbreak specifications and protocols for investigation and reporting, patient isolation guidelines, among others. Extension of the national collection of reference strains and distribution of control/reference CRE/CCRE isolates among CLs.
EE	Implementation of routine laboratory workflow for WGS of bacteria and bioinformatics pipeline. Established coordination among bioinformaticians, microbiologists and epidemiologists for interpretation of WGS data. Establishment of an operational framework for provision of technical support and scientific advice to CLs with regular meetings every 8 weeks.
EL	Implementation of quality assured WGS at the NRL including setup of the ONT sequencing. Strengthened national network of CLs and improved/more frequent communication with the network. Implementation of WGS for CRPa in close collaboration with a network of hospitals. Improved capacity in all major NRL core functions.
LV	Establishment of bioinformatics pipelines for detection and typing of antimicrobial-resistant bacteria. Consolidation of collaboration with the regional laboratory network and other central laboratories. Establishment of a database linking epidemiological data with the sequencing data and phenotypic profiles of bacterial isolates. Participation in AMR surveillance activities at European level including active use of EpiPulse.
LT	Implementation of WGS using Illumina and ONT and bioinformatics pipeline for *K. pneumoniae*, *E. coli*, and *A. baumannii.* Publication of genomic data on *K. pneumoniae* in a peer-reviewed paper in Eurosurveillance. Engagement with stakeholders and decisionmakers at the Ministry of Health, Hygiene Institute and National Public Health Center. Dissemination of guidelines for screening and isolation of patients with CCRE in hospitals across the country.
RO	Implementation of quality assured WGS for reference testing of antimicrobial-resistant bacteria and improved capacity for performing WGS and bioinformatics analysis. Genomic characterisation of a collection of strains that will be used for reference purposes.

Country	Key outcomes in priority countries
	Implementation of ONT sequencing.
RS	Implementation of laboratory infrastructure, WGS and bioinformatics analysis. Consolidation of collaboration with the local network of AMR laboratories. Establishment of referral of isolates and reporting of data from the network laboratories.
SI	Establishment of national AMR surveillance via reporting at laboratory level including prospective monitoring of CPE. Establishment of essential coordination between two institutions in the country with reference laboratory capacities for WGS. Benchmarking of the quality of the WGS setup against international datasets. Progress of the agenda for AMR preparedness and importance of NRL services in the country through engagement with stakeholders and decisionmakers.

While recognising the usefulness of developing action plans, some NRLs found it difficult as this project management methodology was completely new to them, and they would have benefited from receiving additional training on how to work with it. To some NRLs the format and detailed content of the action plan template was too complex. Despite these limitations, the NRLs found that the benefits of developing the action plans extended beyond the project, as they provided guidance for setting objectives and prioritising and planning activities to ensure that the NRLs fulfilled their roles and functions.

3.2.2. Bespoke support to priority countries including country visits and pilot genomic surveillance studies

Key outcomes:

The PCs engaged in a wide range of activities including regular bespoke consultations, pilot genomic surveillance studies and country visits, among others. NRLs in 12 PCs and two ACs completed pilot genomic surveillance studies and two additional NRLs initiated pilot studies but did not complete the data analysis within the timeline. Participation in the wide range of activities strengthened capabilities and capacities for genomic surveillance and outbreak investigations in most PCs.

To ensure adequate level of bespoke support, each PC was assigned a dedicated ‘country team’ that included 2-3 persons from the EURGen-RefLabCap team with complementary expertise. The dedicated country teams would be the primary contact of the NRL and provide them with technical advice throughout the project.

The close collaboration between the country teams and the PCs were initiated when the country teams invited the 13 PCs to receive the first of two country visits. The primary objective of the first visits was two-fold: 1) to allow the NRLs and the dedicated country team to meet face-to-face and develop their collaboration, and 2) to review, further improve and launch the implementation of the action plans for capacity building. The NRLs were encouraged to also involve relevant colleagues, collaborators and important stakeholders in the visits to aid progressing specific aims of the EURGen-RefLabCap action plans that required collaboration outside the NRLs. Scientific advisors from ECDC were also invited to attend the visits as observers.

The first round of country visits also served the purpose of being a fact-finding mission that allowed the EURGen-RefLabCap team to collect more detailed information on the NRLs

formal designation and connectedness with the national health systems, their current delivery of NRL core functions, strengths and weaknesses and gaps, and their plans for implementation of WGS for genomic surveillance and outbreak detection purposes. In addition, the PCs were encouraged to describe the current NRL service configuration in terms of yearly activity, personnel and their expertise, the NRL facilities, funding for NRL services, turnaround times (from receiving samples to reporting) and laboratory quality management systems.

Visiting the NRLs, meeting their teams, seeing the laboratories and equipment, and in many instances also meeting management, collaborators and key stakeholders allowed the visiting country teams to develop an in-depth understanding of the current capacities as well as gaps, challenges and needs for support in each country. Likewise, it also allowed the NRLs to gain a better understanding of the range of networking and support activities, training events and financial support for certain tasks offered by the EURGen-RefLabCap project.

The good collaboration between the country teams and the PCs established during the first visits continued through regular online bespoke consultations. The country teams organised online meetings with each PC, with varying frequencies depending on each PC's preference (for example bi-weekly or monthly meetings). The aim of the online meetings was to follow up on the implementation of the activities described in the action plans and to plan future steps, to solve specific problems encountered by the PCs and to exchange information (updates on EU legislation, upcoming courses and conferences, interesting literature, updates on bioinformatics tools, etc.). Where relevant, for example when discussing specific laboratory procedures and software, the country teams invited additional colleagues from DTU and SSI to attend the meetings and share their expertise. ECDC representatives attended some of these meetings when expertise on international AMR surveillance was particularly relevant, for example when deciding on the inclusion criteria for the pilot genomic surveillance studies and discussing the results of these studies (see below). The NRLs also invited other national colleagues and collaborators to the meetings if it was relevant to the topics on the agenda.

These regular online meetings were supplemented by bespoke support via email. The bespoke support for the PCs continued regularly until the end of the project.

The PCs highly appreciated the bespoke consultations and encouragement they received throughout the project although attending the regular online meetings was sometimes challenging as it required a balance with their routine work. However, the flexibility of these meetings in terms of content and frequency eased this burden.

An important support activity was the launch of pilot genomic surveillance studies in January 2023. The purpose of the pilot studies was to generate preliminary data to demonstrate the usefulness of WGS to improve detection of CRE, CRAb and/or CRPa clusters, and thereby support healthcare facilities to design appropriate control measures. Additionally, with the pilot studies, the NRLs had the possibility to assess the feasibility of the methodology.

Invitation to conduct these studies with the support of EURGen-RefLabCap was extended also to the ACs. The EURGen-RefLabCap team prepared a project description template in which PCs and ACs had to briefly describe the objective of the study and the materials and methods, including collection of isolates, patient metadata, microbiology testing data, epidemiological data, methods for WGS and bioinformatics analyses, plans for data handling and storage, and the study timeline.

The country coordinators in 12 PCs (BA, BG, HR, CY, CZ, EE, EL, LV, LT, RO, RS, SI) and two ACs (PT, ES) designed the pilot genomic surveillance studies in collaboration with the country teams and ECDC. Eleven PCs and three ACs succeeded in conducting WGS and at least initiating bioinformatics analysis of the obtained sequence data by the deadline set four months before the end of EURGen-RefLabCap project for administrative purposes (Table 6). BA and MD initiated pilot studies, but could not perform WGS by the deadline

due to delays in reagent procurement procedures. Nonetheless, the laboratories in these countries are committed to implement WGS as soon as they receive reagents.

Table 6: WGS data generated in the context of the EURGen-RefLabCap pilot genomic surveillance studies

Country	Pathogen	No. of isolates sequenced (sequencing technology)	Scope of the study
BG	CCRE	94 (Illumina) 23 (ONT)	Characterisation of *Klebsiella pneumoniae* ST6260 that is a multi-drug resistant clone harbouring *bla*$_{NDM-5}$ and/or *bla*$_{OXA-232}$ and *rmtB,* among other AMR genes, that has rapidly spread across healthcare facilities throughout the country. The aim is to generate information useful to control this large outbreak.
HR	CCRE	24 (Illumina)	Elucidation of the acquired and chromosomal resistance determinants and characterisation of the epidemiological relationship amongst the NDM-producing *Escherichia coli* collected in the framework of national AMR surveillance network. The aim was to complement results from current national surveillance.
CY	CCRE	45 (Illumina)	Preliminary exploratory study of CCRE isolates collected from regional hospitals to understand what clones and what AMR determinants are circulating in the country. The main aim was to guide future national surveillance.
CZ	CRAb CRPa	41 (Illumina) 83 (Illumina)	Epidemiological investigation of carbapenem-resistant *Acinetobacter* spp. and *Pseudomonas* spp. collected from regional hospitals from the whole country. The aim was to characterise recently detected carbapenem-resistant non-fermenting rods such as *P. aeruginosa* carrying the *bla*$_{NDM-1}$ gene, or strains of *A. baumannii* complex producing carbapenemases of the NDM and VIM type.
EE	CCRE CRAb CRPa	147 (Illumina) 114 (Illumina) 32 (Illumina)	Characterisation of CCRE clones circulating in healthcare facilities in the country with the largest clone being represented by *K. pneumoniae* ST7462 harbouring *bla*$_{OXA-48}$ or *bla*$_{OXA-232}$. Characterisation of the main CRAb lineage circulating in the country (ST2/*bla*$_{OXA-23}$). Preliminary findings of *P. aeruginosa* ST260 harbouring *bla*$_{VIM-1}$. The aim is to generate data to control outbreaks and guide future AMR surveillance.
EL	CRPa	37 (ONT)	Description of the epidemiology of CRPa in hospital patients and genomic characterisation of carbapenem resistance mechanisms to guide hospital infection prevention and control practices for CRPa.
LV	CCRE	318 (Illumina)	Description of changing epidemiology of CCRE, with increasing occurrence of *K. pneumoniae* ST147 harbouring *bla*$_{NDM-1}$ and/or *bla*$_{OXA-48}$ that has spread across healthcare facilities throughout the country. The aim is to generate evidence to support use of WGS for CCRE surveillance and outbreak detection.
LT	CCRE	106 (Illumina)	Description of at least three clonal outbreaks caused by *K. pneumoniae* producing OXA-48 in healthcare facilities across the country. The aim was to gain insight on the recent nationwide increase of OXA-like *K. pneumoniae* clinical isolates and provide information to public health authorities and CLs to design measures to prevent and control CCRE spread.
RO	CCRE	43 (ONT)	Genomic characterisation of extended-drug-resistant *K. pneumoniae* strains co-harbouring *bla*$_{NDM}$ and *bla*$_{OXA-48}$ genes. The aim is to develop capacity for genomic surveillance of priority pathogens using WGS.

RS	CCRE	24 (Illumina) 24 (ONT)	Genomic characterisation of *K. pneumoniae* isolates collected from hospital patients during routine surveillance and for diagnostics purposes over a 12-month period. The aim is to strengthen the capacity for outbreak investigation and national surveillance on antimicrobial-resistant pathogens using WGS, and to produce a proof of concept for identifying outbreaks and potential transmission between different wards.
SI	CCRE	110 (Illumina)	Genomic characterisation of carbapenemase-producing *E. coli* isolates received by two NRLs in a 12-month period. The goal is to ensure compatibility of the pipelines and results at different institutions for future collaboration.
PT	CCRE	45 (Illumina) 45 (ONT)	Genomic characterisation of CCRE from hospital outbreaks and national surveillance. The study aims to provide preliminary data that can guide further, more in-depth investigations in the role of plasmids.
ES	CCRE	24 (ONT)	Study of the role of IncL plasmids in the dissemination of *bla*$_{\text{VIM-1}}$ and *bla*$_{\text{OXA-48}}$ among different CCRE clones and species, through the analysis of the genetic structure of IncL plasmids. The aim was to characterise the population structure and resistance mechanisms of carbapenemase-producing *K. pneumoniae* isolated from intensive care unit patients co-infected with HAI.

Each PC and AC had the opportunity to apply for financial support up to 14 000 euro to reimburse costs associated with the pilot study. Eleven PCs (BA, BG, HR, CY, CZ, EE, EL, LV, LT, RO, SI) and three ACs (MD, PT, ES) submitted a report. Nine PCs and two AC applied for and received the financial support, which was used to cover personnel, software, laboratory reagents or other costs related to the studies.

In parallel with the launch of the pilot genomic surveillance studies, the EURGen-RefLabCap project supported each PC and AC with logistical and financial resources (up to 3 200 euro per country) to purchase a sequencing device (MinION) from ONT and/or associated reagents to build or strengthen WGS capacity in the PCs and ACs. In total, 16 laboratories from 12 PCs (BA, BG, HR, CY, CZ, EE, EL, LV, LT, RO, RS and SI) and three ACs (MD, PT and ES) engaged in this opportunity and were able to receive materials from ONT. In most cases the laboratories chose to purchase ‘Starter Packs’ which contained a MinION sequencing device as well as the necessary reagents to perform WGS.

The purchase of ONT material and training in long-read sequencing were very well received by the NRLs in the PCs and ACs, all of whom have now begun to actively work to implement the methodology locally. The support activity also highlighted important issues with the uneven distribution of reagents across European countries. The purchase of ONT material was not straightforward in some countries, raising concerns about the sustainability of the use of ONT once the reagents procured through the EURGen-RefLabCap have been used.

In the final year of the project, the country teams carried out a second round of country visits to consolidate cooperation with the PCs. Representatives from ECDC were invited as observers to these visits. These visits allowed detailed discussions of the planned capacity building activities and discussions of the pilot studies and improvement work with the national networks of CLs.

The EURGen-RefLabCap team also implemented activities to support NRLs to build and/or maintain a collection of reference material resources. A list of reference strains with relevant phenotypes and/or genotypes available free of charge at DTU and SSI was published on the EURGen-RefLabCap website. A Material Transfer Agreement template was prepared to allow rapid shipment of these strains upon request. During the project reference strains were shipped to HR, EE and LT. A list of other biorepositories providing reference materials was also created on the project website.

Additional resources such as lists of bioinformatics tools and databases relevant for analysis of CCRE, CRAb and CRPa were added on the EURGen-RefLabCap website to support NRLs to analyse and visualise genomic information in a communicable manner.

3.2.3. Implementation of activities to support the national reference laboratories to build capacity in networks of clinical laboratories

Key outcomes:

The NRLs in 27 countries conducted a mapping survey of laboratory capacity for detection of CCRE, CRAb and CRPa in their networks of CLs. The surveys identified strengths and weaknesses in the national setups and enabled the NRLs to improve their national or regional advisory role in developing capacity for detection and characterisation of the priority pathogens within their networks of CLs. The NRLs in 23 countries (AT, BA, BG, HR, CY, CZ, EE, FR, DE, EL, XK, LT, LU, LV, MT, MD, NO, PL, RO, RS, SI, ES and SE) further analysed the situation and initiated capacity building activities within their networks of CLs.

In addition to providing networking and support activities to the NRLs, the EURGen-RefLabCap project aimed at implementing support activities to strengthen the coordinating and advisory role of the NRLs to build capacities in the networks of CLs within each country.

This area of support was initiated by the development of a draft questionnaire for the NRLs to distribute within their national networks of CLs for mapping the capacities in the CLs and networks. The NRLs were introduced to the draft questionnaire and to the methodology to conduct the national mapping surveys. The draft questionnaire addressed ten areas of CL capacity:

- Information about the national networks
- Criteria for submission of clinical samples to the CLs
- Diagnostic testing
- Quality of laboratory services
- Reporting/management of test results from the CLs
- Participation in national/international surveillance
- Referral of samples to the NRLs
- Membership of laboratory network
- Staffing situation
- Specific support in demand by the CLs from the NRLs

The draft questionnaire was discussed, and the final version was agreed with the participants at the on-site workshop at SSI (in June 2022) (see below). This questionnaire could furthermore be translated into other preferred languages, and the NRLs could choose to add up to 10 other questions of relevance to each country.

At the outset of this activity, many countries already had well-functioning networks of CLs that included ongoing collaboration with their relevant NRLs. However, in some countries the NRLs had to build networks or establish contact with other existing networks before they could carry out the survey.

The NRLs that joined the activity provided a national report summarising the results from their country. PCs and ACs received 9 400 euro to cover costs associated with conducting the survey and writing the reports. The individual country reports were then used to create an overview report of the key findings highlighted by the NRLs. Based on these key findings and suggestions from the NRL, the EURGen-RefLabCap team proposed options for actions at national level and highlighted needs at European level (Annex 6.3).

To support the NRLs in their local capacity building efforts, a number of training events were organised within the EURGen-RefLabCap project. This included an on-site train-the-trainer

workshop at SSI in June 2022 aimed at exchanging knowledge and experience on best practice enabling NRLs to build capacity in their own national networks, initially through forming or consolidating the networks of CLs, conducting the mapping survey and completing the planned improvements.

Moreover, an external provider, Implement Consulting Group delivered training to the PCs on project management, development of business cases and sustainability plans, and stakeholder engagement via virtual sessions (May and September 2022). An overview of participation in the activities is presented in Annex 6.1 and the presentations from these activities are available in the EURGen-RefLabCap website (Annex 6.2).

Prior to the mapping exercise, existing national or regional networks of CLs had been inactive for periods of time and/or did not include all CLs that would be needed for sufficient coverage of populations/healthcare facilities in the AMR surveillance systems in the countries. In total, NRLs in 11 PCs (BG, HR, CZ, CY, EE, EL, LT, LV, RO, RS and SI) strengthened the network of CLs in their country as a result of the mapping exercise and other project activities. Improvements in CL networks were possibly also obtained in the other participating countries without PC-status. However, there was no one-to-one follow-up through interviews with these countries.

The NRLs in HR, EE, EL, LT, LV, RO and RS highlighted (in structured interviews) that they either revived or expanded existing CL networks and/or obtained significant improvements in collaboration with the networks of CLs in their countries as a result of the NRLs conducting the mapping exercise. A new national network of CLs was established in CY.

The mapping exercises provided the NRLs up-to-date information on diagnostic capacity, quality of laboratory services, reporting of test results, referral of samples to the NRLs, staffing situation and other operational details that allowed the NRLs to summarise the strengths and weaknesses in the setups for detecting and preventing AMR in their networks. Especially, having a more frequent communication, sharing data and referral isolates to the NRLs were mentioned among the results of the improved collaboration. As a consequence, more aspects of the NRL technical/scientific advisory role and coordinating roles towards clinical laboratories are now being implemented and further formalised in these countries.

The NRL in CZ organised a 3-day workshop with the Czech network of 30 CLs aimed at improving the capacity to detect CRE/CCRE. Moreover, the NRL in CZ distributed control/reference strains of CRE/CCRE to all network laboratories.

In some countries, subsets of CLs are administered under different geographical regions or health sectors (primary/secondary care, private/public sector, ministry of health/public health/defence) and are therefore not necessarily part of the same networks as the NRLs. This creates particular challenges when building laboratory capacity across these administrative boundaries. Such barriers to network formation and collaboration were addressed in SI, RO and RS. In SI, the two national expert laboratories that provide reference functions, agreed to coordinate the genomic monitoring of CPE in healthcare settings across the country. In RO, an existing network of CLs in military hospitals, managed by the Public Health Institute, was established as a CL network of military hospitals for the NRL under the Ministry of Defence that agreed to collaborate, refer isolates and share data with the NRL. In RS, health services are delivered in geographical regions (territories) with local administrations, a network of CLs were selected in the local region that agreed to collaborate, refer isolates and share data with the NRLs.

3.3. Development of guidance

3.3.1. Mapping of phenotypic and genomic methods used

Key outcomes:

European-level and NRL-level gaps in capacity for genomic surveillance of the WS1 pathogens were identified and recommendations for improvement were provided, which guided the design of other activities in the EURGen-RefLabCap project.

An important objective of the EURGen-RefLabCap project was to support the NRLs in the modernisation of diagnostic and molecular typing methods using WGS for genomic surveillance and outbreak detection/investigation of the WS1 pathogens (CCRE), and at a later stage for WS2 pathogens (CRAb and CRPa).

In July 2021, a questionnaire was distributed to the 37 European NRLs that participated in the project for mapping capacity for phenotypic, molecular and genomic methods available at the NRLs for CCRE pathogens (Section 3.1.2). The questionnaire included sections focusing on capacity for phenotypic and genotypic bacterial species identification and AST and strain typing of CCRE, including description of the methods used at the different NRLs, their use of adequate QC materials and their current capacity for data management and sharing. The responses to those sections were used for the creation of a report titled '*Report on the gaps in WGS capacity and molecular testing equipment, software and analytical skills at national laboratory level*'. The report identified the different needs of the NRLs to improve WGS approaches or implement it in their settings and contained targeted recommendations for the NRLs from the PCs to improve their specific capacity. The findings were shared with the participants through a webinar in April 2022 (Annex 6.2) and the report was shared with all participants for email consultation and clarifications. The feedback from the participants was included in the document.

Overall, most NRLs in Europe had the capacity to perform either phenotypic or molecular species identification and AST for CCRE. Improvements should include, but were not limited to, the acquisition and use of adequate QC materials and the implementation of modern molecular methods for detection of AMR in settings which at the time only relied on phenotypic methods. Depending on specific local capacity, NRLs could consider the following capacity-building actions:

- The NRLs that had WGS-based analysis of CCRE already implemented in their settings should ensure the adequate performance of their WGS approach and bioinformatics analysis pipelines, for example by complying with harmonised WGS protocols prepared in the EURGen-RefLabCap project and by participating in EQA exercises
- The NRLs with access to WGS platforms but not yet using them for analysis of CCRE should receive the adequate training, for example support for adapting existing DNA extraction, library preparation protocols and bioinformatics pipelines towards CCRE. A broader range of training could be necessary for countries with less experience with sequencing approaches. This training could be promoted by the EURGen-RefLabCap project through the organising of different training activities
- The NRLs with no access to WGS platforms would benefit from all previous recommendations, however the first step would be securing access to WGS technologies, for example by drafting agreements with other departments that are using the machines, outsourcing the procedures, implementing partnerships with other institutions or purchasing WGS machines. This could be promoted by the creation of individual action plans at the NRLs.

Later, a review was conducted to summarise current EU and international guidance and bioinformatics development initiatives in the area of molecular detection and WGS-based surveillance methods applicable to CCRE. This document collected the work of local or global organisations that integrate WGS-based analysis in their programmes for disease surveillance and management, as well as initiatives regarding the development of new bioinformatics tools and networks for data analysis. Moreover, the review contained descriptions of the most used bioinformatics tools and databases relevant for the analysis of CCRE isolates, and additionally the findings of important benchmarking studies evaluating and/or comparing the performance of those approaches.

The review highlighted that there was a lack of uniformity between different institutions' approaches, since they had different goals and used varied WGS platforms, bioinformatics pipelines and data sharing strategies. The large number of available possibilities for WGS and bioinformatics approaches could potentially hinder comparison of results and integration of data obtained through diverse methods, and add to the challenge of harmonisation and standardisation of protocols. A summary of the recommendations from the review, aiming at the standardisation of WGS-based surveillance and achievement of a harmonised European solution was:

- Accounting for future priority pathogens and priority AMR profiles for surveillance, meaning that the suggested WGS approach should be applicable to CCRE, but not restricted exclusively to those pathogens
- Choosing well-defined AMR-genotypes for validation of the WGS-approach, specifically a subset of well-studied ARGs and point mutations, with proven correlation between their presence and resistance phenotypes
- Establishing the control parameters to be used, including data management QC parameters and including WGS QC parameters
- Establishing the thresholds for the QC parameters
- Defining a set of bioinformatics tools and databases as potential candidates to be included in the harmonised approach, that should be curated, open-access, benchmarked and available as online interfaces but also be downloadable for local use.

The conclusions from the questionnaire distributed to the NRLs and from the review of existing initiatives in WGS were used to guide the design of other activities in the EURGen-RefLabCap project such as the creation of harmonised WGS protocols (Section 3.3.2), creation of guidance documents for internal QC (Section 3.3.3), development of training workshops (Section 3.4.1), and preparation of simulated outbreak exercises (Section 3.4.2) and EQA exercises (Section 3.4.3).

3.3.2. Development of standard WGS protocols

Key outcomes:

The EURGen-RefLabCap team, the countries participating in the project and the ECDC have jointly agreed on protocols with guidance for WGS-based genome analysis methods for national surveillance and integrated outbreak investigations of CCRE, CRAb and CRPa. The WGS protocols provided a foundation for the implementation and quality improvement of WGS for AMR surveillance and outbreak detection purposes in all NRLs.

The EURGen-RefLabCap team drafted two protocols with guidance for common WGS-based genome analysis methods for national surveillance and integrated outbreak investigations of CCRE (in 2022) and of CRAb and CRPa (in 2023).

These documents provide a framework for performing WGS using short-read paired-end massive parallel synthesis sequencing on Illumina platforms (Illumina, Inc., San Diego, CA, USA), which are the most frequently used sequencing platforms in public health laboratory in Europe at the time of writing, and purposely avoid providing fixed step-by-step instructions since WGS is a multi-step procedure with several equally good options for performing each step. Moreover, there was wide variation in the implementation of WGS for surveillance and outbreak investigation of CCRE, CRAb and CRPa in the EURGen-RefLabCap network, ranging from countries that were completely new to WGS to countries that routinely used WGS and had established analytical workflows and pipelines.

The protocols provide guidance for obtaining high quality DNA, performing library preparation and sequencing of the DNA, performing bioinformatics analysis (taxonomic analysis, bacterial typing, detection of genetic determinants of AMR, cluster analysis) and applying best practices for data management. In addition, the protocols outline specific QC strategies, QC parameters and associated thresholds.

After consultation with ECDC, the WGS protocols were emailed to the EURGen-RefLabCap network to launch a two-week consultation period during which NRLs from all countries were encouraged to liaise with national experts, provide comments and suggest changes. Two countries (CZ and FI) provided feedback on the CCRE protocol and six countries (HR, FI, GE, HU, NO, ES) provided feedback on the CRAb and CRPa protocol.

The WGS protocols were also presented orally to the entire network via a webinar in December 2021 for the CCRE guidance and a presentation at the 2nd network meeting in June 2023 for the CRAb and CRPa guidance. The WGS protocols are available at the EURGen-RefLabCap website (Annex 6.3).

3.3.3. Support to implementation of internal quality control schemes

Key outcomes:

Two guidance documents for IQC focusing on Enterobacterales *and on CRAb/CRPa were produced and webinars were hosted presenting the most important considerations for IQC. The documents and presentations from the webinars were useful locally at the NRLs but also an important resource to be further distributed within the national networks of CLs.*

The deliverable '*Guidance document on internal quality control schemes for clinical and reference laboratory antimicrobial susceptibility testing and molecular detection of antimicrobial resistance*' focusing on Enterobacterales was created, reviewed by HaDEA and ECDC, and circulated between the participants of the EURGen-RefLabCap project for email consultation. A webinar for presentation of the guidance document and to promote the review by the project participants was hosted by DTU in November 2022 (Annex 6.2). After email consultation, the document was revised to prepare the third and final version. The document contains a section describing international standard operating procedures (SOPs) guiding IQC strategies, another section describing the laboratory methods to be followed when performing standard AST for Enterobacterales including direct links to relevant supporting documents such as those published by EUCAST and a section for quality control for molecular detection of resistance mechanisms.

The deliverable '*Guidance document on internal quality control schemes for clinical and reference laboratory antimicrobial susceptibility testing and molecular detection of antimicrobial resistance*' focusing on *Acinetobacter* spp. and *Pseudomonas* spp. was created, reviewed by HaDEA and ECDC, and circulated between the participants of the EURGen-RefLabCap project for email consultation. A webinar for presentation of the guidance document and to promote the review by the project participants was hosted by DTU in April 2023 (Annex 6.2). There were no further edits to the document after email

consultation. The document contains similar sections to the first guidance document but focusing on *Acinetobacter* spp. and *Pseudomonas* spp., and additionally contains appendices with practical examples of documentation to be used for IQC purposes.

The IQC guidance documents are available at the EURGen-RefLabCap website (Annex 6.3).

The documents can support the NRLs in the maintenance of proper quality assurance of their own procedures, and furthermore can be distributed within the national networks of CLs to promote the accuracy of results produced at national level.

3.4. Training activities

3.4.1. Laboratory training courses, workshops and webinars

Key outcomes:

The training activities organised by the EURGen-RefLabCap project covered a large spectrum of scientific knowledge and contributed to building capacity in Europe for WGS-based analysis of CCRE, CRAb and CRPa. The engagement in the activities was high and the feedback received from the participants was overall very positive. The activities increased the NRL's hands-on capacity and technical knowledge, and furthermore contributed to the strengthening of national networks of CLs.

In addition to the network meetings, workshops and webinars described in other sections, the EURGen-RefLabCap project organised six webinars (Annex 6.2) and four on-site workshops (Annex 6.1) with the specific goal of providing specialised training to the participants:

- **Webinar on demystifying genomics**, September 2022

  This webinar had the goal of encouraging discussions within the project network regarding the practical issues of implementation and routine use of WGS technologies and bioinformatics analyses in the NRLs. It included presentations about implementation of WGS at SSI, about the ResFinder and multi-locus sequence typing (MLST) bioinformatics tools, and two presentations with national examples of implementing WGS into the NRL workflows (LV and IE).

- **Technical training workshop on Illumina sequencing**, November and December 2022

  This workshop aimed at providing hands-on training WGS and bioinformatics for building capacity for national detection and surveillance of AMR. The workshop started with a virtual session focusing on the biochemical principles of WGS and on the structure and QC of WGS data. This was followed by two days of on-site training at DTU with laboratory training for library preparation for Illumina sequencing, and with several different exercises on the use of bioinformatics tools.

- **Webinar on how to plan an EQA**, January 2023

  This webinar aimed to provide an overview of what is required for conducting an EQA to support the NRLs in providing EQAs to their own networks for strengthening national capacity for diagnostics and surveillance. This included general considerations for planning an EQA, the planning and launch of activities, and documenting the activities for quality assurance purposes. The webinar included references to several relevant ISO-standards.

- **Webinar on bioinformatics approaches for plasmid analysis**, May 2023

  The objective of the webinar was to provide specific training for bioinformatics analysis of bacterial plasmids, that had not been the focus of previous training activities. It contained two presentations of bioinformatics approaches for closing and analysing plasmids. These were provided by project participants (CZ and ES).

- **Train-the-trainer workshop on pedagogical strategies**, June 2023

  This workshop aimed at the development of pedagogical and didactic capacities for the NRLs, in order to support them with communication and training within their own national networks. The workshop took place on-site at DTU as two half-day sessions. It included presentations regarding the practical organisation of on-site and virtual training courses, exercises and workshops.

- **Best practice workshop on Nanopore sequencing**, November and December 2023

  The objective of the workshop was to provide participants with knowledge and tools to generate and use WGS data for surveillance and characterisation of bacterial pathogens through the use of long-read sequencing technologies (ONT), with a course content broad enough to ensure that all participants, even with their heterogeneous expertise, gained new and relevant knowledge. This workshop started with a virtual session focusing on the biochemical principles of long-read WGS, comparison with other technologies, the applications of Nanopore sequencing, overview of the laboratory protocols and the structure and QC of WGS data. This was followed by two days of on-site training at DTU with laboratory training for DNA extraction and QC, library preparation for Nanopore sequencing and preparation of flowcells. Later on, the training continued as a virtual session with examples of QC methods and bioinformatics analyses.

  Before the workshop, the participants were invited to send bacterial isolates to be used as training material during the workshop. In total, 57 samples were received (51 live bacterial samples and six DNA samples) and these were successfully sequenced during the workshop. The participants received emails providing access to password-protected folders in a secure site (www.sciencedata.dk) where they could access the sequence data of their country. Afterwards, six additional samples were received to be sequenced and added to this genome collection, yielding a total of 63 European samples sequenced in the context of the EURGen-RefLabCap project. These were provided by nine laboratories in eight countries (BG, HR, LV, LT, MD, RS, SI (two laboratories) and ES). It is expected that these genomes will be analysed together in the future and the results and conclusions can be published as a scientific publication, since permission was recovered from almost all participants to use the sequence data.

- **Webinars on data sharing and management**, April 2024

  This activity combined two webinars and had the objective of promoting data sharing at European level and encouraging good data management practices to ensure the usefulness of WGS data. It included two presentations. The first presentation was provided by ECDC about ‘Data sharing for European Surveillance’. It focused on the overall status of the implementation of the molecular surveillance roadmap for the EU/EEA countries, as well as the importance and the privacy-related aspects of data sharing. The second presentation was provided by USDA and SFU, Canada about ‘ISO and how to deal with data management of metadata’. It showed an overview of the metadata component of ISO 23418 ‘Whole genome sequencing for typing and genomic characterisation of bacteria - General requirements and guidance’ and included discussion on the mechanisms for implementing the standard for data management, as well real-world implementations as practical examples of how to operationalise the metadata specification in different settings.

- **Webinar on EU opportunities for funding and procedures**, May 2024

  This webinar aimed at presenting further opportunities for funding that could be of interest to the countries. It focused on 'The new EURL and the expected funding and activities under the service contract, and other EU opportunities for funding and procedures'. It was provided by the EC.

- **Webinar on** International Pathogen Surveillance Network **(IPSN)**, June 2024

  This webinar was provided by WHO. It focused on The International Pathogen Surveillance Network (IPSN), a WHO-hosted network bringing together the pathogen genomics community to improve equitable access to genomic surveillance in public health. It covered the IPSN's vision and mission, its structure and membership, the operational bodies, and current areas of work.

- **Technical training workshop Data for Action**, September 2024

  The objective of this workshop was to continue building capacity for genomic epidemiology with a focus on digital infrastructure and communication of WGS data for national and international surveillance and outbreak detection. This one-day workshop took place on-site at DTU. The national WGS pilot projects of the PCs were used as 'cases' in break-out group discussions. It also included presentations by ECDC with strategies for selection of bacterial isolates for sequencing, demonstrating the use of EpiPulse and presenting the Virtual Academy.

- **Webinar series on setting up an AMR surveillance program using WGS**, October/ November 2024

  The EURGen-RefLabCap participants were invited to a four-workshop series on the basics of One Health whole genomic surveillance of AMR. The series, which featured experts from a wide variety of preeminent global institutions such as the US CDC, ECDC, EFSA, FAO, and the US FDA shared the building blocks of setting up an AMR surveillance program using WGS from "how to decide what to sequence" to "using your phenotypic analysis to decide what to sequence" to "what metadata do you need to make your AMR WGS valuable" and finally "how to communicate your sequencing results".

The **engagement in the specialised training activities** was overall high and most of the countries that were invited for on-site training or the webinars participated in them. Specifically, 27 countries participated in the technical training workshop focusing on Illumina sequencing, 13 countries participated in the train-the-trainer workshop focusing on pedagogical strategies (which only targeted PCs and ACs), 17 countries participated in the best-practice workshop on Nanopore sequencing (which only targeted PCs and ACs), and 27 countries participated in the technical training workshop focusing on translating data into action.

Participants had the opportunity to provide **feedback** to most of these activities through feedback surveys. These surveys contained sections for evaluating the relevance of the activity and the knowledge gained during the activity, for rating the individual sessions, and free-text fields for further comments. The feedback for each training activity was very positive (Table 7).

Table 7: Average ratings (scores) assigned to each training activity by the participants

Support activities	No. of replies	Relevance of the activity	Knowledge gained	Individual sessions
Webinar on demystifying genomics	7	8 / 10	7 / 10	8 / 10
Training workshop on Illumina sequencing	29	9 / 10	9 / 10	9 / 10
Webinar on how to plan an EQA	5	9 / 10	10 / 10	NA
Webinar on plasmid analysis *	25	4 / 5	NA	NA
Train-the-trainer workshop	21	9 / 10	9 / 10	9 / 10
Training workshop on Nanopore sequencing	13	9 / 10	9 / 10	9 / 10
Training workshop Data for Action	14	4.5 / 5	4.6 / 5	4.2 / 5

NA: Not applicable

**This webinar was only rated through the second electronic questionnaire used to prepare the 'short evaluation reports' (Section 4.1).*

Some of the comments received in the feedback surveys highlight how useful these activities were for the participants:

- *"The seminars you create are very useful, informative and easy to understand. Thanks for the experience you share!"*
- *"Great organization, eager to go back and discuss additional aspects of WGS and antibiotic resistance."*
- *"Very well organized, great experience!"*
- *"So far the training and educational activities have been excellent and very helpful and useful."*
- *"Group work was very interesting and useful."*
- *"So far everything's has been very useful for us and I hope you will continue with the webinars."*
- *"Congratulation for excellent organisation. I would like to praise that there was a lot of time for discussion and exchange of experiences."*
- *"Very grateful for the possibility to participate in the RefLabCap activities. Many thanks to the EURGen-RefLabCap team!"*

During the structured interviews the NRLs (BA, BG, HR, CY, CZ, EE, EL, LV, LT, RO, RS, SI) continued to highlight the importance of the technical training workshops, explaining that these contributed to the implementation, development or modernisation of their WGS protocols and strategies for bioinformatics analysis. Some NRLs expanded on that discussion by describing concrete actions that were taken as a direct effect of the workshops:

- Acquisition of ISO standards after hearing about those during training (LV)
- Implementation of ONT sequencing at the NRL (CZ, EL, LV)
- Implementation of optimized laboratory protocols at the NRL (RS)
- Scheduling a meeting with the national network of CLs to discuss ECDC protocols (LV)

Overall, the specialised training activities contributed to bridge the gaps that were identified in the early stages of the project. These activities helped the NRLs to acquire specific

knowledge with hands-on applicability, increasing their capacity for sequencing and analysis of sequence data for characterisation and surveillance of the pathogens targeted in the project. Furthermore, they contributed to the strengthening of national networks of CLs.

3.4.2. Simulated outbreak exercises

Key outcomes:

The simulated exercises developed capacity in the NRLs to use WGS to detect and investigate outbreaks by performing typing and species-specific characterisation of resistance and virulence factors and by evaluating the presence of plasmids. The hands-on use of new bioinformatics webtools for outbreak investigations was valuable especially for the participants with little or no experience with WGS data analysis. The exercise material and webtools provided a valuable resource for conducting similar exercises at country level or testing local or national bioinformatics pipelines and facilitated collaboration between different departments and laboratories.

The simulated exercises in the EURGen-RefLabCap project were designed as virtual multidisciplinary workshops aimed at microbiology and healthcare epidemiology experts from the NRLs. The objective was to provide a foundation for performing various steps of outbreak investigations with a focus on the use of WGS-based methods for bacterial typing and cluster analyses, as well as active involvement of epidemiological data for selection of isolates for sequencing. Five multidisciplinary workshops were planned and 19-28 countries participated (Table 8, Annex 6.1). Each workshop focused on an established or emerging problematic clone belonging to the WS1 (CCRE) or WS2 (CRAb or CRPa) pathogens. The simulated exercises were conducted as functional exercises for investigating outbreak scenarios and were designed to be as similar to actual events as possible. The epidemiological case data were released over time in the form of injects of sequencing data (both raw and assembled sequencing data) and fictitious patient metadata to simulate a possible outbreak situation. The sequence data were obtained from public repositories and were mainly generated by Illumina (short-read sequencing data) but also included ONT data (long-read sequencing data).

The first simulated exercise was conducted in September/October 2022 and was focused on detailed introduction of bioinformatics tools for phylogenetic analyses and genomic characterisation of AMR in bacteria. A demonstration of these analyses was performed using publicly available genomic data for *E. coli*. This workshop laid the foundation for investigating simulated outbreak scenarios in upcoming simulated exercise.

The second simulated exercise was conducted in May 2023. The participants investigated a travel related hospital outbreak caused by hypervirulent *K. pneumoniae* harbouring bla_{NDM-1} with suspected patient-to-patient transmission. The exercise focused on both genomic and epidemiological analyses and was based on an extensive genomic dataset and related metadata. The participants learned various methods such as detection of AMR and hypervirulence genes and used this information for outbreak detection.

The third simulated exercise was held in September 2023. The participants investigated a hospital outbreak caused by *P. aeruginosa* harbouring bla_{KPC-2}. The exercise was based on a fictitious scenario of a hospital outbreak with patient-to-patient transmission and a potential environmental source, with a focus on the role of plasmids in the transmission of AMR. The WGS data and associated metadata of samples from the ICU, several wards and environments in a hospital were used for the investigation.

The fourth simulated exercise was conducted in January 2024. The participants worked on solving several travel related outbreaks and local outbreaks caused by bla_{OXA-23} producing

A. baumannii with both inter-hospital and intra-hospital transmission events. The participants were asked to first investigate the retrospective patient and surveillance data from six local hospitals obtained over a year. As a result, the participants learnt about the steps required before the WGS analyses, including how to select the isolates for WGS using the surveillance and patient metadata.

The fifth simulated exercise was planned in November 2024. This was the last simulated exercise in the project and involved the investigation of a multispecies outbreak caused by CCRE. The participants analysed the prevalence of different CCRE in a hospital. Moreover, participants investigated if there is evidence of plasmid-mediated transfer of carbapenemase between species. By doing this analysis, participants learnt how to detect plasmid-borne genes and use this information for investigating plasmid outbreaks.

Table 8: Participation of countries in virtual multidisciplinary workshops on outbreak investigation using simulated exercises

Simulated exercise	Date	Scenario	No. of participating countries
1st (WS1)	Sep 2022	Introduction to the bioinformatics tools for analyses of WGS data that simulate an outbreak caused by *bla*$_{OXA-181}$ and *bla*$_{NDM-5}$-carrying *E. coli*.	26
2nd (WS1)	May 2023	Investigation of a travel-related hospital outbreak caused by *bla*$_{NDM-1}$-carrying *K. pneumoniae* belonging to ST147.	28
3rd (WS2)	Sep 2023	Investigation of an outbreak caused by multiple clones of *bla*$_{KPC-2}$-carrying *P. aeruginosa* with a possible spread of plasmids.	19
4th (WS2)	Jan 2024	Investigation of a multi-hospital outbreak caused by *bla*$_{OXA-23}$-carrying *A. baumannii*.	21
5th (WS1)	Nov 2024	Investigation of a multi-species outbreak (CCRE) involving spread of plasmids carrying *bla*$_{OXA-232}$, *bla*$_{NDM-1}$ or *bla*$_{NDM-5}$.	20

The high engagement in the simulated exercises was accompanied by very positive feedback from the participants. Participants had the opportunity to complete feedback surveys which contained questions about the overall relevance of the analyses included in the exercise and regarding knowledge gained during the exercise. The feedback for each simulated exercise was very positive and many laboratories found that the analyses included in the exercises were relevant and have provided new knowledge regarding outbreak investigation using WGS (Table 9).

Table 9: Average ratings (scores) assigned to each simulated exercise by the participants

Simulated exercise	No. of replies	Overall relevance	Knowledge gained
1st (WS1)	10	9.3 / 10	8.2 / 10
2nd (WS1)	17	9 / 10	9 / 10
3rd (WS2)	13	9.8 / 10	9.4 / 10
4th (WS2)	13	9.2 / 10	8.5 / 10
5th (WS1)	16	9.6 / 10	8.6 / 10

Most of the comments received in feedback survey were acknowledgements for the usefulness of the exercises. Specifically, participants with little or no experience in bioinformatics highly valued the demonstration and training of new webtools and WGS analyses . Some participants found these exercises as a good medium to start and establish

a collaboration between different departments such as the microbiology and epidemiology departments. Some of the comments received in feedback surveys were suggestions regarding the future exercises and these were taken into account while defining the objectives of the upcoming exercises.

The simulated exercises had a concrete positive impact for the participating laboratories. During the structured interviews all the participating NRLs (BA, BG, HR, CZ, EE, EL, LV, LT, RO, RS, SI) highlighted that the simulated exercises were very useful to improve the knowledge regarding strategies for cluster analysis and interpretation of those results and that they contributed to the optimisation of local methods for WGS and bioinformatics analysis. The NRLs also explained that the exercises demonstrated the practical applications of WGS for real-time surveillance or investigation of the priority pathogens. NRLs moreover mentioned that the simulated exercises contributed to establishing or strengthening the collaboration with the epidemiology departments of the institutions.

Overall, the simulated exercises in EURGen-RefLabCap provided training in WGS analyses for outbreak investigations using publicly available software including those with web interfaces. The use of those interfaces in the exercises was useful especially for participants with little to no experience in bioinformatics. Some of the participants which were not familiar with these tools and methods are now able to use them in their settings without the need of a sophisticated bioinformatics infrastructure. Moreover, these exercises are a valuable resource as a guidance for conducting similar exercises at the country and regional level. The materials used in these exercises are available on project website (Annex 6.1).

3.4.3. External quality assessment exercises

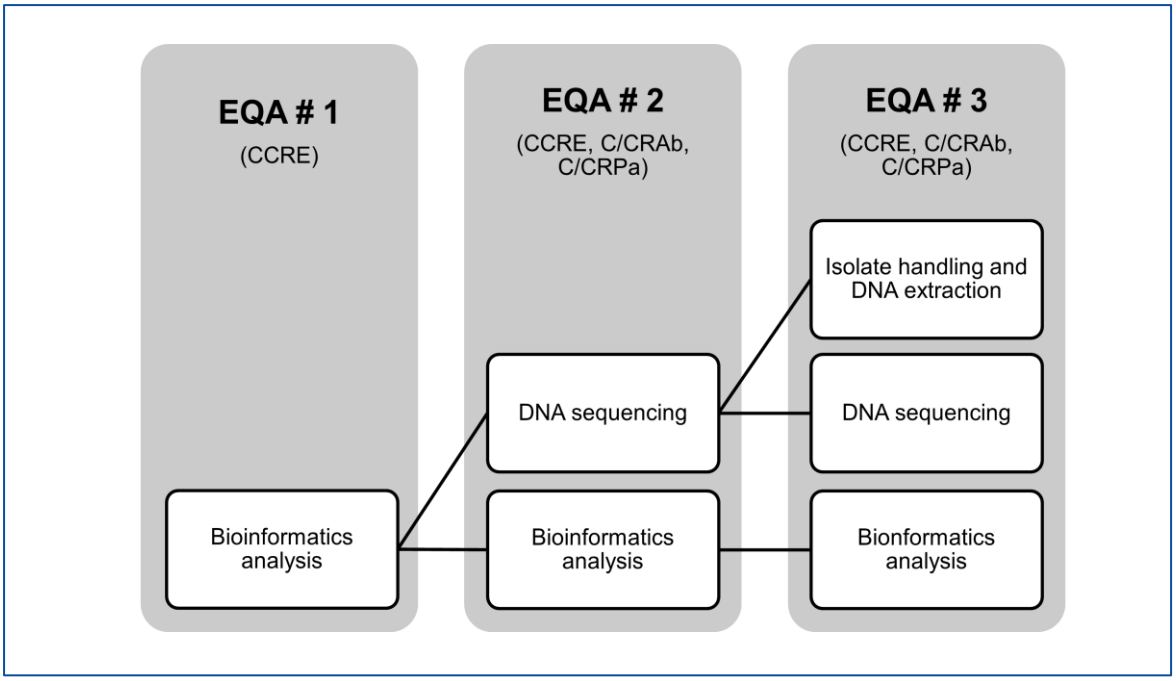
Key outcomes:

The EQAs provided an overview of WGS-based approaches implemented by NRLs in Europe for the surveillance of important healthcare-associated pathogens and identified technical gaps in the implementation of WGS for surveillance in healthcare settings in Europe.

The aim of the EURGen-RefLabCap EQAs was to compare the bioinformatics approaches used by the NRLs in Europe for analysis and surveillance of important healthcare-associated pathogens. The benchmarking of these approaches is important to identify potential problems or variation between the applied WGS and bioinformatics pipelines. Moreover, the EQAs help in identifying local, national, and European opportunities for quality improvement and harmonisation of analysis of WGS data. The EURGen-RefLabCap EQAs were not aimed at assessing the NRLs' capacity or ability to accurately perform confirmatory testing, typing, or surveillance procedures.

Three EQAs (October 2022, May 2023 and May 2024) were conducted in order of increasing challenge (Figure 3). The first EQA focused on CCRE. In this EQA, the NRLs received WGS data to evaluate their current bioinformatics approaches. The second EQA focused on CCRE, CRPa, and CRAb. In this EQA, laboratories received pre-isolated DNA to assess the quality of the WGS data produced by the NRLs, and to benchmark the approaches used for the WGS analysis. The third EQA focused on CCRE, CRPa, and CRAb. In addition to the pre-isolated DNA, the NRLs also received live bacterial cultures to evaluate the procedures for handling of live cultures and DNA extraction, and to assess local capacity for those initial steps of the sequencing process.

Figure 3: Overview of the pathogens and analyses included in three EQAs conducted in the EURGen-RefLabCap



CCRE: Carbapenem- and/or Colistin-Resistant Enterobacterales. C/CRAb: Carbapenem- and/or colistin-resistant *Acinetobacter baumannii.* C/CRPa: carbapenem- and/or colistin-resistant *Pseudomonas aeruginosa.*

The bioinformatics analyses assessed in all three EQAs were: prediction of sequence type (ST) based on MLST, detection of plasmid replicon types, detection of genes and chromosomal point mutations (PMs) mediating AMR, and *in silico* prediction of the AMR profiles. In the second and third EQA, the evaluation of the quality of the raw sequencing data generated by the participants was also performed.

The engagement in the EURGen-RefLabCap EQAs was high and increased throughout the years. In the first EQA, 27 NRLs participated, while in the second and third EQA, 30 and 31 NRLs participated, respectively (Annex 6.1).

The reports containing aggregated results from all the participants were produced for each EQA and published following consultation with HaDEA, ECDC and the EQA participants (Annex 6.3). The results of each EQA were also presented in webinars or during on-site meetings, in June 2023, November 2023 and September 2024 (Annex 6.2).

The results of the EQAs showed that the methods used by most NRLs for WGS, and bioinformatics analyses achieved satisfactory results (Table 10). However, issues were identified in the WGS analyses and the quality of WGS data produced by some NRLs (Table 11). These issues were communicated to those NRLs individually, including recommendations for improvement. Furthermore, other issues were identified, which should not be interpreted as a lack of knowledge and bioinformatics capacity by the NRLs. For example, omission of important genetic AMR determinants (such as point mutations) was mainly caused by lack of consensus between different databases and was also an important problem detected in the EQAs. The majority of NRLs accurately predicted MLST using WGS. The issues identified in MLST prediction were mainly due to the participants using a different MLST scheme for a particular species rather than the MLST scheme in the EQA protocol.

For the detection of genes and mutations conferring AMR, most of the NRLs correctly identified the expected genetic determinants for each species. Similarly, most of the NRLs correctly predicted the AMR profiles using WGS data. However, many NRLs also reported genetic AMR determinants which confer resistance to antimicrobials that are not relevant

for the species. Moreover, many NRLs did not report important antimicrobials even though the acquired genetic mechanism of resistance was detected. These issues appeared to be associated with the insufficient knowledge about certain mechanisms of resistance in the species included in the EQAs. Another explanation for the discrepancies in reporting could be misinterpretation of the EQA protocols.

The majority of the NRLs accurately characterised the plasmids (i.e. detected replicon genes) from WGS data. A common issue identified in the plasmid identification from WGS data was the lack of specific and comprehensive replicon database for *A. baumannii* and *P. aeruginosa*.

Additionally, discrepancies were identified in commonly used databases for the detection of genetic AMR determinants, plasmid replicons and *in silico* prediction of AMR profiles. Many of these discrepancies were due to the lack of databases targeting *A. baumannii* and *P. aeruginosa*. For example, there is no database for mutations conferring AMR in *A. baumannii* and *P. aeruginosa* in ResFinder, which is the most commonly used tool.

Detailed descriptions of problematic issues and recommendations are presented in the EQA reports.

Table 10: Average scores obtained by the NRLs for the bioinformatics analyses in the EURGen-RefLabCap EQAs

	1st EQA	2nd EQA	3rd EQA
Pathogens included	***E. coli*, *K. pneumoniae***	***E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa***	***E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa***
EQA component	**Average of scores for all participants (%)**		
Prediction of MLST	96.4	90	95.5
Detection of plasmid replicons	74	87.8	81.3
Detection of genetic AMR determinants (genes and mutations)	83.3	86	79.4
In silico prediction of AMR profile	14.8	87.3	85.2
Total	**77.6**	**86.8**	**81.7**

Note: the average scores cannot be compared across EQAs due to that fact that materials are different in each EQA.

In the 2nd and 3rd EQAs, the laboratories were also evaluated and scored based on the quality of the sequencing data that they generated in their laboratories using the provided EQA material. This evaluation was performed using a complex scoring scheme including 10 QC parameters, originally developed for the EU Reference Laboratory for AMR (EURL-AR) genomic proficiency tests and further adjusted for the EURGen-RefLabCap EQAs. The scoring system is fully described in the reports from the EQAs. Most of the sequence data

that NRLs generated using their routine methods showed sufficient quality for implementing the WGS-based analyses in their settings (Table 11). Further explanations of the problems observed during the QC of the sequences were provided individually to the NRLs to help improve the quality of their results.

Table 11: Average scores obtained for the quality of WGS data generated by all NRLs in the EURGen-RefLabCap EQAs

EQA material	Average of scores for all participants (%)		
	1st EQA	2nd EQA	3rd EQA
Sequence data from live cultures	NA	NA	91.8
Sequence data from pre-isolated DNA	NA	93.1	92.7
Total	**NA**	**93.1**	**92.3**

NA: Not applicable.

In general, the EURGen-RefLabCap EQAs emphasised that there is a need for the NRLs to enhance their understanding of mechanisms of AMR. There is also a need for the NRLs to be more familiar with recommended bioinformatics tools and contents of the databases used in the analyses. There were other issues identified with the tools and databases commonly used by the NRLs (such as missing important genetic AMR determinants for *A. baumannii* and *P. aeruginosa*). Furthermore, there is a need for harmonisation regarding the analysis of WGS data for public health purposes in Europe for improved cross-border surveillance. The EQA exercises raised awareness of the importance of implementing common approaches to WGS data analysis, not only at country level but also at global level. Therefore, emphasis should be placed on achieving international harmonisation of bioinformatics approaches.

Participants had the opportunity to provide feedback to the EQAs through feedback surveys. The surveys contained questions about the usefulness of the EQA and regarding corrective actions implemented as a result of the individual EQA evaluation reports received by the laboratories. The feedback for each EQA was very positive and many laboratories used the output to implement corrective actions (Table 12).

Table 12: Average ratings (scores) assigned to each EQA by the participants

EQA	No. of replies	Usefulness of the EQA	No. (%) of participants that implemented corrective actions
1st EQA	17	9 / 10	10 (59%)
2nd EQA	3	8 / 10	2 (67%)
3rd EQA	12	9 / 10	6 (50%)

Most of the comments received in the feedback surveys were suggestions regarding the design of the EQAs, comments about the the protocols or webtool for submission of results, comments with scientific viewpoints, and suggestions regarding the information available in the evaluation reports. The comments were used to improve subsequent EQAs.

The EQAs had a beneficial impact for the participating laboratories. During the structured interviews all the participating NRLs (BA, BG, HR, CZ, EE, EL, LV, LT, RO, RS, SI)

highlighted that the EQAs were instructive and helpful for the development of new bioinformatics pipelines and for the quality assurance and subsequent adjustment of already existing WGS and bioinformatics approaches. NRLs moreover mentioned that the EQAs contributed to improved their critical analysis of WGS-based results. The NRLs in four countries (SI, HR, EL and RO) specifically mentioned that the participation in the EQAs was one of the most important achievements related to the implementation of their project action plan, and one significant concern was how to continue to participate in genomic EQAs after the EURGen-RefLabCap project was finished.

4. Evaluation

4.1. Impact of activities on the capacity for national reference laboratories core functions in priority countries

The EURGen-RefLabCap team identified the following indicators to evaluate the improvements in capacity for NRL core functions in PCs during the EURGen-RefLabCap project:

Indicators for NRL Core Function 1 (Reference diagnostics)

1.1 Availability of Illumina sequencing of AMR bacteria at the NRL
1.2 Availability of ONT sequencing of AMR bacteria at the NRL
1.3 Capacity for bioinformatics analyses of Illumina sequence data of bacteria at the NRL
1.4 Capacity for bioinformatics analyses of ONT sequence data of bacteria at the NRL
1.5 Participation in any genomic EQAs for bacteria

Indicators for NRL Core Function 3 (Scientific advice)

3.1 Provision of technical support and scientific advice to CLs and hospitals on the interpretation and relevance of laboratory findings for AMR surveillance and outbreak detection
3.2 Provision of technical support and scientific advice to public health authorities on findings related to AMR surveillance and outbreak detection

Indicators for NRL Core Function 5 (Monitoring, alert and response)

5.1 Availability of local digital infrastructure to store WGS data
5.2 Availability of national digital system with information on test results for AMR surveillance
5.3 Availability of WGS-based national or regional surveillance system for outbreak detection
5.4 Use of EpiPulse to share and discuss findings on CCRE and/or CRAb/CRPa and contribution of WGS data for ECDC Rapid Risk Assessments

These indicators were based on the criteria used for the selection of PCs (Section 3.1.4). For some indicators, NRLs were already engaged in activities before the start of the project and participation in the EURGen-RefLabCap provided further support and motivation. For other indicators, participation in EURGen-RefLabCap triggered the initiation of activities. Conversely, there were also some indicators for which NRLs could not allocate resources during the project (Table 14).

The main progresses were observed for indicators in the ‘Reference diagnostics’ NRL core function, with nearly all PCs having developed capacity for WGS and bioinformatics analysis by the end of the project. Modernisation of diagnostic and molecular typing tests using WGS was a main task in this project, and the outcome achieved show that a four-year framework and an approach adapted to the context of each NRL were appropriate to lead to implementation of new practices. Progresses for indicators of ‘Monitoring, alert and response’ NRL core function were also observed at the end of the project, although additional work is needed in this area. This was somewhat expected since the indicators for progress in this NRL core function, which were mainly about digital infrastructure and

systems for sharing and storage of WGS and health data, require collaboration across many national stakeholders besides the NRL, and over a long period of time.

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Table 13: Status of capacity in PCs for selected indicators of NRL core functions at the beginning (based on results of mapping survey, see section 3.1.2) and at the end (based on self-evaluation by each NRL) of the EURGen-RefLabCap project. Only PCs that have been active throughout the project are shown.

NRL core functions	Indicators	Beginning of EURGen-RefLabCap (2021)												End of EURGen-RefLabCap (2024)											
		Country												Country											
		BA	BG	HR	CY	CZ	EE	EL	LV	LT	RO	RS	SI	BA	BG	HR	CY	CZ	EE	EL	LV	LT	RO	RS	SI
Reference diagnostics	1.1 – WGS (Illumina)																								
	1.2 – WGS (ONT)																								
	1.3 – Bioinformatics (Illumina)																								
	1.4 – Bioinformatics (ONT)																								
	1.5 – Genomic EQAs																								
Scientific advice	3.1 – Advice to CLs and hospitals																								
	3.2 – Advice to public health authorities																								
Monitoring, alert and response	5.1 – Infrastructure for WGS data storage																								
	5.2 – National integrated digital system																								
	5.3 – WGS-based surveillance																								
	5.4 – EpiPulse and ECDC Rapid Risk Assessments																								

For all indicators except 1.5: green, implemented; yellow: in development; no colour, not in focus at the given time. For indicator 1.5: green, participation in full EQA (i.e. from live bacterial cultures to WGS data analysis); yellow, participation in WGS data analysis only.

4.2. Participant feedback on the utility of the support activities

Halfway through the project, the EURGen-RefLabCap team collected feedback from the participating countries to assess the networking and technical support activities delivered in the first two years of the project. In November 2022, the countries were asked to evaluate the utility and impact of the support activities provided to all 37 countries. An electronic questionnaire was used to collect feedback (ratings) on key support activities (network meetings and workshops, capacity and mapping surveys, protocols and guidance documents, training events and targeted activities for PCs). The results of the feedback survey were summarised in the 'Short evaluation report with lessons learned and areas for further work' (January 2023).

The levels of participation, ratings and free text comments indicated that participants were engaged in the **network meetings** and found them valuable. The on-site events were rated slightly higher than the virtual events. In retrospect, the EURGen-RefLabCap team believes that the face-to-face events in 2022 laid the foundation for an efficient dialogue in the later virtual activities through the establishment of a professional relationship.

Completing and organising **the NRL capacity and CLs mapping surveys** placed a considerable workload on the NRLs. Despite this, the feedback was positive, as the surveys had helped them to better understand the situation in their countries, and had helped the NRLs in PCs to develop action plans for capacity building and facilitated a more active scientific advisory role of the NRL towards their national networks of CLs.

The high ratings of the protocol for WGS-based national CCRE surveillance and outbreak investigation highlighted that this type of step-by-step guidance is very important and valuable to the NRLs. In particular, the PCs expressed a great need for this type of protocols to support the implementation of WGS at their NRLs.

The utility of the **NRL action plans** was rated highly in most PCs. However, the capacity and ability to complete planned activities and implement steps in WGS-based surveillance varied a lot between the NRLs. Challenges were associated with lack of resources and staffing and lack of previous experience using an 'action plan approach'. Despite these challenges, all PCs participated in the joint and bespoke capacity building activities and dedicated considerable amounts of time and resources to the project.

The first **EQA** (on bioinformatics analysis of sequence data) was rated highly or extremely useful by the majority of the participating NRLs. For many participants this was their first exposure to performing bioinformatics analysis and there was clearly a need for more EQAs in this area.

The **simulated outbreak exercises** were also highly rated by the participants especially in PCs and ACs. Overall, the participants showed great interest and need for the simulated exercises and the EURGen-RefLabCap team received positive feedback.

At midterm of the project, it was concluded that the format and number of activities should not be changed in the second half of the project. Especially, a new round of country visits should be given a high priority, as the previous visits were highly valued. Technical support and training in WGS and bioinformatics analysis and additional EQAs were also priorities for the remainder of the project.

A second feedback survey was conducted in March 2024 to evaluate the most recent support activities. The results of the feedback survey were summarised in the 'Short evaluation report with lessons learned and areas for further work' (August 2024).

From this second evaluation, a clear picture of appreciation from the PCs and ACs and a continued need for support activities in these countries emerged through the ratings. The PCs rated very highly the EQAs on DNA sequencing and bioinformatics analysis, the simulated outbreak exercises and conducting surveys of their networks of CLs (Table 13).

It is evident that the PCs and ACs greatly benefitted from all types of support activities and that they had continuing needs for further support activities, more than the other countries. In some instances, the ACs rated individual support activities higher than the PCs, possibly reflecting a higher degree of readiness to benefit from certain support activities than the PCs. For example, specialised bioinformatics training might prove more useful to countries that already have access to the technology instead of those who are not familiar with its biochemical background. The relatively small difference between the average ratings of the PCs/ACs compared to the remaining countries suggests that all respondents valued the support activities regardless of country designation.

The opinion of the EURGen-RefLabCap team is that it has been very beneficial to offer some of the support activities to all countries, while offering different, more targeted activities and bespoke support to only the PCs and ACs. However, the participation of the countries with higher capacities for all NRL functions has certainly also been valuable and has contributed to improving the capacity in all countries, as the more experienced participants generously shared their knowledge, expertise and experience with the entire network.

The project team further noticed that, in the second evaluation, the ratings of activities were not influenced by the format of delivery. For example, the virtual simulated outbreak investigation exercises, EQAs and the on-site technical training workshops were rated equally high. This is interpreted as positive feedback on the overall delivery of the training plans and content developed at the outset of EURGen-RefLabCap project.

Table 14: Comparison of average ratings (scores) in each group of countries (second evaluation)

Support activities	PCs	ACs	Other countries*
Network meeting 2023: NRL roles in outbreak investigations	4.6	4.4	3.9
Network meeting 2023: Cross-border spread of CRE	4.3	4.6	3.8
Network meeting 2023: Opportunity to meet face-to-face	4.9	4.6	4.4
Virtual simulated exercise 1 - May 2023: Instructions	4.6	4.5	4.1
Virtual simulated exercise 1 - May 2023: Learning	4.6	4.7	4.2
Virtual simulated exercise 2 - September 2023: Instructions	4.5	4.8	4.1
Virtual simulated exercise 2 - September 2023: Learning	4.5	4.5	4.2
Virtual simulated exercise 3 - January 2024: Instructions	4.6	4.8	4.1
Virtual simulated exercise 3 - January 2024: Learning	4.6	4.8	4.2
Availability of recordings after exercises	4.4	4.5	4.2
Standard WGS protocol CRPa and CRAb	4.4	4.8	4.6
EQA June 2023: Prenotification	4.6	4.4	4.0
EQA June 2023: Protocol	4.6	4.8	4.2
EQA June 2023: Webtool for results	4.3	4.2	3.9
EQA June 2023: Individual participants' reports	5.0	4.6	4.3
EQA June 2023: Learning	4.9	4.4	4.4
Survey on molecular/genomic methods for CRAb: Learning	4.0	4.0	3.5
Survey on molecular/genomic methods for CRPa: Learning	4.1	4.7	3.4
Survey on molecular/genomic methods: Report	4.1	4.7	3.9
Conducting survey of own networks of CLs	4.7	4.4	3.4
Overview report of country specific surveys	4.2	4.2	3.6
Webinar January 2023: How to plan an EQA	3.7	4.5	3.3

Webinar April 2023: Guidance on IQC	4.3	4.3	3.6
Webinar May 2023: Plasmid analysis	4.0	4.2	3.9
Average rating	**4.4**	**4.5**	**4.0**

**Other countries, countries without any specific designation for this project. Scale (1-5): 1, not useful; 2, somewhat useful; 3, useful; 4, very useful; 5, extremely useful.*

In addition to the feedback collected in the two feedback surveys, the participants of the third network meeting engaged in group discussions on improvement of a) the capacity mapping surveys, b) the simulated outbreak exercises and c) the WGS protocols.

At this last network meeting there was opportunity to discuss ideas for improvements to the presentation, distribution and use of the mapping survey results. These included:

- improved illustrations of the survey results
- distribution of brief summary tables and/or oral summaries by video
- interactive on-line data charts with filtering and extraction functions for multiple uses (e.g. benchmarking functions and presentations to local stakeholders)
- problem areas identified in the surveys should be highlighted and solutions proposed
- subsets of results could be communicated on websites, scientific publications, newsletters and national reports after consent by all respondents.

While it was agreed that the survey results should always be shared with all survey participants, there was no consensus on sharing the full datasets or reports in the public domain. It was acknowledged that using the survey results for benchmarking of capacities could be useful, but it was a major concern that the NRLs and national health systems were not comparable across all countries. However, if specific technical areas of the surveys were selected for publication, publicising data in the selected areas could be agreed with the countries that had more restrictive policies on sharing of information.

During the discussions about the simulated outbreak exercises, some important points were agreed. According to the participants, the simulated outbreak exercises:

- described and demonstrated the use of new webtools and methods that are easily implemented at local laboratories without the need of a sophisticated computer infrastructure
- encouraged a stronger collaboration between different departments and laboratories and provided a valuable resource for conducting similar exercises at country level
- were well-structured exercises, very useful and well fitted for public health needs. The scientific information provided in webinars on AMR in the priority pathogens was excellent, but maybe the burden of work could be reduced.

The participants further suggested that future simulated outbreak exercises should address the following:

- how to investigate plasmid outbreaks
- how to choose a reference genome for phylogenetic analysis
- how to work with epidemiological data in outbreak investigations to better understand WGS data
- how to use visualise epidemiological and sequencing data analysis.

The findings from the group discussions on the WGS protocols provided interesting insights into the different ways in which the countries used these documents. Some countries with established WGS capacity used them for comparison with their own procedures, which

helped to build confidence in the accuracy of their results. Some other countries, that were new to WGS, used the protocols to build their own workflows and pipelines.

Suggestions for improving the WGS protocols included adding information on plasmid analysis and having a central institute develop a pipeline and provide training in its use, if full harmonisation is to be achieved. Harmonisation of data analysis methods and bioinformatics tools was considered difficult by some participants, but there was consensus on the need to harmonise QC parameters (e.g. read depth), typing schemes, AMR databases, metadata and terminology in order to compare results.

Some participants suggested that the WGS protocols could be accompanied by supporting documents explaining the interpretation of results from different AMR databases and suggestions for presenting results to clinicians.

Finally, the participants agreed that the WGS protocols should be regularly reviewed within the network and updated at least annually.

4.3. Major outcomes and lessons learned

Increased awareness of best practice, hands-on skills and technical knowledge as result of training activities, guidance on WGS, EQAs and simulated outbreak exercises

The participation in training activities for all participants and the additional training activities for PCs/ACs only was consistently very high throughout the project period and the participant feedback very positive on all activities. Altogether, the training activities increased the NRL's hands-on skills and technical knowledge and thereby enhanced the capability for best practice in the NRLs throughout Europe. The NRLs in BA, BG, HR, CY, CZ, EE, EL, LV, LT, RO, RS and SI highlighted that the technical training workshops contributed to the implementation, development or modernisation of their WGS protocols and strategies for bioinformatics analysis.

The series of EQAs (on bioinformatics analysis of sequence data) was also rated highly by the NRLs and for many participants, this was their first opportunity to perform bioinformatics analysis themselves. The NRLs in BA, HR, CZ, EE, EL, LV, LT, RO, RS and SI highlighted that the EQAs were very valuable for the development of new bioinformatics pipelines and subsequent adjustment of existing WGS and bioinformatics approaches and also contributed to improvement of their approaches to bioinformatics analysis and commitment to quality assurance. Four countries (SI, HR, EL and RO) specifically stated that the EQAs was one of the most important achievements during the project and that future developments in their NRL would benefit tremendously from participation in more EQAs.

Moreover, the simulated outbreak exercises were also highly rated especially by the PCs and ACs as they provided an opportunity to practice newly acquired bioinformatics skills and apply them to simulated outbreak situations. Step-by-step guidance on WGS-based national surveillance and outbreak investigation, provided in the initial stages of the project, were also highly valued especially among the implementers of the WGS technology.

Lessons learned 1: The combination of participation in 'best practice training activities for all participants' and the 'additional training activities for PCS/ACs', EQAs (on bioinformatics analysis of sequence data), simulated exercises and having access to step-by-step guidance was a strength in the setup of the capacity building activities.

Increased awareness of NRL roles and functions, and development and implementation of NRL action plans

Creation of individual NRL action plans in the PCs raised awareness about the NRL roles and core functions (defined by ECDC) and helped the NRLs to identify current gaps in roles/functions, and to plan and implement actions to reach the expected capacity. The NRLs in the 13 PCs (BA, BG, HR, CY, CZ, EE, EL, IT, LV, LT, RO, RS, SI) prepared and implemented an action plan to strengthen their capabilities and capacities to detect and control the spread of CCRE. The action plans were used as management tools for new developments, and in some countries, they were also used to engage with stakeholders to argue their case for resources and position in their health systems. The process of creating the action plans in these NRLs resulted in heightened awareness of the NRL core functions as a technical framework for providing laboratory reference services.

Lessons learned 2: Comparison of the indicator ratings of provision of NRL roles and core functions obtained by the PCs at the beginning of the project to those obtained at the end of the project highlights that largest progress in capacity has been made especially in the reference diagnostic area including the transition to using WGS in all PCs. Progress in capacity for monitoring, alert response functions, including WGS-based surveillance, has also been obtained but to a lesser degree and capacity gaps persist (e.g. in digital infrastructure, coverage of surveillance systems and reporting of data and alerts nationally and to ECDC monitoring systems.

Modernisation of reference testing in priority countries facilitated by bespoke operational support

The individual NRL action plans developed were used as a basis for targeting the bespoke operational support towards the implementation of WGS and bioinformatics analysis, as well as guiding activities to close the identified gaps in NRLs roles and core functions in the countries. The country visits in the PC have been instrumental in successfully delivering the bespoke technical and operational support to the PCs. Regular bespoke consultancy sessions have kept the momentum of the improvement work going and ensured steady progress. In some PCs, organisational barriers to implementation of items of the action plans were also discussed during the country visits. In addition, some NRLs used the opportunity of these visits to engage with key stakeholders in their own or external organisations (i.e. ministries of health, public health organisations, hospitals and other NRLs/CLs) by inviting them to the visits.

Lessons learned 3: Importantly, the pilot genomic surveillance studies allowed the PCs and ACs to apply newly acquired knowledge and technical skills in WGS and bioinformatics analysis and generate for some the first genomic data on AMR in the priority pathogens in their country. Moreover, the NRLs experienced first-hand the potential of genomic epidemiology for AMR surveillance and outbreak detection and inspired them to establish closer national and international collaborations to continue building capacity in this area.

Improved capacity for WGS-based routine analysis

The NRLs in HR, CY, EE, EL, LT, RO and RS developed expertise and capacity in performing WGS and bioinformatic analysis and initiated implementation of quality assured WGS testing for surveillance and outbreak detection purposes. The NRLs in BG, CZ, LV and SI, who already used WGS, further developed and improved bioinformatics pipelines for analysing Illumina and/or ONT data.

Lesson learned 4: The development of expertise for performing and implementing WGS into routine reference services were of high priority for all PCs. A continuing need for capacity building support activities for WGS persists in some NRLs beyond the project.

Improved capacity for WGS-based national and international surveillance

WGS-based surveillance systems were developed in NRLs of CY, CZ, EE, EL, LV, LT, RS and SI covering selected regions/health systems or countries. The NRLs in BG, LV and LT further reported key findings on emerging resistance to the European surveillance portal for infectious diseases (EpiPulse) and the NRLs in LT and EL published preliminary national genomic data in scientific journals. Reporting of alerts on outbreaks to the European surveillance portal for infectious diseases, EpiPulse, has heightened the contribution to the AMR surveillance in Europe.

The NRL in LT published national genomic data on CCRE generated in the WGS surveillance pilot study in the journal 'Eurosurveillance' and the NRL in EL published preliminary national genomic data on CRPa in the journal 'Microorganisms' (see reference 10 and 11). Additionally, the NRLs in LT and LV participated in the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2024 to present findings on a hybrid plasmid sustaining a multi-cluster, multi-hospital outbreak of OXA-48 producing *K. pneumoniae* in Lithuania and on genomic clusters of carbapenemase-producing *K. pneumoniae* circulating in healthcare facilities in Latvia (see reference 12 and 13).

Lesson learned 5: The newly developed capacity for genomic surveillance of AMR pathogens will from now on enable countries, for which epidemiology of priority pathogens was largely unknown prior to this project, to detect and investigate outbreaks enhancing the effectiveness of national and international surveillance. While all NRLs were engaged in developing capacity for WGS and quality assured bioinformatic analyses, there remain gaps in the sharing of genomic AMR surveillance data between institutions at national/regional levels and the uploading of data into European alert and monitoring systems. In addition, concerns of insufficient policy and financial support during and beyond the EURGen-RefLabCap project were expressed by most of the countries.

Acquisition of WGS equipment for completion of WGS surveillance pilot studies

Lesson learned 6: The lack of funds for acquisition of equipment and consumables is a barrier in most of the countries that had not yet implemented WGS for surveillance and outbreak purposes. The PCs and ACs were offered financial support to conduct the pilot studies and additional support to purchase ONT materials. These amounts enabled a number of countries to purchase materials or equipment they needed.

Improved NRL advisory role and collaboration with national networks of clinical laboratories

Leasson learned 7: The introduction to a capacity mapping exercise, that the NRLs were encouraged to conduct among members of networks of CLs in their own countries, resulted in new or strengthened engagement of the NRLs with their respective networks. This facilitated building a new or stronger advisory role of the NRLs towards the CLs that persisted after the mapping exercise. Some NRLs experienced improved or more formalised collaboration with their networks of CLs, more regular communication, and increased referral of samples to the NRL.

The NRLs in CY, HR, CZ, EE, EL, LV, RO and RS highlighted key outcomes in association with development of the NRL role towards the local networks of CLs. The NRLs obtained the following outcomes regarding networks of CLs:

- Formation of national network of CLs and enhanced collaboration (CY),
- More formalised collaboration with national network of CLs (HR),
- Providing laboratory training to personnel from the national CLs for detection and characterization of CCRE, including distribution of reference strains (CZ),

- Establishment of framework for provision of technical support and scientific advice to national network of CLs (EE),
- Strengthened national network of CLs, improved and more frequent communication with the network (EL),
- Consolidation of existing regional network of CLs and other central laboratories (LV),
- Establishment of collaboration with a local network of CLs (RO), and
- Consolidation of existing local network of CLs (RS)

Strengthened cross-border collaborations

The multiple networking activities, including network meetings and on-site training workshops, provided in the project have enabled the NRL representatives to meet and establish collaborations with colleagues from other countries either at personal levels or more formally between NRLs and countries. Participation in all networking and support activities was high for all activities and the ratings of the utility and execution were also consistently high.

Leasson learned 8: From participant feedback surveys it is concluded that the PCs and ACs greatly benefitted from all types of networking and support activities, and also that they had continuing needs for further support activities.

In a few instances collaborations were initiated between groups of countries with connected epidemiological situations, for example in geographical regions with a risk of spread of antimicrobial-resistant pathogens. The most prominent collaboration that has been established to date include the Baltic States (EE, LV, LT) that formed a three-nations NRL network with the aim of strengthening AMR surveillance in the geographical region. Multinational networks like these are greatly supporting the development of cross-border surveillance in line with the aim of the EU regulation 2022/2371 on the serious cross-border threat to health. Other collaborations between neighbouring European countries have been explored but not yet formalised. In the long term, strong international networks are essential to maintaining NRL capacities of all countries.

4.4. Options for actions

At European level, continued efforts to sustain and support quality controlled WGS-based systems for characterization of priority pathogens and for national and international surveillance should be provided to all countries. It is recommended that the majority of the support activities delivered in the EURGen-RefLabCap project are continued in other EU-funded projects, including the European Reference Laboratory for public health on AMR (EURL-PH-AMR).

In particular, at European level, this should include :

- the continued delivery of training exercises in laboratory techniques, WGS and bioinformatics analysis,
- regular EQA/proficiency testing exercises for benchmarking the outcomes and quality of the WGS-data analysis.

Moreover, evidence-based protocols on WGS and bioinformatics developed in the project should be updated as more scientific knowledge and bioinformatics tools and approaches become available.

Due to gaps in the monitoring, alert and response functions, training of the NRLs should involve areas of best practice on reporting phenotypic and genomic data into national surveillance systems and on the issuing of alerts on relevant pathogens and outbreaks to the European surveillance portal for infectious diseases, EpiPulse.

Provision of scientific and technical support to the EU disease networks concerning AMR, including EURGen-Net and EARS-Net, should also be continued.

Moreover, participation of the European NRLs in other regionally or internationally relevant research and development projects and initiatives should be continued to be facilitated to maintain knowledge and expertise.

At individual country level, gaps remain in infrastructures essential to data storage, handling, sharing and reporting. Countries should continue to build, upgrade and maintain scalable infrastructure for WGS data handling and storage, consolidate and secure reporting of NRL testing results to the national networks of CLs and public health and health authorities. In some countries, digitalisation of laboratory data in NRL and/or CLs is still lagging behind, and improvement of local, regional and national integrated digital systems are required.

Finally, strengthening the engagement with key stakeholders and decision-makers to obtain funding and structural support for NRL services, national surveillance and preparedness for cross-border threats to health is essential to the provision of all NRL roles and functions in the future.

5. Conclusions

The EURGen-RefLabCap project provided networking and support activities to the NRLs in EU and other European countries to support the declared EU and global goal of controlling the threat to human health caused by healthcare-associated antimicrobial-resistant pathogens.

The specific objective to support the coordination and operation of NRLs by providing networking and capacity-building activities was met through the delivery of activities within **three main areas**:

1) building capacity in the NRLs for diagnosing, typing, monitoring and reporting on AMR;
2) strengthening the role of the NRLs in building laboratory capacity for AMR in the networks of CLs in their respective countries; and
3) modernising diagnostic testing and molecular typing of AMR using WGS.

The development of capacities and capabilities in all three main areas of the project has improved AMR surveillance in Europe.

The active engagement of all NRLs in the network and capacity building activities throughout the four-year project period and the positive feedback received on all activities highlights the continuing need for networking and technical support activities for all NRLs.

Lessons learned from delivering support activities in the EURGen-RefLabCap project will be carried over to the planning and delivery of support activities in the European Reference Laboratory for public health on AMR (EURL-PH-AMR) designated to a consortium led by SSI and composed of DTU (including the existing EURGen-RefLabCap DTU/SSI team) and the Clinical Microbiology Laboratory, Region Kronoberg - EUCAST Development Laboratory, Sweden.

6. References

1. European Centre for Disease Prevention and Control. Assessing the health burden of infections with antibiotic-resistant bacteria in the EU/EEA, 2016-2020. Stockholm: ECDC; 2022. doi: 10.2900/73460
2. World Health Organization. Facts sheets. Antimicrobial resistance. 21 November 2023. Antimicrobial resistance (who.int)
3. European Commission. A European One Health Action Plan against Antimicrobial Resistance (AMR). 2017. amr_2017_action-plan_0.pdf (europa.eu)
4. Decision No 1082/2013/EU of the European Parliament and of the Council of 22 October 2013 on serious cross-border threats to health and repealing Decision No 2119/98/EC Text with EEA relevance. Decision - 2013/1082 - EN - EUR-Lex (europa.eu)
5. World Health Organization. Global Action Plan on Antimicrobial Resistance. 2015. Global action plan on antimicrobial resistance (who.int)
6. Council Recommendation on stepping up EU actions to combat antimicrobial resistance in a One Health approach 2023/C 220/01. Recommendation on stepping up EU actions to combat antimicrobial resistance (AMR) in a One Health approach (2023/C 220/01)
7. Regulation (EU) 2022/2371 of the European Parliament and of the Council of 23 November 2022 on serious cross-border threats to health and repealing Decision No 1082/2013/EU (Text with EEA relevance). EU Regulation (2022/2371)
8. European Centre for Disease Prevention and Control. Core functions of microbiology reference laboratories for communicable diseases. Stockholm: ECDC; 2010. 1006_TER_Core_functions_of_reference_labs.pdf (europa.eu)
9. WHO Regional Office for Europe/European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2022 – 2020 data. Copenhagen: WHO Regional Office for Europe; 2022. Antimicrobial resistance surveillance in Europe 2022 - 2020 data (europa.eu)
10. Emergence of OXA-48-producing Klebsiella pneumoniae in Lithuania, 2023: a multi-cluster, multi-hospital outbreak. Greičius P, Linkevicius M, Razmuk J, Sinotova J, Alm E, Svartström O, Bortolaia V, Kudirkienė E, Roer L, Hendriksen RS, Tamoliūnaitė G, Palm D, Monnet DL, Kohlenberg A, Griškevičius A.. Euro Surveill. 2024 Apr;29(16):2400188. doi: 10.2807/1560-7917.ES.2024.29.16.2400188. PMID: 38639094; PMCID: PMC11027475.
11. Emergence of NDM-1-Producing Pseudomonas aeruginosa Nosocomial Isolates in Attica Region of Greece. Pappa O, Louka C, Karadimas K, Maikousi E,Tzoukmani A, Polemis M, Panopoulou A-D, Daniil I, Chryssou S, Mellou K, Kjeldgaard JS, Zarkotou O, Papagiannitsis C, Tryfinopoulou K. Microorganisms 2024, 12(9), 1753; https://doi.org/10.3390/microorganisms12091753
12. Hybrid plasmid caused a rapid increase of OXA-48 *K. pneumoniae* in Lithuania. Oral presentation. Greicius P. European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE). Stockholm, Sweden, 20-22 November 2024.
13. Identification of the main genomic clusters of carbapenem-resistant *Klebsiella pneumoniae* in the healthcare network by whole genome sequencing in Latvia. Poster presentation. Niedre-Otomere B. European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE). Stockholm, Sweden, 20-22 November 2024.

7. Annexes

7.1. Lists of participation in EURGen-RefLabCap activities

Table I. Number of countries invited/participated in the kick-off and network meetings

		Kick-off meeting (virtual)	1st network meeting (virtual)*	2nd network meeting (on-site)*	3rd network meeting (on-site)*	No. activities attended/ Total activities
	Countries participated/ countries invited	32/37	35/37	32/37	29/37	
	Date	May-21	Dec-21	Jun-23	Sep-24	
	For more information in section	3.1.1	3.1.3	3.1.3	3.1.3	
EURGen-RefLabCap designation	**Country**					
	AL	x	x	x	x	4/4
	AT	x	x	x	x	4/4
PC	BA	x	x	x	x	4/4
	BE*	x	x	x	x	4/4
PC	BG	x	x	x		3/4
PC	CY		x		x	2/4
PC	CZ	x	x	x	x	4/4
	DE	x	x	x		3/4
	DK	x	x	x	x	4/4
PC	EE	x	x	x	x	4/4
PC	EL	x	x		x	3/4
AC	ES	x	x	x	x	4/4
	FI	x	x	x	x	4/4
	FR	x				1/4
PC	HR		x	x	x	3/4
	HU	x	x	x	x	4/4
	IE	x	x	x	x	4/4
	IS	x	x	x	x	4/4
PC	IT	x	x	x		3/4
PC	LT	x	x	x	x	4/4
	LU	x	x	x	x	4/4
PC	LV*	x	x	x	x	4/4

		Kick-off meeting (virtual)	1st network meeting (virtual)*	2nd network meeting (on-site)*	3rd network meeting (on-site)*	No. activities attended/ Total activities
AC	MD	x	x	x		3/4
	ME*		x	x	x	3/4
	MK	x	x	x		3/4
	MT	x	x	x		3/4
	NL		x	x	x	3/4
	NO	x	x	x	x	4/4
AC	PL	x	x	x	x	4/4
AC	PT	x	x		x	3/4
PC	RO*		x	x	x	3/4
PC	RS*	x	x	x	x	4/4
	SE	x	x	x	x	4/4
PC	SI	x	x	x	x	4/4
	SK	x			x	2/4
	TR	x	x	x	x	4/4
	XK	x	x	x		3/4

Note: PCs: priority countries received the most support, ACs: additional countries received support for implementation of WGS and were included in most activities for the PCs
*: The country did not assign a contact for WS2
The agendas and presentations are available on https://www.eurgen-reflabcap.eu/meetings

Table II. Number of countries that were invited/participated in workshops

		Attended the train-the-trainer workshop on capacity building activities (on-site)	Attended the technical training workshop on Illumina sequencing (on-site)	Attended the train-the-trainer workshop on pedagogical strategies (on-site)	Attended the best practice workshop on nanopore sequencing (on-site)	Attended the technical training workshop on data for action (on-site)	No. activities attended/ Total activities
Countries participated/ countries invited		33/37	27/37	13/17	16/17	27/37	
Date		Jun-22	Dec-22	Jun-23	Dec-23	Sep-24	
For more information in section		3.2.3	3.4.1	3.4.1	3.4.1	3.4.1	
EURGen-RefLabCap designation	**Country**						
	AL	X	X				2/3
	AT	X	X				2/3
PC	BA	X	X	X	X	X	5/5
	BE*	X	X			X	3/3
PC	BG	X		X	X		3/5
PC	CY	virtual	X		X	X	4/5
PC	CZ	X	X	X	X	X	5/5
	DE	X					1/3
	DK	X	X			X	3/3
PC	EE	X	X	X	X	X	5/5
PC	EL	virtual	X		X	X	4/5
AC	ES	X	X		X	X	4/5
	FI	X				X	2/3
	FR	X					1/3
PC	HR	X	X	X	X	X	5/5
	HU	X	X			X	3/3
	IE	X	X			X	3/3
	IS		X			X	2/3
PC	IT	X	X	X	virtual		4/5
PC	LT	X	X	X	X	X	5/5

		Attended the train-the-trainer workshop on capacity building activities (on-site)	Attended the technical training workshop on Illumina sequencing (on-site)	Attended the train-the-trainer workshop on pedagogical strategies (on-site)	Attended the best practice workshop on nanopore sequencing (on-site)	Attended the technical training workshop on data for action (on-site)	No. activities attended/ Total activities
	LU	X	X			X	3/3
PC	LV*	X	X	X	X	X	5/5
AC	MD	virtual	X	X	X	X	5/5
	ME*					X	1/3
	MK	X					1/3
	MT	X	X				2/3
	NL					X	1/3
	NO	X	X				2/3
AC	PL	X	X	X		X	4/5
AC	PT	X			X	X	3/5
PC	RO*	virtual	X	X	X	X	5/5
PC	RS*	X	X	X	X	X	5/5
	SE	virtual					1/3
PC	SI	X	X	X	X	X	5/5
	SK					X	1/3
	TR	virtual	X			X	3/3
	XK	X	X			X	3/3

Note: PCs: priority countries receive the most support, ACs: additional countries received support for implementation of WGS and were included in most activities for the PCs.
Light blue colour: The activity was only for PC and AC, other countries were not invited to participate in the activity
Virtual: The country participated in the virtual part of the workshop only
*: The country did not assign a contact for WS2
The agendas and presentations are available on https://www.eurgen-reflabcap.eu/workshops

Table III. Number of countries that were invited/participated in virtual simulated outbreak exercises and the external quality assessments (EQA)

		Virtual simulated exercises (CCRE outbreak)	Virtual simulated exercises (CCRE outbreak)	Virtual simulated exercises (CRAb outbreak)	Virtual simulated exercises (CRPa outbreak)	Virtual simulated exercises (CCRE outbreak)	1st EQA on CCRE	2nd EQA on CCRE and CRAb/CRPa	3rd EQA on CCRE and CRAb/CRPa	No. activities attended/ Total activities
Countries participated/ countries invited		26/37	28/37	17/32	21/32	26/37	27/37	28/37 (CCRE) 26/32 (CRAb/CRPa)	31/37 (CCRE) 27/32 (CRAb/CRPa)	
Date		Sep-22	May-23	Sep-23	Jan-24	Oct-24	Fall 2022	Summer 2023	Summer 2024	
For more information in section		3.4.2	3.4.2	3.4.2	3.4.2	3.4.2	3.4.3	3.4.3	3.4.3	
EURGen-RefLabCap Designation	**Country**									
	AL		x	x	x	x				4/5
	AT	x	x	x	x	x	x	x	x	8/8
PC	**BA**	x	x	x	x	x	x	x	x	8/8
	BE*	x	x			x	x	x	x	6/6
PC	**BG**		x				x	x	x	4/8
PC	**CY**	x		x						2/8
PC	**CZ**		x	x	x	x	x	x	x	7/8
	DE	x	x				x	x	x	5/8
	DK	x	x	x	x	x	x	x	x	8/8
PC	**EE**	x	x	x	x	x	x	x	x	8/8
PC	**EL**	x		x	x	x	x	x	x	7/8
AC	**ES**	x			x	x	x	x	x	6/8
	FI	x	x		x	x	x	x	x	7/8
	FR	x	x				x	x	x	5/8
PC	**HR**				x	x	x		x	4/8
	HU	x	x		x	x	x	x	x	7/8

		Virtual simulated exercises (CCRE outbreak)	Virtual simulated exercises (CCRE outbreak)	Virtual simulated exercises (CRAb outbreak)	Virtual simulated exercises (CRPa outbreak)	Virtual simulated exercises (CCRE outbreak)	1st EQA on CCRE	2nd EQA on CCRE and CRAb/CRPa	3rd EQA on CCRE and CRAb/CRPa	No. activities attended/ Total activities
	IE						x	x	x	3/8
	IS	x						x	x	3/8
PC	IT	x	x		x		x	x	x	6/8
PC	LT	x	x	x	x	x	x	x	x	8/8
	LU	x	x	x	x	x	x	x	x	8/8
PC	LV*	x	x			x	x	x	x	6/6
AC	MD		x	x		x	x	x	x	6/8
	ME*									0/6
	MK		x	x	x	x				4/8
	MT	x	x		x	x	x	x	x	7/8
	NL					x		x	x	3/8
	NO	x	x	x	x	x	x	x	x	8/8
AC	PL	x	x	x	x	x	x	x	x	8/8
AC	PT	x	x	x	x	x			x	6/8
PC	RO*		x			x	x	x	x	5/6
PC	RS*	x	x			x	x	x	x	6/6
	SE	x					x	x	x	4/8
PC	SI	x	x	x	x	x	x	x	x	8/8
	SK		x						x	2/8
	TR	x	x		x			x		4/8
	XK	x	x	x		x				4/8

Note: PCs: priority countries receive the most support, ACs: additional countries received support for implementation of WGS and were included in most activities for the PCs.
*: The country did not assign a contact for WS2
Light blue colour: WS2 activity, the country was not invited because no contact was assigned for WS2
The training material for the simulated exercises are available on https://www.eurgen-reflabcap.eu/simex
The EQA reports are available on https://www.eurgen-reflabcap.eu/eqa

Table IV. Number of countries that were invited/participated in the national mapping survey of local clinical laboratories, the pilot study (only PC and AC) and received financial support for specific activities (only PC and AC)

		National report on the mapping survey of network of local clinical laboratories	National report on the pilot study	Received ONT material	Received financial support for mapping survey	Received financial support for pilot study	No. activities attended/ Total activities
Countries participated/ countries invited		27/37	15/17	15/17	14/17	14/17	
Date		2022/2023	2023/2024	2024	2023/2024	2024	
More information in section		3.2.3	3.2.2	3.2.2	3.2.3	3.2.2	
EURGen-RefLabCap designation	**Country**						
	AL						0/1
	AT	X					1/1
PC	BA	X	X	X	X	X	5/5
	BE*						0/1
PC	BG	X	X	X	X	X	5/5
PC	CY	X	X	X	X	X	5/5
PC	CZ	X	X	X	X	X	5/5
	DE	X					1/1
	DK	data					0/1
PC	EE	X	X	X	X	X	5/5
PC	EL	X	X	X	X	X	5/5
AC	ES	X	X	X	X	X	5/5
	FI	data					0/1
	FR	X					1/1
PC	HR	X	X	X	X	X	5/5
	HU	data					0/1
	IE						0/1
	IS	data					0/1

		National report on the mapping survey of network of local clinical laboratories	National report on the pilot study	Received ONT material	Received financial support for mapping survey	Received financial support for pilot study	No. activities attended/ Total activities
PC	IT						0/5
PC	LT	X	X	X	X	X	5/5
	LU	X					1/1
PC	LV*	X	X	X	X	X	5/5
AC	MD	X	X	X		X	4/4
	ME*						0/1
	MK						0/1
	MT	X					1/1
	NL						0/1
	NO	X					1/1
AC	PL	X			X		2/5
AC	PT		X	X		X	4/5
PC	RO*	X	X	X	X	X	5/5
PC	RS*	X	X	X	X		4/5
	SE	X					1/1
PC	SI	X	X	X	X	X	5/5
	SK						0/1
	TR						0/1
	XK	X					1/1

Note: PCs: priority countries receive the most support, ACs: additional countries received support for implementation of WGS and were included in most activities for the PCs.
Light blue colour: The activity was only for PC and AC, other countries were not invited to participate in the activity
Data: The country submitted data only
*: The country did not assign a contact for WS2

Table V. Number of priority countries (PC) developing an action plan for laboratory activities, participating in the virtual workshop on project management, receiving NRL visits by the EURGen-RefLabCap support teams, and giving a structured interview of the outcome and future needs

	Developed an action plan on laboratory activities supported by the project	Attended the workshop on project management - part 1 (Virtual)	Attended the project management course - part 2 (Virtual)	Received a 1st NRL visit	Received a 2nd NRL visit	Participated in a structured interview of outcome and future needs	No. activities attended/ Total activities
Date	2022	May-22	Sep-22	2022/2023	2023/2024	Fall 2024	
More information in section	3.2.1	3.2.2	3.2.2	3.2.2	3.2.2	4.2	
Country							
BA	x	x			x	x	4/6
BG	x	x	x	x	x	x	6/6
CY	x	x		x	x	x	5/6
CZ	x	x		x	x	x	5/6
EE	x	x	x	x	x	x	6/6
EL	x	x	x	x	x	x	6/6
HR	x	x	x	x		x	5/6
IT	x	x		x			3/6
LT	x	x		x	x	x	5/6
LV*	x	x	x	x	x	x	6/6
RO*	x	x	x	x	x	x	6/6
RS*	x	x	x	x	x	x	6/6
SI	x	x	x	x	x	x	6/6

Note: PCs: priority countries receive the most support
*: The country did not assign a contact for WS2

Table VI. Number of countries that were invited/participated in the national mapping survey

		Survey on NRL capacity and gaps in WGS for CCRE	Survey on NRL capacity for CRAb/CRPa	No. activities attended/ Total activities
	Countries participated/ countries invited	37/37	23/32	
	Date	Jul-21	Nov-22	
	More information in section	3.1.2	3.1.2	
EURGen-RefLabCap designation	Country			
	AL	x	X	2/2
	AT	x	X	2/2
PC	BA	x		1/2
	BE*	x		1/2
PC	BG	x	X	2/2
PC	CY	x	X	2/2
PC	CZ	x	X	2/2
	DE	x	X	2/2
	DK	x	X	2/2
PC	EE	x	X	2/2
PC	EL	x	X	2/2
AC	ES	x	X	2/2
	FI	x	X	2/2
	FR	x	X	2/2
PC	HR	x	X	2/2
	HU	x		1/2
	IE	x		1/2
	IS	x	X	2/2
PC	IT	x		1/2
PC	LT	x	X	2/2
	LU	x		1/2
PC	LV*	x		1/2
AC	MD	x		1/2
	ME*	x		1/2

		Survey on NRL capacity and gaps in WGS for CCRE	Survey on NRL capacity for CRAb/CRPa	No. activities attended/ Total activities
	MK	x		1/2
	MT	x	X	2/2
	NL	x	X	2/2
	NO	x	X	2/2
AC	**PL**	x	X	2/2
AC	**PT**	x		1/2
PC	**RO***	x		1/2
PC	**RS***	x		1/2
	SE	x	X	2/2
PC	**SI**	x	X	2/2
	SK	x	X	2/2
	TR	x		1/2
	XK	x	X	2/2

Note: PCs: priority countries receive the most support, ACs: additional countries received support for implementation of WGS and were included in most activities for the PCs.
*: The country did not assign a contact for WS2
Light blue colour: WS2 activity, the country was not invited because no contact was assigned for WS2

7.2. Lists of webinars

Overview of webinars offered to all NRLs during the project. The majority of the presentations from the webinars can be found here: https://www.eurgen-reflabcap.eu/webinars.

Table VI. List of webinars offered to all NRLs during the project

Webinars	Date	No. of participants	More information in section
CCRE protocol	Dec-21	35	3.3.2
Survey on NRL capacity for CCRE	Dec-21	35	3.1.2
Highlights of existing WGS initiatives regarding CCRE	Dec-21	35	3.3.1
Survey on NRL capacity for phenotypic, molecular and WGS-based characterisation of CCRE	Apr-22	22	3.1.2
'Guidance document on internal quality control schemes' for CCRE	Nov-22	-*	3.3.3
Demystifying genomics, how do we get started	Sep-22	-*	3.4.1
How to plan an EQA	Jan-23	28	3.4.1
Survey on genotypic methods used at the NRLs for CRAb/CRPa typing	Mar-23	35	3.3.1
Epidemiological data on CRAb/CRPa in the EU/EEA	Mar-23	35	3.1.2
'Guidance document on internal quality control schemes' for CRAb/CRPa	Apr-23	-*	3.3.3
Bioinformatics approaches for plasmid analysis	May-23	-*	3.4.1
1st EQA results	Jun-23	32	3.4.3
WGS protocol for CRAb/CRPa	Jun-23	32	3.3.2
2nd EQA results	Nov-23	51	3.4.3
Data sharing and management - 'ISO and how to deal with data management of metadata'	Apr-24	32	3.4.1
Data sharing and management - 'Data sharing for European Surveillance'	Apr-24	32	3.4.1
EU opportunities for funding and procedures	May-24	49	3.4.1
International Pathogen Surveillance Network (IPSN)	Jun-24	35	3.4.1
3rd EQA results	Sep-24	48	3.4.3
Webinar series on setting up an AMR surveillance program using WGS How to decide what to sequence Using your phenotypic analysis to decide what to sequence What metadata do you need to make your AMR WGS valuable How to communicate your sequencing results	Oct/Nov-24	-*	3.4.1

*The number of participants was not recorded.

7.3. List of deliverables completed and approved by HaDEA

Overview of all deliverables in the contract for the EURGen-RefLabCap project. All deliverables were completed and have been approved by HaDEA. Some of the deliverables are publicly available on the project website and the link is included below.

Table VII. Overview of all deliverables in the contract for the EURGen-RefLabCap project

Project management documents	Link to publicly available documents / More information (Section)
Kick-off meeting - due to COVID-19 the meeting was held virtual	
Draft Inception report	
Final Inception Report, revised after the kick-off meeting	
Narrative progress report	
Interim report at M24 linked to interim payment	
Mid-term meeting Luxembourg	
Draft Final Report	
Final report *	
Deliverables (Area 1) Building capacity in the NRLs for to improve NRL core functions for AMR	**Link to publicly available documents / More information (Section)**
Established reference laboratory network for the EURGen-RefLabCap project	3.1.1
Website live	https://www.eurgen-reflabcap.eu/ 3.1.1
Summary report of main issues and gaps in information on capability and capacity of public health reference laboratory functions for CRE/CCRE and EURGen-RefLabCap questionnaire 2021	3.1.2
Report on identified gaps and identification of priority countries, including the list of identified 'priority countries'	3.1.2
Work plan for technical support activities for all countries in the network	3.1.3
Two short evaluation report with lessons learned and areas for further work	4.2
Minimum 8-maximum 12 individual action plans for each of the priority countries, produced in English and in the national language of the countries concerned	3.2.1

*Approval of the final report is ongoing

Deliverables (Area 2) Activities for strengthening the role of the NRLs to build capacities in local networks of CLs	Link to publicly available documents / More information (Section)
NRL reports for each country on capacity of CLs	3.2.3
Overview report of all countries on capacity of CLs	https://www.eurgen-reflabcap.eu/protocols-and-guidelines 3.2.3
Plan for further support to NRLs to develop national capacity building activities for regional and local labs	3.2.2
Deliverables (Area 3) Activities for modernising diagnostic and molecular typing tests for the specified pathogens using WGS	**Link to publicly available documents / More information (Section)**
Report on the review of the existing initiatives in WGS and preliminary recommendations for further work in the frame of this contract	3.3.1
Report on the gaps in WGS capacity at National level	3.3.1
Proposal for common WGS-based genome analysis methods and standard protocols for national CCRE surveillance and integrated outbreak investigations.	https://www.eurgen-reflabcap.eu/protocols-and-guidelines 3.3.2
Validated common WGS-based genome analysis methods and standard protocols for national CCRE surveillance and integrated outbreak investigations for use by participating NRLs for public health	https://www.eurgen-reflabcap.eu/protocols-and-guidelines 3.3.2
Plan for bespoke operation support to priority countries addressing the identified gaps for workstream 1 WGS over the course of the contract, including at minimum one country visit per priority country	3.2.2
Training plan for all countries	3.1.3
Guidance document on internal quality control schemes	https://www.eurgen-reflabcap.eu/protocols-and-guidelines 3.3.3
Proposed quality assurance scheme(s);	3.4.3
Execution of annual quality assurance exercises for year 2, 3 and 4	https://www.eurgen-reflabcap.eu/eqa 3.4.3
Report of proposals for two additional priority pathogens for strengthening laboratory capacity	3.1.5
Contact details of members of the expanded network created for the purposes of this contract	3.1.1
Proposal for common WGS-based genome analysis methods and standard protocols for national surveillance and integrated outbreak investigations for the two selected antimicrobial-resistant bacterial pathogens CRAb and CRPa	https://www.eurgen-reflabcap.eu/protocols-and-guidelines 3.3.2
Validated common WGS-based genome analysis methods and standard protocols for national surveillance and integrated outbreak investigations for use by participating NRL for CRAb and CRPa	https://www.eurgen-reflabcap.eu/protocols-and-guidelines 3.3.2
Report on the mapping of current molecular or genomic AMR prediction and strain methods used in NRLs for CRAb and CRPa	3.1.2
Plan for bespoke operation support for priority countries	3.2.2

Training plan	3.1.3
Guidance document on internal quality control schemes	https://www.eurgen-reflabcap.eu//protocols-and-guidelines 3.3.3
Proposed quality assurance scheme(s)	3.4.3
Execution of annual quality assurance exercises for year 3 and 4	https://www.eurgen-reflabcap.eu/eqa 3.4.3

7.4. EURGen-RefLabCap action plan

Table VIII. Aims and objectives proposed to the PCs to design an EURGen-RefLabCap action plan to strengthen capabilities and capacities to detect and control the spread of CCRE in healthcare settings

NRL CORE FUNCTION 1 - Reference diagnostics	
Overall aim	**Objectives**
The NRL is committed to quality assurance.	To participate in EQAs.
	To facilitate EQA for CLs.
	To hold accreditation for characterisation of CRE and/or CCRE.
The NRL performs phenotypic AST of CRE/CCRE according to reference methods.	To perform phenotypic AST according to reference methods including use of control strains from a reliable source.
	To have an internal quality control scheme/quality management system.
The NRL performs WGS-based typing of CRE/CCRE.	To have defined laboratory and bioinformatic approaches and methods.
	To perform quality control testing of WGS.
	To upload WGS data for sharing purposes.
	To report WGS data to the CLs.
	To use controls from a reliable source for polymerase chain reaction (PCR).
The NRL offers diagnostic confirmation services (i.e. validates diagnostic test results, provides advice and support).	To provide reference services for CRE/CCRE.
	To ensure correct species identification.
	To use international guidelines for confirmatory testing and further characterisation of CRE/CCRE at the NRL.
	To offer WGS-based reference services.
The NRL has a system for investigation of profiles of atypical and unusual CRE/CCRE isolates.	To create a standard operating procedure to identify profiles of atypical and unusual CRE/CCRE isolates.
	To collect and test profiles of atypical and unusual CRE/CCRE isolates.

NRL CORE FUNCTION 2 - Reference material resources	
Overall aim	**Objectives**
The NRL has and hosts a collection of reference materials / bio-bank.	To create a collection of reference materials / bio-bank.
	To maintain a collection of reference material for phenotypic AST of CRE/CCRE.
	To maintain a collection of reference material for genotypic AMR detection in CRE/CCRE.
The NRL provides reference material to local and regional CLs.	To establish a logistic framework and agreements for sharing reference material with CLs.
NRL CORE FUNCTION 3 - Scientific advice	
Overall aim	**Objectives**
The NRL provides scientific advice and technical support to public health authorities (e.g. ministries, risk managing agencies, etc.).	To communicate regularly with public health authorities.
	To issue national guidance on CL testing and risk-based screening of patients for CRE/CCRE.
	To issue a national plan for containment of CCRE (it can be a national action plan on CRE/CCRE or part of an AMR action plan or part of national guidance on infection prevention and control).
	To contribute to production of national guidance on infection prevention and control of CRE/CCRE.
The NRL provides scientific advice and technical support to the CLs.	To communicate regularly with CLs.
	To issue national protocol for CLs on phenotypic AST for carbapenem and colistin.
	To monitor compliance with AST protocols.
	To issue national protocol for CLs on genotypic characterisation of CCRE.
	To monitor compliance with protocols for genotypic characterisation of CRE/CCRE.
	To organise training activities for the CLs based on needs and for the implementation of new guidance/policy.
NRL CORE FUNCTION 4 - Collaboration	
Overall aim	**Objectives**
The NRL participates in regional/international public health microbiology networks.	To be part of international and regional CCRE networks.
	To be a member of the National Antimicrobial Susceptibility Testing Committee (NAC).
	To participate in international networks/initiatives for development/evaluation of CRE/CCRE diagnostic and reference testing methods.
The NRL participates in other regionally or internationally relevant projects and initiatives, including R&D to underpin the quality, scope and development of NRL core activities.	To participate in international CRE/CCRE (epidemiology/surveillance) networks, including EURGen-Net.

NRL CORE FUNCTION 5 - Monitoring, alert and response	
Overall aim	**Objectives**
The NRL provides input to national recommendations for public health notification (and other reporting).	To establish harmonised criteria for selection of CRE/CCRE for referral to the NRL.
	To specify a case definition for a CRE/CCRE infected/colonised patient for reporting purposes
	To specify an outbreak definition for CRE/CCRE infected/colonised patients.
	To agree on mandatory reporting of CRE/CCRE by the CLs to the NRL.
	To agree on CRE/CCRE listing as 'notifiable disease' under national public health regulations.
The NRL provides data on CRE/CCRE to national laboratory surveillance systems and national networks.	To implement a laboratory management software for collection and storage of CRE/CCRE data that allows export and reporting.
	To implement a national integrated digital system allowing interoperability between CLs, NRLs and public health authorities.
	To publish CRE/CCRE surveillance data in periodic reports.
The NRL provides data on CRE/CCRE to international networks.	To submit data to EARS-Net.
The NRL provides support and data to multi-sectoral and One-Health collaboration and coordination.	To identify who needs support and data to multi-sectoral and One-Health collaboration and coordination.
	To implement a One-Health system for AMR monitoring in the country.
The NRL actively contributes to provision of data-based early warning systems.	To use the CRE/CCRE data in the national integrated digital system for surveillance and early warning purposes.
The NRL actively provides support in preparedness and outbreak response.	To define the NRL roles and responsibilities in outbreak support.

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