Provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in Salmonella species and Campylobacter species in human samples

SC 2019 74 09 FWD AMR-RefLabCap
Deliverable G4.2 Final report

Written by Statens Serum Institut
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European Health and Digital Executive Agency (HaDEA)

Unit A2 - EU4Health/SMP Food
HaDEA COV2
Place Rogier, 16, B-1049 BRUXELLES
Belgium
Email : HaDEA-HP-TENDER@ec.europa.eu

EUROPEAN COMMISSION

Directorate-General Health and Food Safety (DG SANTE)

Directorate B — Public health, Cancer and Health security
Unit B3 — Health monitoring and cooperation, Health networks
L-2920 Luxembourg
Email : SANTE-CONSULT-B3@ec.europa.eu

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Acronyms and abbreviations

AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
DG SANTE	The Commission's Directorate-General for Health and Food Safety
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
EU	European Union
EURL	European Union reference laboratory
EQA	External quality assessment
FWD AMR-RefLabCap	Food- and Waterborne Diseases Antimicrobial Resistance - Reference Laboratory Capacity
HaDEA	European Health and Digital Executive Agency
IQC	Internal quality control
MoH	Ministry of Health
Mdn	Median value
NRL	National reference laboratory in public health
PCR	Polymerase chain reaction
PH	Public health
QC	Quality control
WGS	Whole-genome sequencing

Abstract

The service contract aimed to strengthen coordination, support, and capacity in national reference laboratories (NRLs) in public health (PH) for antimicrobial resistance (AMR) in *Salmonella* and *Campylobacter* across Europe. It focused on capacity building, enhancing the NRLs' roles, and modernising diagnostic and typing tests using whole-genome sequencing (WGS). The project established a comprehensive laboratory network and significantly improved the NRLs' roles and capacity for AMR in both pathogens through targeted support, especially for NRLs with the greatest needs. Key documents and protocols were developed, and external quality assessment schemes and ring trials were organised. These efforts modernised WGS-based diagnostic and typing tests, resulting in more uniform AMR testing. The advancements strengthened laboratory capabilities, improved AMR monitoring, and enhanced PH preparedness.

Future support for NRLs should build on these achievements by refining laboratory procedures, standardising data interpretation, improving communication, and addressing resource gaps through targeted mentoring, comprehensive training, and adequate funding. Clear legal frameworks and external support are crucial for better coordination and support of national laboratory networks. Interdisciplinary collaboration can be improved through joint trainings, data sharing frameworks, and formal agreements. Expanding support to other food and waterborne pathogens and fostering cross-border collaboration will further enhance PH readiness.

Résumé

Le contrat de service visait à renforcer la coordination, le soutien et les capacités des laboratoires nationaux de référence (LNR) dans le domaine de la santé publique (SP) pour la résistance aux antimicrobiens (RAM) de *Salmonella* et *Campylobacter* à travers l'Europe. Il s'est concentré sur le renforcement des capacités, amélioration du rôle des LNR et la modernisation des tests diagnostiques et de typage via le séquençage du génome entier (SGE). Un réseau complet de laboratoires a été établi améliorant considérablement le rôle et les capacités des LNR concernant la RAM des deux pathogènes grâce à un soutien ciblé, particulièrement pour les LNR les plus nécessiteux. Des documents clés, des protocoles, des systèmes externes d'assurance qualité et des essais circulaires ont été développés. Ces efforts ont harmonisé les tests basés sur le SGE, améliorant la surveillance de la RAM et les résultats de la SP.

Le soutien futur aux LNR devrait affiner les procédures de laboratoire, standardiser l'interprétation des données, améliorer la communication, adresser les déficits de ressources par un mentorat ciblé, une formation complète et un financement adéquat. Des cadres juridiques clairs et un soutien externe sont essentiels pour la coordination et le soutien des réseaux de LNR. La collaboration interdisciplinaire peut être améliorée grâce à des formations communes, le partage des données et des accords formels. Une extension du soutien à d'autres pathogènes d'origine alimentaire et hydrique et la promotion de la collaboration transfrontalière va améliorer davantage la préparation de la SP.

Executive summary

Context

This final report summarises the 4-year work carried out by Statens Serum Institut (SSI) and the Technical University of Denmark (DTU) under the service contract with the European Health and Digital Executive Agency (HaDEA) (Service Contract 20197409).

The general objective of this action was to support the countries (European Union and European Economic Area (EU/EEA), Bosnia-Herzegovina, Serbia and Moldova) to enhance the validity and accuracy of surveillance data reported at European level in order to inform concerted actions against antimicrobial resistance (AMR) and enable better detection and control of cross border threats to humans from AMR.

The purpose of the service contract was to strengthen coordination, support and capacity in national reference laboratory (NRL) in public health (PH) functions for AMR in *Salmonella* and *Campylobacter* from human samples within Europe. The specific project objectives were:

(i) Building capacity in all functions and key roles required for NRLs in the field of AMR in *Salmonella* and *Campylobacter* from human samples.

(ii) Supporting the role of NRLs, and associated structures, to strengthen regional and local laboratory capacities for AMR within the countries.

(iii) Modernisation of diagnostic and molecular typing methods using whole-genome sequencing (WGS) to ensure improved and more uniform diagnostics and characterisation of AMR across Europe.

Methodology

The project organisation included four teams: a management team and three teams that focused on different aspects of the project: networking, training and methods.

The following methods were used to deliver projects tasks, activities and deliverables:

- Timetables for task and deliverable implementation
- Internal meetings (SSI, DTU) and meetings with stakeholders (HaDEA, The Commission's Directorate-General for Health and Food Safety (DG SANTE), European Centre for Disease Prevention and Control (ECDC))
- Quality control (QC), risk assessment and management

- Project evaluation
- Progress reports, interim reports, draft final report and final report.

The above methods and their outcome were described in 14 general deliverables produced by the project management team.

Main results and outcomes

To achieve the projects purpose and objectives, the work was divided into two specific tasks:

Task 1: Generic networking and capacity building activities to strengthen NRLs functions for AMR surveillance of human *Salmonella* and *Campylobacter* infections with a specific focus on countries where capacities were less well developed.

Task 2: Activities to support the role of NRLs to work with and build capacities in regional and local laboratories in their own countries for the two pathogens: *Salmonella* and *Campylobacter* from humans.

The project team produced 22 deliverables covering the two specific tasks, which included activities related to networking, capacity building, method modernisation, and evaluation.

Laboratory network establishment and networking. The Food- and Waterborne Diseases Antimicrobial Resistance - Reference Laboratory Capacity (FWD AMR-RefLabCap) network was established in April 2021. By the project's end, it included 46 laboratories across 37 countries. Of the 37 countries, 32 were EU/EEA and EU Health Programme countries, and five were candidate or potential candidate countries with observer status in the project. The project facilitated a virtual kick-off meeting, three network meetings, including two in-person meetings in Copenhagen, to discuss project activities, collaborations, and updates. It also organised two online meetings with representatives from relevant European Union reference laboratories (EURLs) in food and feed control area. Representatives from ECDC, HaDEA and DG SANTE participated in all meetings. A dedicated project website was developed to include all materials from project trainings and events, and other relevant information.

Capacity building activities. These activities included trainings, support to countries with the greatest needs and support for national surveillance and local capacity building.

In 2022, two practical hands-on courses on phenotypic AMR testing methods for *Salmonella* and *Campylobacter*, and three virtual multidisciplinary training

workshops for PH professionals to enhance collaboration and integrate WGS into AMR surveillance and outbreak investigations were organised.

National AMR surveillance recommendations were developed and countries' capacities were assessed. On the basis of the assessment, twelve "priority countries" with greatest needs for capacity building and three "additional countries" facing challenges to identify and manage outbreaks of *Salmonella* and *Campylobacter* were selected. All 15 countries received tailored support, including action plan development, meetings, visits, and financial support.

National surveillance was supported by developing guidance to the national reference laboratories for establishment and coordination of a national network of laboratories for AMR surveillance and conducting a survey to assess and develop existing networks. Local capacity building included mapping regional and local laboratory capacities, supported by financial aid, and organising virtual and physical trainings based on identified needs.

Method modernisation. The activities in this area included development of guidance documents and annual external quality assessment (EQA) exercises and ring trials.

Four key documents guiding AMR surveillance in *Salmonella* and *Campylobacter* were produced:

i) a WGS review and protocol for detecting and tracing AMR genes;
ii) an updated EU protocol for harmonised monitoring of AMR incorporating WGS for genetic monitoring of AMR;
iii) a draft national model protocol for AMR surveillance in line with EU definitions;
iv) a guidance document on internal QC schemes for antimicrobial susceptibility testing (AST) and AMR detection.

These documents provided comprehensive methodologies, QC strategies, and practical advice for laboratory networks.

Three EQA exercises and three ring trials were organised to evaluate participants' ability to produce high-quality sequence data and to evaluate bioinformatics pipelines for AMR determination, respectively. The outcome of all EQA exercises and ring trials were summarised in anonymised reports containing result evaluation and recommendations for improvements.

Evaluation and impact

Participation in project activities. Various activities, including webinars, workshops, and training sessions were organised, with laboratories' participation averaging at 75%.

Participant feedback on specific project activities. To collect NRLs feedback on project activities, in total eight evaluation surveys were conducted.

NRLs valued the network meetings, actively engaging in discussions and presentations. Knowledge sharing was enhanced through an online communication platform and knowledge exchange groups, however low engagement suggests a need of better approaches in the future.

Project trainings were perceived useful to most participants, although occasionally time constraints limited the participation. To enhance participation, preparatory materials, recorded sessions, and online content were provided. NRLs expressed a need for more hands-on sessions on WGS data analysis and AMR gene identification, indicating future training needs.

Priority countries often faced challenges due to insufficient laboratory resources and other constrains at the national level which affected their support. Despite these obstacles, tailored guidance led to significant progress in WGS implementation and improvements in AMR surveillance in most countries.

NRLs appreciated support in mapping the capacities of national laboratory networks. For the future support, they indicated a need for guidance for surveillance sampling plans and communication with local laboratories. Further, they asked for joint trainings for epidemiologists and microbiologists, discussions on specific pathogens, and involving health policymakers.

Guidance documents produced during the project were well-received, although differences in PH systems and resources require further support and consultations.

Annual EQA and ring trial exercises were valuable for enhancing skills and ensuring consistency, quality, and data comparability across the countries.

Impact of project activities. Overall, the NRLs indicated the highest project impact on reference diagnostics, and a lower impact on monitoring, alert, and response function of NRL. The project's tailored support to the priority countries resulted in significant improvements in their NRLs functions by:

- Increased phenotypic AMR testing for *Salmonella* and *Campylobacter*, with all NRLs fully adopting these tests. WGS adoption grew, phasing out older fingerprinting techniques. By the project's end, 12 out of 15 NRLs had WGS capabilities, which were significantly used for AMR determination and cluster detection.
- The implementation of WGS improved outbreak detection and collaboration with epidemiologists and food and veterinary sectors.
- Reporting to the PH administration showcasing the usefulness of WGS and informing future needs.
- The establishment of national networks of local/regional laboratories and surveillance systems, enhanced communication and collaboration among

laboratories, and increased referral of isolates to the NRLs. Guidelines and training were provided to support the national laboratory networks.
- Establishing new collaborations, e.g. with the FWD AMR-RefLabCap team, other NRLs, and ECDC, sharing WGS experiences, and obtaining new knowledge on *Salmonella* and *Campylobacter*.

Next steps for future support

Based on the NRLs' expressed needs and wishes, suggestions for future support to NRLs in four main areas were identified.

The utilisation of WGS may be increased if more resources were available to produce WGS data and more trainings were provided to interpret and to communicate WGS results, hindering full WGS integration into PH actions. NRLs that have integrated WGS noted the lack of harmonisation and accreditation across Europe. Future support to NRLs may include longer mentoring visits, more training and technical support, and funding options to overcome these barriers.

Interdisciplinary and cross-sector collaboration and capacity building should be further improved to address challenges, such as insufficient knowledge and skills in sequencing data interpretation and lack of formalised collaborations. This may be achieved by providing trainings, frameworks and solutions for integrated data sharing and communication, and formalised collaborations.

The NRLs' role should be further strengthened to improve the coordination and support of national laboratory networks. This can be facilitated by providing clear legal frameworks at the EU and national levels and exploring possibilities for external support to raise awareness among healthcare authorities and policymakers, such as through ECDC visits and audits.

There is also a need for further opportunities to enhance EU wide knowledge sharing and collaboration on specific topics. This could be achieved by creating platforms for informal data sharing and consultations, organising online presentations and workshops, and fostering exchange visits between NRLs.

The future support should continue and expand the activities for *Salmonella* and *Campylobacter* as well as for other food- and waterborne pathogens which will further enhance readiness for various PH threats.

Conclusions

The project established a comprehensive laboratory network that created opportunities for knowledge and experience sharing among NRLs across Europe. The capacity of NRLs and associated structures to address AMR in *Salmonella* and *Campylobacter* from human samples was significantly improved

through a series of targeted activities. To modernise diagnostic and molecular typing tests using WGS, the project developed several key documents and protocols. This, coupled with organisation of EQAs and ring trials, led to more uniform standards for AMR testing and characterisation across Europe. The impact of these improvements is significant, as they will contribute to improved PH outcomes by ensuring timely and accurate detection and reporting of resistant strains nationally and at the EU level.

Résumé analytique

Contexte

Ce rapport final résume les travaux menés pendant quatre ans par le Statens Serum Institut (SSI) et l'Université technique du Danemark (DTU) dans le cadre du contrat de service conclu avec l'Agence exécutive européenne pour la santé et le numérique (HaDEA) (contrat de service 20197409).

L'objectif général de cette action était d'aider les pays (Union européenne et Espace économique européen (UE/EEE), Bosnie-Herzégovine, Serbie et Moldavie) à améliorer la validité et la précision des données de surveillance communiquées au niveau européen afin d'éclairer les actions concertées contre la résistance aux antimicrobiens (RAM) et de permettre une meilleure détection et un meilleur contrôle des menaces transfrontalières pour l'homme liées à la RAM.

Le but du contrat de service était de renforcer la coordination, le soutien et la capacité des laboratoires nationaux de référence (LNR) dans les fonctions de santé publique (SP) pour la RAM chez les *Salmonella* et *Campylobacter* à partir d'échantillons humains en Europe. Les objectifs spécifiques du projet étaient les suivants

i) Renforcer les capacités dans toutes les fonctions et les rôles clés requis pour les LNR dans le domaine de la résistance aux antimicrobiens chez les *Salmonella* et *Campylobacter* à partir d'échantillons humains.
ii) Soutenir le rôle des LNR et des structures associées pour renforcer les capacités des laboratoires régionaux et locaux dans le domaine de la résistance aux antimicrobiens dans les pays.
iii) Moderniser les méthodes diagnostiques et de typage moléculaire à l'aide du séquençage du génome entier (WGS) afin d'améliorer et d'uniformiser les diagnostics et la caractérisation de la RAM dans toute l'Europe.

Méthodologie

L'organisation du projet comprenait quatre équipes : une équipe de gestion et trois équipes axées sur différents aspects du projet : la mise en réseau, la formation et les méthodes.

Les méthodes suivantes ont été utilisées pour réaliser les tâches, les activités et les résultats du projets :

- Des calendriers pour la mise en œuvre des tâches et des résultats
- Des réunions internes (SSI, DTU) et des réunions avec les parties prenantes (HaDEA, Direction générale de la santé et de la sécurité

alimentaire de la Commission (DG SANTE), Centre européen de prévention et de contrôle des maladies (ECDC)).
- Contrôle de la qualité (CQ), évaluation et gestion des risques
- Évaluation des projets
- Rapports d'avancement, rapports intermédiaires, projet de rapport final et rapport final.

Les méthodes susmentionnées et leurs résultats ont été décrits dans 14 documents généraux produits par l'équipe de gestion du projet.

Principaux résultats et réalisations

Pour atteindre le but et les objectifs du projet, le travail a été divisé en deux tâches spécifiques :

Tâche 1: Activités génériques de mise en réseau et de renforcement des capacités visant à renforcer les fonctions des LNR en matière de surveillance de la résistance aux antimicrobiens des infections humaines de *Salmonella* et de *Campylobacter*, avec un accent particulier sur les pays où les capacités sont moins bien développées.

Tâche 2: activités visant à soutenir le rôle des LNR dans la collaboration et le renforcement des capacités des laboratoires régionaux et locaux dans leur propre pays pour les deux agents pathogènes : *Salmonella* et *Campylobacter* chez l'homme.

L'équipe du projet a produit 22 résultats couvrant les deux tâches spécifiques, qui comprenaient des activités liées à la mise en réseau, au renforcement des capacités, à la modernisation des méthodes et à l'évaluation.

Mise en place d'un réseau de laboratoires et la mise en réseau. Le réseau Food- and Waterborne Diseases Antimicrobial Resistance - Reference Laboratory Capacity (FWD AMR-RefLabCap) a été mis en place en avril 2021. À la fin du projet, il comprenait 46 laboratoires répartis dans 37 pays. Sur ces 37 pays, 32 étaient des pays de l'UE/EEE et du programme de santé de l'UE, et cinq étaient des pays candidats ou candidats potentiels ayant le statut d'observateur dans le cadre du projet. Le projet a facilité une réunion virtuelle de lancement, trois réunions de réseau, dont deux réunions en personne à Copenhague, pour discuter des activités du projet, des collaborations et des mises à jour. Il a également organisé deux réunions en ligne avec des représentants des laboratoires de référence de l'Union européenne (EURL) dans le domaine du contrôle des denrées alimentaires et des aliments pour animaux. Des représentants de l'ECDC, de la HaDEA et de la DG Santé ont participé à toutes les réunions. Un site web dédié au projet a été développé pour inclure tout le matériel des formations et des événements du projet, ainsi que d'autres informations pertinentes.

Activités de renforcement des capacités. Ces activités comprenaient des formations, un soutien aux pays ayant les besoins les plus importants et un soutien à la surveillance nationale et au renforcement des capacités locales.

En 2022, deux cours pratiques sur les méthodes phénotypiques d'analyse de la RAM pour *Salmonella* et *Campylobacter*, et trois ateliers de formation multidisciplinaires virtuels pour les professionnels de la santé publique ont été organisés afin d'améliorer la collaboration et d'intégrer le WGS dans la surveillance de la RAM et les enquêtes sur les épidémies.

Des recommandations nationales en matière de surveillance de la RAM ont été élaborées et les capacités des pays ont été évaluées. Sur la base de cette évaluation, douze « pays prioritaires » ayant les plus grands besoins en matière de renforcement des capacités et trois « pays supplémentaires » confrontés à des difficultés pour identifier et gérer les épidémies de *Salmonella* et *Campylobacter* ont été sélectionnés. Tous les 15 pays ont bénéficié d'un soutien adapté, comprenant l'élaboration d'un plan d'action, des réunions, des visites et un soutien financier.

La surveillance nationale a été soutenue par l'élaboration d'orientations pour les LNR afin de créer un réseau national de laboratoires pour la surveillance de la RAM et par une enquête visant à évaluer et développer les réseaux existants. Le renforcement des capacités locales comprenait la cartographie des capacités des laboratoires régionaux et locaux, soutenu par une aide financière, ainsi que l'organisation de formations virtuelles et présentielles adaptées aux besoins identifiés.

Modernisation des méthodes. Les activités dans ce domaine comprenaient l'élaboration de documents d'orientation, des exercices annuels d'évaluation externe de la qualité (EQA) et des essais circulaires.

Quatre documents clés guidant la surveillance de la RAM chez *Salmonella* et *Campylobacter* ont été produits :

i) un examen et un protocole WGS pour la détection et le traçage des gènes de la RAM,
ii) un protocole actualisé de l'UE pour la surveillance harmonisée de la RAM intégrant le WGS pour la surveillance génétique de la RAM,
iii) un projet de protocole modèle national pour la surveillance de la RAM conformément aux définitions de l'UE, et
iv) un document d'orientation sur les systèmes de CQ internes pour les tests de sensibilité aux antimicrobiens (TSA) et la détection de la RAM.

Ces documents fournissent des méthodologies complètes, des stratégies de contrôle de qualité et des conseils pratiques pour les réseaux de laboratoires.

Trois exercices d'EQA et trois essais circulaires ont été organisés pour évaluer la capacité des participants à produire des données de séquençage de haute qualité et pour évaluer les pipelines bioinformatiques pour la détermination de la RAM, respectivement. Les résultats de tous les exercices d'EQA et des essais circulaires ont été résumés dans des rapports anonymisés contenant l'évaluation des résultats et des recommandations d'amélioration.

Évaluation et impact

Participation aux activités du projet. Diverses activités, notamment des webinaires, des ateliers et des sessions de formation, ont été organisées, avec une participation moyenne des laboratoires de 75 %.

Commentaires des participants sur des activités spécifiques du projet. Huit enquêtes d'évaluation ont été menées pour recueillir les commentaires des LNR sur les activités du projet.

Les LNR ont apprécié les réunions du réseau, participant activement aux discussions et aux présentations. Le partage des connaissances a été amélioré grâce à une plateforme de communication en ligne et à des groupes d'échange de connaissances, mais le faible taux d'engagement suggère la nécessité d'améliorer les approches à l'avenir.

Les formations du projet ont été perçues comme utiles par la plupart des participants, bien que des contraintes de temps aient parfois limité la participation. Pour y remédier, des supports préparatoires, des sessions enregistrées et du contenu en ligne ont été fournis. Les LNR ont exprimé un besoin accru de sessions pratiques sur l'analyse des données WGS et l'identification des gènes de la RAM, soulignant des besoins de formation futurs.

Les pays prioritaires ont souvent été confrontés à des difficultés liées à l'insuffisance des ressources des laboratoires et à d'autres contraintes au niveau national, qui ont affecté leur soutien. Malgré ces obstacles, des conseils adaptés ont permis de réaliser des progrès significatifs dans la mise en œuvre des WGS et d'améliorer la surveillance de la RAM dans la plupart des pays.

Les LNR ont apprécié le soutien apporté à la cartographie des capacités des réseaux de laboratoires nationaux. Pour l'avenir, ils ont indiqué qu'ils avaient besoin de conseils pour les plans d'échantillonnage de surveillance et la communication avec les laboratoires locaux. En outre, ils ont demandé des formations conjointes pour les épidémiologistes et les microbiologistes, des discussions sur des agents pathogènes spécifiques et l'implication des décideurs en matière de santé.

Les documents d'orientation produits dans le cadre du projet ont été bien accueillis, même si les différences entre les systèmes et les ressources du

secteur de la santé publique nécessitent un soutien et des consultations supplémentaires.

Les exercices annuels d'EQA et d'essais circulaires ont permis d'améliorer les compétences et de garantir la cohérence, la qualité et la comparabilité des données dans tous les pays.

Impact des activités du projet. Dans l'ensemble, les LNR ont indiqué que l'impact du projet était plus important sur les diagnostics de référence et le plus faible sur la fonction de surveillance, d'alerte et de réponse des LNR. Le soutien sur mesure apporté par le projet aux pays prioritaires a permis d'améliorer considérablement les fonctions des LNR grâce aux éléments suivants :

- Augmentation des tests phénotypiques de résistance aux antimicrobiens pour *Salmonella* et *Campylobacter*, tous les LNR ayant pleinement adopté ces tests. L'adoption du WGS a progressé, éliminant progressivement les anciennes techniques d'empreintes digitales. À la fin du projet, 12 des 15 LNR disposaient de capacités WGS, qui ont été largement utilisées pour la détermination de la RAM et la détection des grappes.
- La mise en œuvre du WGS a amélioré la détection des épidémies et la collaboration avec les épidémiologistes et les secteurs alimentaire et vétérinaire.
- Les rapports avec les administrations de santé publique ont démontré l'utilité du WGS en informant sur les besoins futurs.
- La mise en place de réseaux nationaux de laboratoires locaux/régionaux et de systèmes de surveillance a amélioré la communication et la collaboration entre les laboratoires, ainsi que le renvoi des isolats aux LNR. Des lignes directrices et des formations ont été fournies pour soutenir ces réseaux.
- L'établissement de nouvelles collaborations, notamment avec l'équipe FWD AMR-RefLabCap, d'autres LNR et l'ECDC, le partage d'expériences WGS et l'obtention de nouvelles connaissances sur *Salmonella* et *Campylobacter*.

Prochaines étapes pour un soutien futur

Sur la base des besoins et attentes exprimés par les LNR, des suggestions pour un soutien futur dans quatre domaines principaux ont été identifiées.

L’utilisation du WGS pourrait être augmentée si davantage de ressources étaient disponibles pour produire des données WGS et si des formations supplémentaires étaient organisées pour interpréter et communiquer les résultats WGS. Cela limite actuellement une intégration complète du WGS dans la santé publique. Les LNR ayant intégré le WGS ont signalé un manque d’harmonisation et d’accréditation à l’échelle européenne. Un soutien futur pourrait inclure des visites de mentorat prolongées, davantage de formations et

de soutien technique, ainsi que des options de financement pour surmonter ces obstacles.

La collaboration interdisciplinaire et intersectorielle ainsi que le renforcement des capacités doivent être davantage améliorés pour répondre à des défis tels que l'insuffisance de connaissances et de compétences en interprétation des données de séquençage et le manque de collaborations formalisées. Cela pourrait être résolu par des formations, des cadres et des solutions pour le partage et la communication intégrés des données, ainsi que par des collaborations formalisées.

Le rôle des LNR devrait être renforcé pour améliorer la coordination et le soutien des réseaux de laboratoires nationaux. Cela pourrait être facilité par l'établissement de cadres juridiques clairs au niveau de l'UE et au niveau national, ainsi que par l'exploration des possibilités de soutien externe pour sensibiliser les autorités sanitaires et les décideurs politiques, par exemple via des visites et audits de l'ECDC.

Il est également nécessaire de créer davantage d'opportunités pour améliorer le partage des connaissances et la collaboration à l'échelle de l'UE sur des sujets spécifiques. Cela pourrait être accompli par la mise en place de plateformes pour le partage informel de données et des consultations, l'organisation de présentations en ligne et d'ateliers, ainsi que la promotion de visites d'échange entre les LNR.

Le soutien futur devrait continuer et élargir les activités pour *Salmonella* et *Campylobacter* ainsi que pour d'autres pathogènes d'origine alimentaire et hydrique, afin de renforcer la préparation face aux diverses menaces en santé publique.

Conclusions

Grâce à ce projet, un réseau complet de laboratoires a été établi, offrant des opportunités de partage des connaissances et des expériences entre les LNR à travers l'Europe. Les capacités des LNR et des structures associées pour traiter la RAM chez les *Salmonella* et *Campylobacter* à partir d'échantillons humains ont été considérablement améliorées grâce à une série d'activités ciblées.

Pour moderniser les tests diagnostiques et de typage moléculaire à l'aide du WGS, le projet a élaboré plusieurs documents et protocoles clés. Associée à l'organisation d'exercices d'EQA et d'essais circulaires, cette démarche a permis d'harmoniser les standards des tests et de la caractérisation de la RAM à l'échelle européenne.

L'impact de ces améliorations est significatif, car elles contribueront à de meilleurs résultats en matière de santé publique en garantissant une détection

et un signalement rapides et précis des souches résistantes, tant au niveau national qu'européen.

1. Introduction

1.1. Overview of the report

This document constitutes the final report of the "Provision of European Union (EU) networking and support for public health reference laboratory functions for antimicrobial resistance (AMR) in *Salmonella* species and *Campylobacter* species in human samples" with the European Health and Digital Executive Agency (HaDEA) under service contract 20197409. The content of the report is structured as presented in Table 1.

Table 1 – Structure of the report

Section	Content and purpose
Context and purpose of the project	The goal and the aims of the project
Methodology	Organisation of work. Summary of methodologies used to deliver the specific tasks, activities and deliverables. Summary of all tasks and all activities of the project
The main outcomes and results	A short description of work carried out under all tasks and all activities/outputs produced during the entire duration of the contract
Evaluation and impact	Summary of evaluations carried out during the project, participation in project activities, results of participants feedback on project activities and their impact
Next steps for future support	Proposals for further work provided on the basis of project evaluations ("way forward"/ "next steps")
Conclusions	Main conclusions from the project considering overall project purpose and aims
Annexes	Annex 1: List of laboratories that participated in FWD AMR – RefLabCap network
	Annex 2: List of deliverables
	Annex 3: Evaluation of the capacity building activities
	Annex 4: Remaining challenges and needs for future support in priority countries (not publicly available)

1.2. Context and purpose of the project

The service contract was executed jointly by the contractors Statens Serum Institut (SSI)(lead) and the Technical University of Denmark (DTU).

The general objective of this action was to support the beneficiary countries (European Union and European Economic Area (EU/EEA), Bosnia-Herzegovina, Serbia and Moldova) to enhance the validity and accuracy of surveillance data reported at EU level in order to inform concerted actions

against AMR and to enable better detection and control of cross border threats to humans from AMR.

The purpose of the service contract was to provide services to strengthen coordination, support and capacity in national microbiology reference laboratory functions for AMR in *Salmonella* and *Campylobacter* within Europe.

The specific project objectives were:

i) Building capacity in all functions and key roles required for national reference laboratories (NRLs) in public health (PH) in the field of AMR in *Salmonella* and *Campylobacter* from human samples;

ii) Supporting the role of NRLs, and associated structures, to strengthen regional and local laboratory capacities in AMR within the countries;

iii) Modernisation of diagnostic and molecular typing methods using whole-genome sequencing (WGS) to ensure improved and more uniform diagnostics and characterisation of AMR across Europe.

The contract work was done in accordance with the specifications provided in the service contract (Tender text; Annex I to the service contract) and the final inception report (**Deliverable G1.3**) that provided a description of how the services should be provided. Throughout the implementation period the work was closely followed by the contracting authority (HaDEA) and different stakeholders were invited to take part in the coordination of the service contract, in particular the European Centre for Disease Prevention and Control (ECDC) and the European Commission Directorate General for Health and Food Safety (DG SANTE). When relevant, the European Food Safety Authority (EFSA) and the relevant EU reference laboratories in the food and feed control area were also consulted.

The various activities of the service contract have been reported continuously throughout the entire duration of the project. This final report provides an overview and evaluation of all tasks and outputs produced. It includes feedback from NRLs and highlights the main outcomes and achievements of the project, along with suggestions for future activities.

2. Methodology

The project methodology was previously outlined in the final inception report (**Deliverable G1.3**) and is summarised in this section. Progress on specific tasks, activities, results and feedback was reported in general project deliverables (see Table S2 in Annex 2).

2.1. Project organisation

The project organisation included four teams: the management team and three teams that focused on different aspects of the project: networking, training and methods. The management team consisted of the project manager and the leaders of the three other teams. The main focus of the project management team was to ensure a high quality of all deliverables as well as adherence to the time schedule. The networking, training and methods team consisted of team leaders and other team members. The team leaders coordinated the work among the team members and the work between the different teams and tasks. Their responsibility was also to report the progress of all the work to the management team.

2.2. Methodology used to deliver the tasks, activities and deliverables

The project started with a virtual kick-off meeting with the contracting authority and representatives from DG SANTE, ECDC and other relevant bodies (**Deliverable G1.1**). Prior to the meeting, the draft inception report (**Deliverable G2.2**) was provided and during the meeting, it was presented and discussed with the meeting attendees. Feedback from the meeting attendees was incorporated into the final inception report (**Deliverable G1.3**).

Timetables for task and deliverable implementation: timetables in a form of Gantt charts with start and expected finish dates for all tasks and deliverables were developed by the project management team. These tables were used for detailed planning of all project tasks and deliverables and to make immediate changes when needed.

Internal meetings (DTU, SSI, EURGen-RefLabCap): every four weeks, the management team held project management meetings to ensure the timely execution of the tasks, deliverables and risk assessments. Any deviations or delays were discussed and decisions on changes in allocation of resources were taken if necessary to ensure high-quality deliverables. Time was allocated at each meeting to capture immediate feedback from the teams responsible for the specific activities.

Every four weeks the meetings with European Antimicrobial Resistance Genes – Reference Laboratory Capacity (EURGen-RefLabCap) project management team took place to discuss any complementary tasks and their coordination between the two projects.

The leaders of the subject-specific teams had regular meetings with their team members to ensure the coordinating and close collaboration of the relevant personnel working on specific tasks.

Meetings with stakeholders (HaDEA, DG SANTE, ECDC): the management team organised regular progress meetings with all relevant stakeholders where progress on all tasks and deliverables was presented using a developed progress reporting table. During these meetings, the detailed plans of specific tasks and deliverables that needed consultations were presented and discussed. The meetings took place every 2-3 months. Throughout the whole project period 17 meetings were held in total.

Quality control (QC): to ensure high technical and scientific level and quality of all deliverables the project manager ensured that qualified personnel is available for the described work. Replacements were made in case of staff changes. Furthermore, a formalised internal review process and final language proofreading of the draft deliverables was implemented. Specific QC measures were also in place for laboratory procedures, preparation and packaging of reference material, training of staff, organisation of external quality assessment exercises (EQAs), development of surveys, and other relevant measures.

Risk assessment and management: at the start of the project, the management team made a thorough risk analysis of the project which was discussed with the contracting authority and other relevant bodies. The initial risk assessment was followed-up by regular assessments of challenges, possible delaying factors and barriers for producing the expected high-quality deliverables. A template spreadsheet developed during the project was used to track and prioritise the identified risks. The management team carried out systematic risk assessment at least every six months. In the presence of any risks, the management team made decisions, e.g. by allocation more resources, involving other competences, contacting external experts. The challenges and proposed mitigations were reported in the 6-month progress reports.

Project evaluation: templates for evaluation of general activities, physical meetings and teleconferences, as well as proposals for adjustments of future activities were developed at the beginning of the project and applied throughout the course of the project. This procedure ensured the managements team’s possibility of capturing the immediate feedback from the NRLs and the team members and acting on this knowledge as appropriate. The information was further used in the progress reports as well as the interim report for the contracting authority and other relevant bodies.

Progress reports, interim reports, draft final report and final report: throughout the duration of the project, progress reports were delivered every 6

months (**Deliverable G2.1-7**). Progress reporting included progress on planned tasks, deliverables, activities and results as well as a description of the activities planned for the next 6 months. Issues to be discussed with contracting authority, DG SANTE or ECDC, including possible delays and planned remedial action were also covered.

An interim report (**Deliverable G3.1**) was produced halfway through the project. The interim report included a short description of work carried out under all tasks and all activities/outputs produced during the first two years of the project, the main outcomes and results and proposal for further work. A short section was included on the evaluation of the work. In connection to this report, the mid-term meeting (**Deliverable G3.2**) was held with contracting authority, and representatives from DG SANTE, ECDC and other relevant bodies where the content of the report was presented and discussed. The feedback from attendees was incorporated into a revised version of the interim report.

This report, (**Deliverable G4.1**) includes the main outcomes, results and lessons learned together with proposals for future work. A section on the evaluation of work based on the feedback from the PH NRLs is included. The report was provided for contracting authority and representatives from DG SANTE, ECDC and other relevant bodies. Based on the feedback, a final report (**Deliverable G4.2**) will be compiled and submitted according to the requirements for the final report given in the tender specifications page 30-32.

2.3. Summary of all specific tasks and activities of the project

The contractors' detailed methodology for the implementation of specific project tasks and activities was presented in the final inception report (**Deliverable G1.3**). The methodology included an outline and a timetable for implementation of two project tasks and related activities:

Task 1: Generic networking and capacity building activities to strengthen national PH reference laboratory functions for AMR surveillance of human *Salmonella* and *Campylobacter* infections with a specific focus on countries where capacities are less well developed (activities 1.a to 1.q).

Task 2: Activities to support the role of PH NRLs to work with and build capacities in regional and local laboratories in their own countries for the two pathogens: *Salmonella* and *Campylobacter* from humans (activities 2.a to 2.e).

The activities under the two tasks were divided into four main types of related activities: networking, capacity building, method modernisation and evaluation (Table 2). The results and outcomes of all these activities were reported as specific project deliverables as listed Tables S3-S4 in Annex 2 and are described in sections 3 and 4 of this report.

Table 2 – Specific project tasks and their description

Main project activities	Task: activities	Description
Network and networking activities	*1: a, b, c, j*	Network establishment, Website, Meetings with European Union reference laboratories (EURLs) in food and feed control area, Network meetings, other networking activities
Capacity building activities		
Work plan	*1. g*	Work plan that excludes 1. i, l and 2: b, c, e
Priority countries	*1: d, e, f, i*	Requirements, summary report, questionnaire, gap report and priority countries (PCs) selection, action plans
Training activities	*1: k, o*	Hands-on courses on antimicrobial susceptibility testing (AST), multidisciplinary training workshops
Support to NRLs for regional/local capacity building	*2: a, b, c, d*	Mapping exercise, training plan, establishment of national laboratory networks, model protocol
Method modernisation		
Guidance documents	*1: m, n, l* *2. e*	Guidance document for WGS, WGS protocol, updated EU protocol, Internal quality control (IQC) document
EQAs and ring trials	*1: p, q*	Annual EQAs, ring trials
Evaluation	*1. h*	Evaluations carried out during the project and their outcome

3. The main project results and outcomes

In this section, we provide a short description of the results and the outcome of all project activities carried out under Tasks 1 and 2. The list of all deliverables resulting from these activities is provided in Annex 1.

3.1. Laboratory network establishment and networking

3.1.1. Laboratory network (1.a)

The Food- and Waterborne Diseases Antimicrobial Resistance - Reference Laboratory Capacity (FWD AMR-RefLabCap) network of PH reference laboratories for AMR in *Salmonella* and *Campylobacter* in humans was established by April 2021. **Deliverable T1.1** contains a description of the procedure of network establishment. By the end of the project the FWD AMR-RefLabCap network consisted of 46 PH reference laboratories for *Salmonella* and/or *Campylobacter* in 37 countries. Among the 37 countries, 32 represented EU/EEA and EU Health programme countries, and five candidate/potential candidate countries. Participant list is available in Table S1, Annex 1.

3.1.2. Website (1.b)

A project website https://www.fwdamr-reflabcap.eu/ was developed and went on-line on 13 April 2021. The report on the website content was delivered in June 2021 (**Deliverable T1.2**). During the project, the website was continuously updated with the new information. Major improvements to the website structure were performed in March 2023. The current website version contains: a description of the project, contact information, a list of the participating countries and laboratories, resources of relevant materials and guidance documents, information and materials from the past and upcoming EQAs, ring trials and other project events. The website is planned to be accessible until December 2026. After this date, we expect that the most important materials will be made available on the new website of the new EURL in PH for food- and waterborne bacteria.

3.1.3. Meetings with EURLs in the food and feed control area (1.c)

During the project, two online meetings were held with representatives from the EURLs for *Salmonella*, *Campylobacter*, AMR and STEC (cross-EURL working group on NGS) in the food and feed control area as well as representatives

from ECDC, EFSA, HaDEA and DG SANTE. The first meeting was in March 2021 and the second in March 2023. During these meetings, the activities planned in the FWD AMR-RefLabCap project and related to the work of the EURLs and potential collaboration in a project framework were discussed. These meetings provided the opportunity to exchange experiences and coordinate and align the activities, particularly on the development and harmonisation of WGS activities. **Deliverables T1.3.1-2** contain the meeting agendas, minutes and presentations.

3.1.4. Network meetings (1.j)

During the project, three network meetings were organised. Due to the pandemic and the related travel/meeting restrictions the first meeting was held virtually on 30 November and 1 December 2021. The mid-term and the final meetings were held in Copenhagen, Denmark on 26-27 April 2023 and on 29-30 October 2024, respectively. In all meetings, the participants representing all network laboratories, relevant EURLs in food safety, ECDC, HaDEA and DG SANTE were invited. The meetings' agenda typically included: updates from the authorities, past and upcoming project activities, presentations from EURLs in food safety, breakout and panel discussions and countries' presentations on relevant topics. **Deliverables T1.10.1-3** contain the agenda, outcome and evaluation of the meetings. The meeting's agenda and presentations are also available on the project's website.

3.2. Capacity building activities

3.2.1. Work plan for capacity building activities (1.g)

In agreement with the HaDEA, ECDC, DG SANTE and the network members, a work plan for capacity building activities in the project was developed. The plan contained description of all project activities, except activities for priority countries (Task 1.i) and for local capacity building support (Task 2.b). The work plan was presented at the first network meeting and was submitted as **Deliverable T1.7**.

3.2.2. Support to countries with the greatest needs

3.2.2.1. Recommendations of minimum and optimal requirements (1.d)

The *Recommendations of minimum and optimal requirements in the NRLs for Salmonella and Campylobacter AMR surveillance systems at the national level* document was prepared and discussed with ECDC. It was built on the three PH microbiology system dimensions and the five core functions of microbiology reference laboratories described in the EU Laboratory capability monitoring system report (EULabCap) and Core functions of microbiology reference laboratories for communicable diseases, respectively. The final version of the document was submitted as **Deliverable T1.4** and it was presented at the first network meeting.

3.2.2.2. Summary report and draft questionnaire (1.e)

Using minimum and optimal requirements (**Deliverable T1.4**) as a basis, a report summarising existing knowledge of capacity and capability for testing and surveillance of AMR in *Salmonella* and *Campylobacter* in human samples within the network was developed (Part 1). In addition, a questionnaire was designed to assess the current reference laboratory services and capacity (Part II). Both parts were developed in consultation with ECDC, HaDEA and DG SANTE and were submitted as **Deliverable T1.5**.

3.2.2.3. Gap report and identification of priority and additional countries (1.f)

The questionnaire developed as part of **Deliverable T.1.5** (Part II) was distributed among the network to obtain a full overview of the capacities and capabilities in the NRLs, to identify gaps in all countries. Based on this, 12 “priority countries” with greatest needs for capacity building and three “additional countries” facing challenges to identify and manage outbreaks of *Salmonella* and *Campylobacter* were selected. The final comprehensive gap report (**Deliverable T1.6**) contains an outcome of consultation with contracting authority, DG SANTE and ECDC, and includes all the data from the survey and a report on the selection of the priority and additional countries.

3.2.2.4. Action plans and implementation (1.i)

The 12 countries identified in Task 1.f accepted “priority country” status in the project. Following acceptance, a meeting for all priority countries was held to

explain the selection criteria and to introduce a tailored technical and operational support plan for 2022-2024. The minimum and optimal requirements (**Deliverable T1.4**) document was shared with the “priority countries” to serve as a basis for their action plan development. To support individual action plan development, teams of 2-3 experts from the contractor institutions (“country teams”) were dedicated to work with each of the priority countries. In the period of M13-M24 country teams provided individual support to 12 priority countries (Task 1.f) for action plan development for national surveillance of AMR in *Salmonella* and *Campylobacter* including implementation of WGS. The support was provided through individual visits to each of the priority countries during 2022, regular online meetings as well as email correspondence (Task 1.i). All 12 countries submitted individual action plans which are the main part of **Deliverable T1.9**. In the project period of M25-M48, the country teams continued to provide tailored/operational support to the priority countries for implementation of the action plans (Task 1.i).

The task also included financial support to the NRLs from the “priority countries” and the “additional countries” identified in Task 1f. This support could be used to purchase consumables/equipment for implementation of the action plan and/or to support activities dedicated to capacity building in regional/local laboratories in the country. The funding was granted following a simple application procedure agreed with HaDEA in February 2024. By November 2024, 10 NRLs from 10 countries were granted 63.670 EUR in financial support ranging from 5300 to 7494 EUR per country. The money was predominantly used by countries to purchase reagents for WGS and *Salmonella* antisera. A report to HaDEA on the financial support provided to NRLs in relation to this task (reimbursable according to the contract) was submitted on 29 November 2024.

A detailed outcome of the support to the priority countries and the additional countries was presented at the network meetings, described in the progress reports (**Deliverables G2.3-7**), in the interim report (**Deliverable G3.1**) and in section 4.3.2 of this report.

3.2.3. Training activities

3.2.3.1. Hands-on courses (1.k)

Two courses on phenotypic testing methods of *Salmonella* and *Campylobacter* were held at DTU in Denmark on 16-17 May and 18-19 May 2022, respectively. The programme of the courses was tailored based on the outcome of the survey (Task 1.e) and network input during the first network meeting (Task 1.j). The main focus of both courses was best practice training and laboratory exercises for harmonised phenotypic testing of *Salmonella* and *Campylobacter*

using AST (MIC and disk diffusion). In addition, training on species/serovar identification and genotypic determination of AMR using WGS was provided. The organisation, training materials, and evaluation of both courses was provided in **Deliverable T1.11**. Training materials were shared with participants by email and are also available on the project website.

3.2.3.2. Multidisciplinary training (1.o)

The plan for multidisciplinary training for PH epidemiologists and microbiologists was prepared and delivered as **Deliverable T1.15**. According to this plan, three annual multidisciplinary training workshops were held online on 3-4 November 2022, 23-27 October 2023, and 24-28 June 2024. The main aim of these workshops was to improve the collaboration between microbiologists and epidemiologists for integration of WGS to national AMR surveillance and outbreak investigations of human *Salmonella* and *Campylobacter*. These workshops included technical presentations on cluster detection, relevant presentations related to the value of WGS, outbreak investigations at the national and at the European level delivered by the project team, ECDC and the participants. During the 2nd and 3rd workshops tabletop exercises for outbreak investigation for both species were carried out. For the tabletop exercises, participating countries were divided into groups of 5-6 countries of same (2nd workshop) or different (3rd workshop) levels of WGS. The groups could meet on specific days to discuss the exercise and at the same time share their experience and own approaches regarding different steps of outbreak investigation. These discussions were summarised by the project team and any raised issues were discussed on the last day of the workshop. The organisation and participants' feedback on all multidisciplinary workshops were reported in progress reports (**Deliverables G1.4-7**), interim report (**Deliverable G3.1**) and in this report (section 4.2). The presentations from the three workshops were shared with participants by email and are available on the project website.

3.2.4. National surveillance and local capacity building support

3.2.4.1. State-of-play reports (2.a)

Following the work plan (T1.7), the project team carried out four webinars/virtual trainings, developed a questionnaire, and a summary report template with the aim to support countries in mapping and evaluation of regional and local laboratories capacities. All materials from these activities are available on the project website and were described in progress reports (**Deliverables G1.5-7**). Overall, 21 NRLs representing 19 countries conducted the mapping exercise and submitted summary reports in English. All reports were evaluated with the

aim of identifying strengths, weaknesses and needs common to all countries as well as elements which may be particular to specific countries. The evaluation was summarised in a consolidated report. The summary reports in English of each country and a consolidated report were submitted as **Deliverable T2.1** on 12 June 2023. The consolidated report was made available on the project website.

A financial support of up to 5000 EUR in January 2023, to compensate for the expenses related to the mapping exercise was provided to countries conducting the mapping exercise. Twelve out of 21 eligible NRLs applied and received the financial support according to the procedure agreed with HaDEA in November 2022 and re-confirmed in December 2023. A final report of the “Payments to the NRLs for the mapping work” was sent out to HaDEA in March 2024.

3.2.4.2. Plan to support NRLs for local capacity building (2.b)

The plan with a list of activities aimed for supporting the NRLs in building national capacity at the local and regional laboratories over the following three years was developed and submitted as **Deliverable T2.2**. In accordance with the approved plan, the project team carried out a number of trainings: two project management virtual training workshops on making the business cases and sustainability plans in May 2022, a webinar on how to plan an EQA in February 2023, a 2-days physical training workshop in March 2024, and a webinar on how to calculate sample size for surveillance in October 2024. Some of these activities were set in the training plan (Task 2.b) and others were organised based on the countries’ needs expressed following the mapping exercise (Task 2.a). The organisation and participants’ feedback on all activities were reported in progress reports (**Deliverables G1.5-7**), interim report (**Deliverable G3.1**) and in section 4.2 of this report. The materials from all activities were shared with participants by email and are available on the project website.

3.2.4.3. Plan to support establishment of national laboratory network (2.c)

A plan with activities to support the NRLs in establishing and coordinating national (sentinel) network of regional and local laboratories for AMR monitoring in human *Salmonella* and *Campylobacter* was developed and submitted as **Deliverable T2.3**.

Following the approved plan, the project team conducted a survey to ascertain the current NRLs knowledge about their national laboratory networks for national surveillance of AMR in *Salmonella* and in *Campylobacter*, including their capacities for pathogen detection and characterisation. The results from

the survey were used to identify needs for support from FWD AMR – RefLabCap project.

The survey results showed that a national laboratory network for *Salmonella* and for *Campylobacter* was absent in 33% and 44% countries, respectively. Similar percentage of countries did not have regular communication with local/regional laboratories. The survey also showed that in > 40% of the countries AMR testing in *Salmonella* and in *Campylobacter* was performed in local/regional laboratories, and >68% of the respondents did not have knowledge about the capacity for pathogen detection/characterisation in the local/regional laboratories. Insufficient AMR surveillance for *Salmonella* and *Campylobacter* or lack of knowledge about it was reported by 33% and 60% of countries, respectively. The survey results were presented to the network and discussed in a webinar on 3 October 2022 in relation to task 2.a.

To address the identified gaps, the project team included this theme in other activities, in connection to other related activities in other tasks of the project: the work with the priority countries (Task 1.i), train the trainers workshop (Task 2.b) and multidisciplinary training workshops (Task 1.o). Reporting on implementation of specific activities related to this task was provided in relation to the above activities in the progress reports (**Deliverable G1.4-7**) and in the interim report (**Deliverable G2.1**).

3.3. Method modernisation

3.3.1. Guidance documents

3.3.1.1. WGS review and protocol (1.l, 1.m)

A *Report on proposed methodologies set out in a methodological guidance document, including the most important resistance genes for the purpose of detection and tracing epidemic high-risk clones and epidemic plasmids of human Salmonella and Campylobacter isolates with the scientific background supporting the choice* was submitted as **Deliverable T1.12**. This document includes the results of a literature review and mapping of international and national guidance on genomic-based monitoring of AMR in human *Salmonella* and *Campylobacter* infections. This review and other available information formed the scientific basis of the proposed methodologies for AMR gene detection and its integration into routine WGS-based surveillance activities. Based on this document an *Agreed common protocol for whole genome sequencing-based analysis for detection and tracing of epidemic clones of antimicrobial resistant Salmonella and Campylobacter* - to be used for national surveillance and integrated outbreak investigations by PH NRLs - was prepared

considering ECDC and network feedback. The final version of this protocol was submitted as **Deliverable T1.13**. This protocol describes how to perform WGS-based analysis of *Salmonella* and *Campylobacter*. The protocol covers the steps of obtaining high-quality DNA, performing library preparation and sequencing of the DNA and performing bioinformatics analysis. The protocol also suggests specific QC strategies, QC parameters and recommended thresholds and gives recommendations on bioinformatics tools and reference gene databases for the detection and prediction of AMR determinants in *Salmonella* and *Campylobacter*.

3.3.1.2. Updated ECDC protocol (1.n)

The existing EU protocol for harmonised monitoring of antimicrobial resistance in human *Salmonella* and *Campylobacter* was reviewed and amendments have been proposed to encompass the use of WGS. The amendments to the protocol focused on monitoring genetic determinants and integration of these to foodborne outbreak investigations in a One Health approach. The *Proposal for the updates on the EU protocol for AMR monitoring in human Salmonella and Campylobacter infections* was sent for consultation with the network members and the EURL for AMR and a final version prepared based on the feedback received, was submitted as **Deliverable T1.14.1**. A presentation of this document was given to the network in November 2022 in the 1st multidisciplinary workshop online.

3.3.1.3. Model protocol for national surveillance of AMR (2.d)

To support the NRLs in implementation of the updated EU protocol for AMR monitoring, a *Model protocol for national surveillance of AMR in human Salmonella and Campylobacter infections* was developed in consultation with ECDC, the laboratory network, and the EURL for AMR in food and feed control area. The model protocol provides suggestions for how the laboratories that function as NRLs on AMR in *Salmonella* and *Campylobacter* can set up the national surveillance of AMR in their countries in line with the EU case definitions. It covers the procedures beginning from when isolates are obtained at primary diagnostic laboratories to the actual testing performed according to the EU protocol, including suggestions for setting up representative national sampling schemes. The draft and the final versions of the protocol were submitted as **Deliverable T2.4.1** and **Deliverable T2.4.2**, respectively. In addition, it was presented to the network at the first multidisciplinary workshop in November 2022.

3.3.1.4. Guidance document on internal QC schemes (2.e)

The project team has developed *Guidance document on internal quality control schemes for reference antimicrobial susceptibility testing and detection of genetic determinants of antimicrobial resistance for Salmonella and Campylobacter isolates from human samples*. The protocol provides guidance to the NRLs and their countries' local laboratories regarding the methods and processes that should be in place for routine IQC of AST. The document describes the standardised and/or recommended methods for AST of *Salmonella* and *Campylobacter* in Europe, and the proposed methods for detecting relevant AMR determinants. It also provides practical advice for routine IQC and includes examples of control strategies and examples of schemes for registering important testing details. The document was submitted as **Deliverable T2.5** and was made available on the project website. The presentation to the network was made on 23 March 2023.

3.3.2. EQAs and ring trials

3.3.2.1. External quality assessment exercises (1.p)

A plan for three annual EQA exercises on WGS-based resistome profiling and clone identification in resistant *Salmonella* and *Campylobacter* was developed (**Deliverable T1.16**). Following an approved plan, the first annual EQA (EQA1-WGS-AMR) was initiated in May-June 2022 in coordination with the ongoing ECDC EQA schemes for AST in *Salmonella* and in *Campylobacter* (EQA-AST). It included three *Salmonella* and three *Campylobacter* strains, which were distributed to the registered participants. The second and the third EQAs were organised in the period from March to June 2023 and in the period from February to May 2024, respectively. Both EQAs included three strains of each species and the participants received DNA samples for conducting their own sequencing and bioinformatics analyses. The main focus of these EQAs was to evaluate participants' ability to produce good quality sequences for genomic determination of AMR using either own (1st and 2nd EQA) or suggested (3rd EQAS) bioinformatics workflow. After each EQA, the result evaluation and recommendations for sequencing QC and bioinformatics workflows were provided in the anonymised reports, which were submitted as **Deliverables T1.16.1-3.** The annual EQA reports were shared with the participants by email and were made available on the project website. In addition, results were presented and discussed with the network members at the network meetings and webinars.

3.3.2.2. Ring trials (1.q)

The overall idea of annual inter-laboratory ring trials of bioinformatics pipelines for prediction of AMR genes in *Salmonella* and *Campylobacter* was presented in the Work Plan (**Deliverable T1.7**). The first annual ring trial (RingTrial1-WGS-AMR) took place in September-November 2022, the second in March-June 2023, and the last one in March-June 2024. From three to four sequences of an increasing complexity per species were provided to the participants of these three exercises. In all exercises, the participants could use their own bioinformatics workflows for AMR determination. The execution and the anonymised outcome from each of the three ring trials were presented in the annual reports, which were submitted as **Deliverables T1.17.1-3** and were shared with the participants by email and on the project website. In addition, results were presented and discussed with the network members at the network meetings and webinars.

4. Evaluation and impact

4.1. Participation in project activities

During the project, 31 activities, divided as described in Table 2, and corresponding to the various tasks and subtasks of the project (tasks 1a-1q and 2a-2e) were organised. All network laboratories funded by the project, i.e., representing EU/EEA countries and the three additional EU health programme countries, were invited to participate in all project activities (46 laboratories). Five laboratories representing five other EU candidate/potential candidate countries were invited to all activities, but their presence in the physical events, and participation in EQAs and ring trials was not covered by the project.

Table 3 summarises the participation of network laboratories in all project activities. Laboratory participation ranged from 41% to 100%, with an overall average participation rate of 75%.

Table 3 – Countries participation in network activities

Activities	Type of activity	Date	No. of NRLs present/invited (participation %) [A]
1. Network and networking activities			
General introduction (part of 1.j and 1.f)	Video conference	7 September 2021	45/45 (100%)
1st Network meeting (1.j)	Video conference	30 November – 1 December 2021	45/45 (100%)
Mid-term Network meeting (1.j)	Physical meeting	26-27 April 2023	36/46 (78%)
Final Network meeting (1.j)	Physical meeting	29-30 October 2024	27/46 (59%)
2. Capacity building activities			
NRL capacity survey (1.f)	Survey	September 2021	45/45 (100%)
Joint priority countries meeting (1.i)	Video conference	27 January 2022	13/14 (93%)
Hands-on AST course *Salmonella* (1.k)	Laboratory training course	May 17-18 2022	25/31 (81%)
Hands-on AST course *Campylobacter* (1.k)	Laboratory training course	May 19-20 2022	27/31 (87%)
1st Multidisciplinary workshop (1.o)	Virtual training workshop	3-4 November 2022	35/45 (78%)

Table 3 – Countries participation in network activities (continued)

Activities	Type of activity	Date	No. of NRLs present/invited (participation %) [A]
2nd Multidisciplinary training workshop (1.o)	Virtual training workshop	23-27 October 2023	36/46 (88%)
3rd Multidisciplinary training workshop (1.o)	Virtual training workshop	24-28 June 2023	24/46 (52%)
Project management workshop Part I (2.b)	Virtual training workshop	23 May 2022	12/17 (70%)
Project management workshop Part II (2.b)	Virtual training workshop	12 September 2022	9/17 (53%)
Mini-survey (2.c)	Survey	September 2022	39/45 (87%)
1st workshop: Mapping exercise (Introduction) (2.a)	Webinar	3 October 2022	37/45 (82%)
2nd workshop: Mapping exercise (Strategy) (2.a)	Virtual training workshop	26 October 2022	31/45 (69%)
3rd workshop: Mapping exercise (Data analysis, interpretation and dissemination) (2.a)	Virtual training workshop	31 January 2023	31/46 (76%)
How to plan an EQA (2.b)	Webinar	14 February 2023	26/46 (57%)
The outcome of the mapping exercise (2.a)	Webinar	23 November 2023	31/46 (76%)
Train-the-trainers workshop (2.b)	Physical training workshop	12-13 March 2024	17/41 (41%)
How to calculate sample size for surveillance (2: b,c)	Webinar	18 October 2024	31/41 (76%)
3. Method modernisation			
WGS protocol discussion (1.m)	Webinar	6 May 2022	39/45 (87%)
Guidance document on IQC schemes (2.e)	Webinar	23 March 2023	30/46 (65%)
EQA1-WGS-AMR (1.p)	EQA exercise	2022	25/40 (62%)
RingTrial1-WGS-AMR (1.q)	Ring trial exercise	2022	23/40 (58%)
EQA1-WGS-AMR and the RingTrial1-WGS-AMR results (1: p, q)	Webinar	7 February 2023	26/46 (57%)
EQA2-WGS-AMR (1.p)	EQA exercise	March-June 2023	30/41 (73%)
RingTrial2-WGS-AMR (1.q)	Ring trial exercise	March-June 2023	36/41 (88%)

Table 3 – Countries participation in network activities (continued)

Activities	Type of activity	Date	No. of NRLs present/invited (participation %) [A]
Highlights from EQA2-WGS-AMR and RingTrial2-WGS-AMR (1: p, q)	Webinar	1 March 2024	30/46 (65%)
EQA3-WGS-AMR (1.p)	EQA exercise	February-May 2024	30/41 (73%)
RingTrial3-WGS-AMR (1.q)	Ring trial exercise	March-June 2024	32/41 (78%)

[A] when 46 or 45 means all NRLs participating in the project were invited, when 40 or 41 – the NRLs means representing candidate/potential candidate countries were not invited. Of note, the number of laboratories changed since January 2023 when *Campylobacter* NRL was established in one of the countries and joined the project.

4.2. Participant feedback on specific project activities

To collect participants feedback, eight surveys were carried out: six activity-specific surveys, the mid-term evaluation survey (M1-M24) and the final project evaluation survey (M25-M39). The evaluations from these surveys were summarised in the two short evaluation reports delivered as **Deliverables T1.8.1-2** and are summarised in Annex 3, Tables S5-S7. The conclusions from the first evaluation report were also presented in the Interim project report submitted as **Deliverable G3.1.** In this report, we provide a summary of the participants feedback based on the above evaluations, considering the major points that should be addressed in the future work.

In addition, in this report we include results of last two activities-specific evaluations that were carried out in M40-M46, and thus were not included in the above reports: 3rd EQA exercise (Task 1.p), 3rd Ring trial exercise (Task 1.q), 3rd Multidisciplinary training workshop (Task 1.o), webinar on how to calculate sample size for surveillance (Tasks: 1b-c) and Final network meeting (Task 1.j) (Annex 3, Table S8).

Network and networking activities: Participants' feedback highlighted the excellent organisation and value of the meetings, along with suggestions for improvements. Frequently, the participants expressed the need for a discussion forum on laboratory procedures and bioinformatics. To address this, after the first network meeting, we created a project workspace on Slack, a cloud-based team communication platform. Furthermore, during and after the mid-term meeting, the project team initiated working groups on relevant topics to enhance collaboration between the participants. Unfortunately, a low activity level was observed on the Slack channel and in the working groups, presumably due to busy schedule of the participants, reservations to initiate discussions, or a need for more active external coordination. Despite the low participant activity in the

above initiatives, participants were active during the group interactions and country presentations in the mid-term and in the final network meetings, and therefore they were prioritised also in other project activities. Further exploration of methods to encourage ongoing knowledge sharing beyond the project's duration was discussed during the final project network meeting and the outcome is presented in section 5 of the report.

Capacity building activities: capacity building activities included activities that were common to all project participants (1.f, 1.k, 1.o), activities that were dedicated to participants that had biggest gaps in phenotypic and genomic *Salmonella* and *Campylobacter* testing and characterisation namely “priority countries” (1.i), and activities that were dedicated to the priority countries and to the additional countries selected under task 1.f (Tasks: 2.a, 2.b, 2.c).

Among the common project activities, two hands-on training workshops on harmonised phenotypic methods for *Salmonella* and *Campylobacter,* and three multidisciplinary training workshops received overall positive feedback. Considering these trainings, a number of participants expressed a need for additional hands-on sessions on: WGS data analysis, raw data evaluation and handling, AMR gene identification, cluster detection, prediction of antibiotic susceptibility based on the genomic findings and phylogenetic clustering, discrepancies when analysing phenotypic and genotypic data, including more technical comparisons and links to tools. Of note, these needs were mostly expressed by countries that are at the beginning of WGS implementation, which are mostly priority countries. To address these needs priority countries were encouraged to initiate pilot WGS studies and received individual support on sequence data analysis and interpretation, upon request. Furthermore, the need for training in cluster detection was addressed during the annual multidisciplinary training events, which included technical presentations and group discussions for PH microbiologists and epidemiologists on results interpretation for investigation of outbreaks including multistate outbreaks.

Feedback from many participants also highlighted time constraints faced by the participants due to workload and staffing issues, leading to limited engagement in project activities. To enhance participation, several improvements were implemented in the final year of the project, including: the provision of precomputed results in a third multidisciplinary workshop, emphasising the relevance of presentations, sharing preparatory materials, recording online sessions, and disseminating content through the website. Additionally, discussions on event outcomes missed by priority countries were held during regular progress meetings to make sure their inclusion despite their absence in these events.

Among the activities that were specific for the 12 priority countries were joint priority countries meetings, country visits, and tailored consultancy for action plan development and implementation, and financial support carried out under task 1.i.

Among the challenges that were often mentioned by these countries were poor communication between different PH authorities in the country, absence of collaboration between PH and veterinary-food sector, insufficient staffing and financial resources allocated to laboratories to support their performance in all five core functions of NRL. Due to the above challenges, the project's "country teams" had to adapt to existing situations in different countries and to guide the laboratories according to their abilities and needs. It was also difficult for both priority countries and the contractor to receive and to distribute the financial support according to the tender specifications due to complexity of procurement procedures and budget restrictions at the NRLs which led to unused funding. Despite these challenges, towards the end of the project, all countries had made relevant improvements such as initiating/improving AMR testing, starting pilot studies of WGS and participating in WGS-based ring trials and/or EQAs (see section 4.3.2 for details).

In addition, participants expressed a continued need for training and advice to facilitate the transition to WGS-based surveillance for strengthening laboratory capacity in AMR surveillance for *Salmonella* and *Campylobacter* in their country.

The project's support in assessing the capacities of regional and local laboratories (Task 2.a) and in carrying out capacity building at regional and local level (Task 2.b) was well received by both priority and additional countries. Participants recognised the difficulty of assessing capacities and valued the insights gained with the help of project members. Some also highlighted the need for consistent political support for NRLs to improve their efforts.

Participants noted areas where additional support is needed:

i) How to obtain representative samples for AMR surveillance;
ii) How to select samples for WGS-based outbreak detection and investigation.

To address this, a webinar was organised (linked to Task 2.c). Further training needs were also identified: bioinformatic analysis of WGS, development of sampling plans for surveillance and strategies for engaging with local laboratories. For future activities, the participants proposed joint training for epidemiologists and clinical microbiologists on *Salmonella* and *Campylobacter* infections, discussions on *Salmonella* Typhi and Paratyphi, detection of AMR genes in pathogens, and understanding the entire surveillance setup. Additionally, they emphasised the importance of involving health policy makers to ensure sustainability and securing funds for implementation.

Method modernisation activities: to support countries in method modernisation for genetic AMR detection and characterisation in *Salmonella* and *Campylobacter*, the project team developed several guidance documents described in section 3.3.1. Participant feedback and consultations/discussions

with the network revealed that, overall, these documents are useful for all members in the network due to the limited existing national and/or international guidance for AMR surveillance in *Salmonella* and *Campylobacter*. However, some of the network members pointed out that due to the existing differences in PH systems/resources in the countries there is a need of further support/consultations from the project team to be able to adapt the guidance to specific situations, especially when more than one solution is suggested in the documents. The project team addressed the need for such support as part of support for the priority countries (Task 1.i), and remaining countries were further consulted during activities related to local/regional capacity building support (Task 2.b), support for the establishment of national surveillance network (Task 2.c), and training activities of the project.

The participants' proficiency in detecting AMR determinants was tested through their participation in annual EQA exercises (Task 1.p) and ring trials (Task 1.q). Participants emphasised the value of these exercises, noting that discussions and advice from skilled peers significantly enhanced their proficiency on the area. The participation also provided valuable training in WGS analysis, result sharing, and method improvement, ultimately offering reassurance regarding consistency, comparability and quality across the countries.

4.3. Impact of project activities

To assess the impact of the project's activities, as part of the final project evaluation survey, the following evaluations were conducted:

i) an evaluation of the project's impact/effect on the capacity and capability building in NRL's functions for *Salmonella* and *Campylobacter* throughout the whole project period from 2021 to date.

ii) an evaluation of the project's support to priority countries by conducting structured interviews.

The overall strategy and draft content of these evaluations were discussed and agreed with HaDEA, DG SANTE and ECDC in February 2024. The outcome of these evaluations is exclusively presented in this report and has not been presented elsewhere.

4.3.1. Project's impact on the capacity and capability building in NRLs

The evaluation of the project's impact/effect on the capacity and capability building in NRL's functions for *Salmonella* and *Campylobacter* was included in the final project evaluation survey. The respondents indicated the status of their

country in the project as of March 2024 (priority, other, candidate/potential candidate).

Further, they had to rate a beneficial effect/impact of the project on the capacity and capability at their NRL for these reference functions: i) reference diagnostics, ii) monitoring, alert and response, and iii) collaboration and research. The respondents were asked to rate the beneficial effect/impact at a scale from 1 to 5, where 1 represented very low effect/impact and 5 represented very high effect/impact. An option 'not applicable' was chosen, if improvements were not needed at the start of the project.

Evaluation of the project impact on the capacity and capability of the NRL for the reference diagnostics showed high impact to priority countries (Median value (Mdn): 4.5) and medium impact (Mdn: 4.0) to countries with a different status in the project. This was in particular evident in ratings given for the reference diagnostics aspects related to WGS implementation and analyses (Table 4).

Table 4 – Project impact on the capacity and capability for the reference diagnostics function in NRLs

Country status[A]/ Improvements	"Priority countries" (n=14)		Other countries (n=17)		Candidate/potential candidate countries (n=5)	
Median score or "not applicable" response[B]	**Median score**	**NA[C]**	**Median score**	**NA**	**Median score**	**NA**
Salmonella and/or *Campylobacter* detection (culture or PCR-based)	4.0	5	3.0	7	4.0	0
Species identification	4.0	5	2.5	7	4.0	1
Serovar identification	4.5	6	3.0	8	3.0	1
Phenotypic antimicrobial susceptibility testing	4.0	3	3.0	4	4.0	1
WGS implementation	4.5	0	4.0	3	4.0	1
Genotypic detection of antimicrobial resistance genes and point mutations (not including WGS)	4.0	1	4.0	4	3.0	1
Laboratory part of WGS (from DNA extraction to sequencing)	5.0	1	4.0	4	4.0	1
Bioinformatic WGS data analysis	5.0	0	4.0	0	4.0	1
WGS data interpretation for surveillance and outbreak investigations	4.5	0	4.0	1	3.5	1
IQC schemes at the NRL	4.5	2	3.0	2	3.5	1
The outcomes of participation in EQAs/ring trials	5.0	0	4.0	0	4.0	2
Total median score	**4.5**		**4.0**		**4.0**	

[A] Numbers in the brackets indicate number of countries that rated at least one of the listed items.

[B] Median values were calculated to present responses from countries with different status in the project. A indicates the number of respondents that chose "not applicable option" corresponding to improvements were not needed at the start of the project. The colouring is based on score categories: low impact (<2), medium impact (2-4), high impact (>4).

[C] Indicates 'not applicable' which was chosen, if improvements were not needed at the start of the project.

In relation to their ratings, one priority country commented that *"[…] Main focus was in WGS method and supporting clinical microbiology laboratories".* In addition, one country with the "other countries status" highlighted that *"It would be great to continue activities in order to strengthen our NRL and local laboratories capacities regarding surveillance of Salmonella and Campylobacter"* and another noted that *"We only participated in subset of activities based on our needs"*.

A fairly high number of countries chose "not applicable" in relation to other than WGS aspects of reference diagnostics. This indicates that many countries already had well developed procedures for these diagnostic areas of *Salmonella* and *Campylobacter* and thus the support was not relevant (Table 4).

The project impact on the capacity and capability for monitoring, alert and response function in NRLs was rated lower than the impact on the reference diagnostics. However, similarly as for the reference diagnostics, the impact was higher for priority countries (Mdn: 4.0) than for the candidate/potential candidate countries (Mdn: 3.0) and other countries (Mdn:2.5) (Table 5).

Table 5 – Project impact on the capacity and capability for monitoring, alert and response function in NRLs

Country status [A]/ Improvements	"Priority countries" (n=14)		Other countries (n=17)		Candidate/potential candidate countries (n=5)	
Median score or "not applicable" response[B]	**Median score**	**NA[C]**	**Median score**	**NA**	**Median score**	**NA**
Sample collection (referrals) for surveillance and/or outbreak investigations	4.0	3	1.0	6	3.0	0
Providing a national advisory role to support to clinical laboratories in your country	4.0	2	3.0	5	3.0	0
Providing support to outbreak investigations and management in your country	4.0	1	3.0	5	2.0	0
Communication with epidemiologists in surveillance and outbreak situations	4.0	2	3.0	3	3.0	0

Table 5 – Project impact on the capacity and capability for monitoring, alert and response function in NRLs (continued)

Country status [A]/ Improvements	"Priority countries" (n=14)		Other countries (n=17)		Candidate/potential candidate countries (n=5)	
Median score or "not applicable" response[B]	**Median score**	**NA[C]**	**Median score**	**NA**	**Median score**	**NA**
Collaboration with veterinary and food authorities in surveillance and outbreak situations	3.0	2	2.5	5	3.0	0
Developing or improving national guidelines for screening, testing and/or reporting of relevant pathogens	4.0	2	2.0	6	3.0	0
Reporting of laboratory results to users in your country (e.g. in outbreak/cluster analysis, early warning, etc.)	4.0	1	2.0	6	3.0	0
Reporting of laboratory results at the European level	4.0	0	3.0	4	3.0	0
Information and communication systems for data sharing, alert and response	4.0	0	2.5	3	3.0	0
Total median score	**4.0**		**2.5**		**3.0**	

[A]Numbers in the brackets indicate number of countries that rated at least one of the listed items.

[B]Median values were calculated to present responses from countries with different status in the project. A indicates the number of respondents that chose "not applicable option" corresponding to improvements were not needed at the start of the project. The colouring is based on score categories: low impact (<2), medium impact (2-4), high impact (>4).

[C]Indicates 'not applicable' which was chosen, if improvements were not needed at the start of the project.

One priority country commented that *"Moderate effect/impact was rated because these areas are to be improved constantly. During the project these have already improved and work is ongoing to gain high effect."*

Generally low rates received by respondents in candidate/potential candidate countries may be influenced by the fact that their participation in the project activities was not covered by the project.

This function was mostly addressed through the national capacity building activities in Tasks 2.a and 2.b where the main participants were priority countries and only few other countries. This may explain why other countries rated the impact of this function low.

The evaluation further revealed that the project impact on the capacity and capability for collaboration and research function in NRLs was higher for candidate/potential candidate countries (Mdn: 3.5) than for priority countries (Mdn: 3.0) and other countries (Mdn: 2.8) (Table 6).

Table 6 – Project impact on the capacity and capability for collaboration and research function in NRLs

Country status [A]/ Improvements	"Priority countries" (n=14)		Other countries (n=17)		Candidate/potential candidate countries (n=5)	
Median score or "not applicable" response[B]	**Median score**	**NA[C]**	**Median score**	**NA**	**Median score**	**NA**
Participation in national research and/or capacity building projects	4.0	1	3.0	5	4.0	0
Participation in international research and/or capacity building projects (other than FWD AMR-RefLabCap)	3.0	1	2.5	7	2.0	0
Participation in national reference laboratory networks	3.0	3	2.0	9	4.0	0
Participation in international reference laboratory networks	3.0	1	3.0	6	3.0	0
Total median score	**3.0**		**2.8**		**3.5**	

[A]Numbers in the brackets indicate number of countries that rated at least one of the listed items.

[B]Median values were calculated to present responses from countries with different status in the project. A indicates the number of respondents that chose "not applicable option" corresponding to improvements were not needed at the start of the project. The colouring is based on score categories: low impact (<2), medium impact (2-4), high impact (>4).

[C]Indicates 'not applicable' which was chosen, if improvements were not needed at the start of the project.

One of the candidate/potential candidate countries commented that early detection of food and waterborne infections and monitoring pathogen virulence, AMR, and genotype distribution nationwide was enabled in relation to the development of a 5-year National Genomic Surveillance Strategy which specifically focused on COVID-19, but also provide added value by introducing genomic surveillance to existing surveillance systems. One of the other countries also highlighted that *"Networking at the face-to-face meetings helped with this area".* Overall, the results from this evaluation indicate the need for continued networking and capacity building activities to further strengthen collaboration and research in the area of AMR surveillance in *Salmonella* and *Campylobacter*.

As part of the evaluation survey, the participants were asked whether they have a plan for sustaining the improvements obtained since 2021. The results showed that the majority of the NRLs have a plan or the planning is in progress

(Table 7). Survey respondents who have plans to sustain improvements highlighted several key points. Some noted that once new services or techniques are introduced, procurement processes will continue automatically, with new tenders issued as needed. Others emphasised the need for technical support and a qualified workforce to enhance genomic surveillance, aiming to improve inter-sectoral collaboration across PH, food safety, and environmental sectors following the One Health approach. Some plan to optimise genomic analysis for AMR surveillance based on the project suggestions, while others expressed concerns about limited future funding impacting the use of WGS for AMR prediction.

Table 7 – Sustainability plans

Country status	"Priority countries" (n=14)	Other (n=17)	Candidate/potential candidate (n=5)
Yes, we have a plan	5 (36%)	8 (47%)	1 (20%)
Not yet, but planning is in progress	7 (50%)	5 (29%)	3 (60%)
No, we do not have a plan	2 (14%)	4 (24%)	1 (20%)

In the additional comments to the survey, respondents expressed a mix of gratitude and challenges. Some appreciated the scientific support, the friendly atmosphere, and the opportunity to establish lasting professional relationships. They highlighted the value of networking, workshops, and webinars in enhancing their practices and gaining insights from other countries' experiences. However, a few respondents noted that despite participating, they had not seen significant improvements in their laboratory capacity, particularly with WGS. Candidate countries expressed concerns about limited involvement due to staffing issues and lack of funding but hoped for greater participation in the future. Overall, respondents valued the training, resources, and networking opportunities provided by the project, while looking forward to further engagement and support.

4.3.2. The outcome of structured interviews with the priority countries

In August/September of 2024, the last progress meeting with each NRL from the priority countries was dedicated to conduct a structured interview. The interview was aimed to make a final evaluation of the implementation of action plans, project impact on the progress and to identify remaining challenges and needs for support in these countries.

To prepare for these structured interviews, each priority country team was asked to complete a structured interview template by providing some brief

answers/key points to the questions. It was highlighted that the replies should be discussed with the entire team that has been involved in the FWD AMR-RefLabCap work. In the follow-up interviews, there was an opportunity to elaborate on the answers in more detail. The interviews were conducted by the project team members that normally provide bespoke consultancy to the NRL.

The structured interviews consisted of seven sections:

1. NRL Action plan implementation and outcome
2. Remaining challenges and continuation
3. Evaluation of dedicated support activities for priority countries
4. Proposals for further support activities
5. Further evaluation of bespoke consultancy
6. Proposals for improvement of bespoke consultancy activities
7. Evaluation of technical and operational capacity at the NRL

1. NRL Action plan implementation and outcome: the action plans of priority countries focused on all five core functions of microbiology reference laboratory. Below we summarise the most important achievements and outcomes identified by NRLs for each function separately.

Reference diagnostics: The 15 NRLs have mentioned a number of main achievements in various areas of reference diagnostics. One NRL signified their improvement in *Campylobacter* isolation. One NRL highlighted introduction of PCR methods to identify and classify *Campylobacter* strains. Four NRLs started to perform AST and/or enhanced their methods and protocols for AST. Genotypic AMR testing, other than WGS, was initiated by one NRL. Achievements in WGS capacity building at NRL or outsourced WGS was mentioned by nine NRLs. Bioinformatics training and infrastructure improvements were noted in eight NRLs, including building data analysis pipelines and acquiring necessary equipment. Lastly, participation in EQAs was noted by 4 NRLs, helping them to identify and improve gaps in their genomic and AST capabilities.

Reference materials: NRLs did not note achievements for this function.

Monitoring, alert and response: among the main achievements in this area, one NRL highlighted that *"the project helped us establish a national reference center for campylobacteriosis […]"*. One NRL established a national sentinel surveillance system for AMR, three NRLs formed and one started to form a national network of microbiology laboratories, and one formed a sentinel network of laboratories. All these NRLs enhanced their communication and collaboration with these networks. Three NRLs noted an increased referral of *Salmonella* and/or *Campylobacter* isolates from the regional and local

laboratories to the NRLs. Five NRLs signified activities that they initiated to support the national laboratory networks. One of the NRLs issued concise guidelines for the isolation, identification, and AST of *Salmonella* and *Campylobacter*, conducted courses for microbiology specialists to improve their qualifications, provided guidance for rapid and accurate identification of *Salmonella* and *Campylobacter* with loop-mediated isothermal amplification (LAMP) for hospital care or outbreak cases. Another NRL published several guidance documents for local and regional laboratories. One NRL applied harmonised protocols for AMR and disseminated them in the network of clinical labs. One NRL was content by the established contacts with national laboratory network and ability to assist with *Campylobacter* diagnostics. Lastly, one of the NRLs noted the organisation of the first proficiency testing round to strengthen the sentinel laboratory network's capacity for AST of *Salmonella*.

Five laboratories highlighted improved outbreak investigations through the implementation of WGS and enhanced collaboration with epidemiologists and/or food and veterinary laboratories. One NRL expanded regular sequencing for outbreak investigations in collaboration with both epidemiologists and the veterinary sector. Another NRL initiated routine sequencing of 20-30% of *Salmonella* isolates, focusing on outbreaks and travel-related cases, and organised regular meetings to discuss achievements in *Salmonella* sequencing. Sequencing of *Salmonella* and *Campylobacter* from clinical, animal, and environmental samples improved interdepartmental communication and collaboration through regular online meetings, leading to better outbreak detection and characterisation. One NRL began outbreak investigations of selected *Salmonella* and *Campylobacter* in collaboration with epidemiologists. Lastly, another NRL improved collaboration among microbiologists, epidemiologists, and bioinformaticians for *Salmonella* investigations.

Collaboration and research: the *Campylobacter* NRL in one country expressed satisfaction with the established contact with the *Salmonella* NRL, highlighting the benefits of sharing experiences, particularly regarding WGS. They also emphasised the value of new and updated information on *Campylobacter* obtained from the project, ECDC, and international colleagues. Another NRL echoed the importance of networking and exchanging experiences with other NRLs. Additionally, two NRLs noted the crucial support from ECDC for WGS, which enabled them to receive their first sequencing data. Lastly, one NRL highlighted a new collaboration with a food-veterinary institute, resulting in joint sequence analyses of *L. monocytogenes* and a forthcoming publication, setting a precedent for other pathogens.

Scientific advice: two NRLs highlighted their activities in providing scientific advice. The *Salmonella* NRL from one priority country issued a final report based on mapping findings to the Ministry of Health (MoH). They also prepared the first annual report on AMR in *Salmonella*, which will be delivered to the national epidemiology department and the MoH next year. Another NRL drafted

a technical report on the findings of the WGS surveillance of *Salmonella*. This report will be adapted for sharing with the MoH to demonstrate the achievements and benefits of WGS, as well as to inform about further needs.

2. Remaining challenges and continuation: all NRLs have identified ongoing challenges and needs to sustain and improve their laboratory capacities across different areas of their work.

Eleven NRLs face common challenges in implementing WGS, including insufficient funding, lack of skilled personnel, and inadequate infrastructure. Some laboratories are slowly progressing with library preparation and sequencing, while others struggle with limited access, staffing shortages, and the need for external funding, such as EU funds. Routine application of WGS for pathogens like *Campylobacter* and *Salmonella* is a goal for many, but maintaining and expanding WGS capabilities requires continuous investment in training, equipment, and bioinformatics support. Additionally, some laboratories are seeking international expertise to address local skill gaps.

Seven NRLs face common challenges in sample/isolate referral for AMR testing, including the need for official national guidelines and improved referral processes. Laboratories often rely on hospital data, which often lacks comprehensive AMR testing, making data interpretation difficult. There is a need for harmonised national guidelines to ensure consistent testing practices. Additionally, increasing the referral of samples from regional and provincial labs is crucial, with some countries working on new regulations to improve this process. Specific needs include recruiting more clinical labs to send isolates, enhancing referral from both public and private labs, and securing resources for AMR testing.

Six NRLs face challenges in outbreak investigation and collaboration, particularly the need for improved communication and structured collaboration with epidemiologists and food-vet authorities. NRLs often struggle with limited collaboration, which hampers the application of WGS to confirm clusters and outbreak sources. There is a need for regular, formalised meetings and standard operating procedures (SOPs) to enhance communication. Specific challenges include complex organisational structures, unclear roles, and responsibilities, and the necessity for better data exchange and collaboration during outbreak situations. Formalising communication and collaboration with stakeholders, including state food and veterinary services, is essential for effective outbreak investigation and data sharing at the European level.

Five NRLs also face general challenges related to staffing and funding. There is a need to build dedicated teams, often starting with training young colleagues and involving them in capacity building activities. Securing stable financing for NRL activities is a priority, as is resource allocation for daily work related to surveillance and outbreak investigation. Developing and agreeing on testing algorithms with epidemiologists impacts future budgets. Direct involvement from

organisations like ECDC can help raise awareness of these gaps and develop plans to address them, e.g. with visits and audits potentially increasing media publicity and support.

Four NRLs face common challenges in WGS data analysis and interpretation. There is a need for better comprehension of bioinformatics results and evaluation, as well as external guidance to interpret cluster thresholds during outbreaks. As sequencing efforts increase, more challenges with data interpretation are anticipated. Specific needs include improving the interpretation of sequencing results for outbreak investigations, including QC, AMR, and virulence factors.

Three NRLs emphasised challenges in data sharing and reporting at the European level, including mismatched case and laboratory data due to unconfirmed epidemiologist reports and unresolved technical issues with reporting systems like Tessy. There is a need for formal collaboration procedures between PH organisations and agricultural ministries to establish common databanks for AMR results. Additionally, technical difficulties in uploading WGS and epidemiologic data to ECDC applications, coupled with a lack of support, hinder effective data sharing and reporting.

Two NRLs expressed the need to gain accreditation for *Campylobacter* species identification, and for AST according to the harmonised protocol for AMR.

Two NRLs face challenges in supporting regional and local labs, particularly in implementing QC in routine activities. There is a need to introduce QC standard operating procedures (SOPs) to local laboratories and organise training activities based on their specific needs and new guidance or policies. Additionally, increased collaboration with regional and local labs is necessary but often hindered by a lack of staff and budget.

Two NRLs highlighted challenges in data sharing and reporting at the national level, with a common need for improved real-time reporting systems and digitalisation of data. There is a need for shared platforms or common interfaces between laboratories and surveillance units to facilitate electronic surveillance and enhance data exchange. Specific needs include developing real-time reporting capabilities and integrating digital tools to streamline data sharing and reporting processes.

One NRL noted the lack of legitimate and official nomination as a National Reference Laboratory.

3. Evaluation of dedicated support activities for priority countries: all NRLs in priority countries provided feedback on dedicated support activities and how the activities affected the reference laboratory capacity.

NRL country visit: NRLs found the project team visits highly beneficial for enhancing their capacities. Common benefits included improved contact with project coordinators, support in implementing action plans, and better understanding of key priorities such as national mapping exercises and strengthening local and regional labs. Specific advantages varied, with some NRLs gaining valuable insights into WGS data analysis, presenting their capacities, and preparing grant applications. The visits also facilitated direct exchanges of expertise, strengthened networking and collaboration, and helped clarify project frameworks. Many NRLs emphasised the importance of face-to-face meetings for effective communication and planning, and some suggested longer or additional visits to further support their needs.

NRL action plan: the development of action plans significantly impacted their capacities by providing structured frameworks and clear objectives. Common benefits included improved strategic planning, better self-assessment, and enhanced communication and collaboration among laboratories. Specific challenges varied, such as the need for more staff and time to implement the plans fully, and initial confusion with the templates. Overall, the action plans were seen as beneficial for tracking progress, setting milestones, and guiding future activities, despite some difficulties in execution and the need for periodic updates and adjustments.

Bespoke consultancy via video calls/e-mail: this activity significantly improved the capacities of NRLs by providing regular support, knowledge exchange, and encouragement. These consultations were invaluable for clarifying uncertainties, particularly in implementing action plans and introducing new surveillance methods. They offered professional and friendly support, detailed explanations, and procedural guidance. While some NRLs found the consultancy more general due to their current stage of equipment procurement, others benefited from discussing specific issues and next steps. The frequency and format of these meetings were generally well-received, with suggestions for shorter but more frequent sessions. Overall, the consultancies were seen as beneficial for making timely corrections, improving collaboration, and building new capacities.

WGS pilot study: the pilot studies, which were funded either from own funds or through ECDC support, significantly improved the capacities of NRLs by enabling them to sequence numerous isolates, build data analysis pipelines, and upload data to platforms like EpiPulse. Common benefits included improved surveillance of outbreaks and AMR genes, and the creation of databases for ongoing monitoring. Specific challenges included the complexity of WGS data analysis for some participants and the need for further technical guidance. The study also facilitated the establishment of laboratory networks, encouraged participation from various labs, and provided valuable data for scientific publications and future surveillance efforts. Overall, the pilot study was

seen as a crucial step in advancing WGS capabilities and improving PH responses.

Mapping exercise of regional/local laboratories capacities (if applicable): the mapping exercise significantly improved the capabilities of NRLs by providing an overview of clinical microbiology capacities, ensuring laboratory quality, and improving communication and feedback. Common benefits included gaining insights into the real situation of infection diagnosis, identifying gaps in methodologies, and fostering collaboration with local stakeholders. Specific advantages varied, such as using survey platforms to create tailored questionnaires, learning to conduct surveys, and establishing networks for better diagnostic testing and sample referral. The exercise also highlighted the need for standardised laboratory methodologies and facilitated the collection of valuable data for future improvements.

Train-the-trainer workshop in March 2024: this activity significantly impacted NRLs by providing practical knowledge, valuable networking opportunities, and insights into addressing and communicating challenges within laboratory networks. Common benefits included the exchange of experiences and solutions to common issues, and the establishment of important contacts with experts from different countries. Specific feedback varied, with some participants finding the workshop highly useful for improving NRL functions and fostering collaborations, while others felt it could have offered more. The availability of workshop materials online was appreciated by those unable to attend in person, ensuring broader access to the information shared.

Financial support for the mapping exercise and for the action plan implementation: the support significantly improved the capacities of NRLs by enabling them to evaluate clinical microbiology capacities, procure necessary materials, and organise their activities. Common benefits included the ability to conduct mapping exercises, order kits for practice, and upgrade diagnostic methods. Specific advantages varied, such as using funds for scientific activities, improving antibiotic susceptibility testing, and supporting the procurement of essential lab materials. However, some NRLs faced challenges with financial procedures and legal constraints, which hindered the realisation of support.

Overall, the financial aid was highly appreciated and recognised as a crucial factor in advancing laboratory capacities.

4. Proposals for further support activities: NRLs were asked to suggest new support activities that were not provided in FWD AMR-RefLabCap during the past 3 years and explain how they could benefit the development of reference laboratory capacity.

The suggested support activities focus on several common themes, such as: the need for improved training in genomic data interpretation, bioinformatics,

and the use of WGS. Many countries highlighted the importance of collaboration with coordinators and organisations such as ECDC to analyse pilot study results, refine sampling strategies, and improve pathogen-specific evaluations. There is also a shared need for better integration of national-level information exchange and expanded support for NRLs to handle a broader range of pathogens.

Specific points include the necessity for affordable laboratory equipment, administrative support to overcome budgetary hurdles, and the organisation of twinning exchanges and mentor visits to develop expertise. Some countries emphasised the importance of ongoing support for sending DNA samples to commercial laboratories for WGS, especially during outbreaks. Additionally, the creation of online platforms for problem-solving and detailed technical support for using platforms like EpiPulse were also suggested to improve overall surveillance and response capabilities.

5. Further evaluation of bespoke consultancy:

The agenda/structure of video meetings: the feedback on the agenda and structure of video meetings was overwhelmingly positive. Common points included the clarity, relevance, and well-structured nature of the agendas, which were seen as appropriate and aligned with the participants' needs. The meetings were consistently pre-scheduled and organised, providing necessary information to meet targets and address relevant issues. Specific points highlighted the detailed and fluid nature of the agendas over time, the usefulness of collaboration with project coordinators, and the recommendation for future consultancies via video calls or email. Some countries also appreciated the bespoke consultancies and the responsiveness of the agendas to their specific needs and actions.

The frequency and length of video meetings: the feedback on the frequency and length of video meetings was generally positive. Common points included that the meetings were held at appropriate intervals and durations, allowing sufficient time to discuss key issues and implement discussed actions without being overly long. Specific points highlighted the meetings' effectiveness despite busy schedules.

Follow-up on video meetings/emails (effectiveness and timeliness): The feedback on follow-up for video meetings and emails was highly positive. Common points included the quick, efficient, and timely nature of follow-ups, with additional information and action points being forwarded promptly. Specific points highlighted the preference for follow-ups via email to address specific queries without taking up meeting time, and the consistent support and guidance provided by the team, which made participants feel secure and well-supported in their new tasks.

Composition of the support team in terms of their expertise: the feedback on the composition of the support team was overwhelmingly positive. Common points included the team's high level of expertise, knowledge, and readiness to assist with queries. The support team was praised for their professionalism, helpfulness, and the ability to provide valuable guidance and consultation. A specific suggestion was made to consider adding a clinical microbiologist to the team to further enrich its composition.

6. Proposals for improvement of bespoke consultancy activities.

Countries suggested several changes to enhance future bespoke consultancy activities. Common points included the need for mapping individual or country-specific needs and providing targeted consultancies, as well as the preference for smaller group meetings for easier contact and discussion. Specific suggestions included longer face-to-face visits for mentoring, involving healthcare authorities in consultancies, and providing formal written statements about project goals. Additionally, on-site expert presence for training, sharing data for feedback, and detailed analysis of NRL capacities were recommended. Some countries were satisfied with the current format and did not suggest changes.

NRLs also explained how the changes could benefit the development of reference laboratory capacity at their NRL. For example, making consultancy need-based and educational, as well as organising smaller group meetings, could enhance collaboration and ease of contact. Longer face-to-face visits to well-established institutions were recommended to gain practical experience. Involving healthcare authorities in the project could ensure better decision-making and funding support. QC and reliability of methods could be improved through expert on-site presence and data sharing. Recommendations from projects and ECDC could help secure funding and explain the importance of activities to public authorities. Some countries were satisfied with the current format, while others suggested simplifying financial support processes for future projects.

7. Evaluation of technical and operational capacity at the NRL: NRLs evaluated the changes in their capacity and capability for *Salmonella* and *Campylobacter* from 2021 to 2024, focusing on phenotypic and genotypic AMR testing, WGS status, and the use of WGS for AMR determination and cluster detection (Table 8). The evaluation revealed an increase in phenotypic AMR testing for both pathogens, with all NRLs in priority countries now performing these tests. Molecular typing of *Salmonella* and *Campylobacter* using methods other than WGS remained stable or increased slightly. Most laboratories maintained or introduced PCR-based methods for AMR gene characterisation and species/serovar identification. However, with the introduction of WGS, alternative methods for cluster detection, such as PFGE and MLVA, were phased out. By the end of the project, 12 out of 15 NRLs had WGS equipment or access to it. A significant proportion, 81% for *Salmonella* and 64% for

Campylobacter, began using WGS for AMR determination and cluster detection. Importantly, most NRLs that implemented WGS noted it is not used routinely. Typically, it is applied only retrospectively to a selected number of isolates, often those associated with outbreaks. The primary reason for its limited use is the lack of dedicated funding for sequencing. The laboratories which did not implement WGS by the end of the project were those that were just recently established, those that are in progress of implementations and those that have major issues in laboratory funding and/or staff.

Table 8 – Technical and operational skills at 15 NRLs representing priority countries before and after-action plan implementation

Type of Implementation [A]	Pheno_AMR_S	Pheno_AMR_C	Geno_AMR_S	Geno_AMR_C	ST_S	ST_C	WGS_AMR_S	WGS_AMR_C	WGS_ST_S	WGS_ST_C	WGS_Status
Before (2021) [B]	11	9	5	1	3	3	0	0	1	1	4
After (2024) [B]	12	12	6	4	4	3	9	7	9	7	12

[A] Description of the columns: Pheno_AMR: NRL performs phenotypic AMR testing (research or surveillance), Geno_AMR: NRL performs genotypic AMR testing (except WGS, research or surveillance), ST: NRL performs molecular strain typing (ST) (except WGS, research or surveillance), WGS_AMR: NRL is using WGS for AMR detection (research or surveillance), WGS_ST: NRL is using WGS for strain typing (ST) (research or surveillance), WGS Status: NRL has WGS equipment or access to it. S and C indicate implementation for *Salmonella* and *Campylobacter*, respectively.

[B] The numbers before and after consider changes in 12 *Salmonella* and 12 *Campylobacter* NRLs in all implementations, except WGS Status where all 15 NRLs are considered.

The status of capacity for selected indicators of NRL core functions at the end of the FWD AMR - RefLabCap project, remaining challenges and needs for future support in each priority country are summarised in Table 9 and Table S9 in Annex 4.

Table 9 - The status of capacity for selected indicators of NRL core functions at the end of the FWD AMR-RefLabCap project

A Gray – not relevant or implemented before 2021, green – implemented in 2021-2024, yellow- in progress, white – not implemented.

B S and C indicate laboratory for *Salmonella* and *Campylobacter*, respectively.

C Currently outsourced sequencing

5. Next steps for the future support

The needs for future support were identified from the analysis of the NRLs' feedback during the project evaluations and from a group discussion on the future needs carried out during the final network meeting of the project. Based on the above, suggestions for expanded future support in the following four areas were identified:

1. Enhancing the utilisation of WGS

Significant progress was made during the project in expanding the use of WGS for AMR testing and cluster detection in *Salmonella* and *Campylobacter* in additional NRLs. However, evidently, WGS is not yet fully integrated into PH actions in many of these laboratories due to limited resources, lack of experience in data interpretation, and challenges in result communication. Additionally, NRLs that have fully integrated WGS, noted the lack of WGS harmonisation and accreditation across Europe. The NRLs expressed the needs for future support which may help to overcome these barriers:

- Facilitating longer, face-to-face mentoring visits to NRLs:
  - To provide training and QC recommendations
  - To provide consultations on adapting relevant EU guidelines to different PH systems and contexts
- Organising further training and technical support for WGS:
  - In-depth data analysis and interpretation
  - Upload of WGS and epidemiological data to EpiPulse and its use
  - Protocols for harmonised use of short- and long- read-based WGS
  - Guidance for WGS accreditation
- Providing funding options, which may include:
  - Simplified financial support processes for future projects
  - Maintenance of external support for WGS and data analysis
  - Opportunities for EU funding.

2. Improving interdisciplinary and cross-sector collaboration and further capacity building

The project stimulated increased collaboration between NRLs, epidemiologists, and veterinary-food safety laboratories for data sharing and joint outbreak investigations using WGS. However, challenges remain, such as epidemiologists and food-veterinary laboratories having insufficient knowledge on the utility of sequencing data and lacking the skills for sequence data interpretation for outbreak investigations and characterisation of AMR. The absence of formalised collaborations in outbreak investigations is also a challenge. To address these, greater efforts are needed in:

- Joint trainings for epidemiologists and microbiologists on outbreak investigation and characterisation of AMR
- Providing a framework for data sharing and interpretation, including improved communication between the microbiologists and epidemiologists
- Facilitating integrated WGS data storage, sharing and analysis between NRLs and food-veterinary laboratories
- Formalising communication and collaboration between governmental food and veterinary services and the NRLs.

3. Strengthening further the NRLs role

The NRLs role in AMR surveillance has been strengthened through WGS implementation and capacity building in the national surveillance network activities of the project. Despite this, the NRLs often noted the existence of vague national laws with undefined NRLs role, obligations by regional and local laboratories regarding providing data and/or isolates to NRL for reference testing as well as complex PH organisation and limited support from the government. To address the above challenges, the NRLs' role should be further reinforced for better coordination and support of national laboratory networks for AMR surveillance and outbreak detection. The improvements in this area may be fostered by:

- Providing a clearer and more comprehensive understanding of the legal framework at European and national levels:
    - To simplify PH structures
    - To better specify the roles and responsibilities within the PH structures
- Exploring the possibilities for external support that could raise the awareness among national healthcare authorities and policymakers:
    - For example, through ECDC visits targeting specific topics/pathogens, and comprehensive analyses of NRL capacities, accompanied by recommendations for improvements.

4. Cross-border collaboration and networking

The activities of the project provided opportunities for cross-border collaboration and networking for AMR surveillance and in outbreak situations. However, the NRLs expressed a need for further opportunities to enhance EU wide collaboration. This may be addressed by:

- Identifying experts on specific topics and creating platforms for:
    - Informal data sharing and consultations
    - Organising online presentations and workshops
    - Fostering exchange visits between the NRLs.

Ultimately, the NRLs highlighted that it is important to continue and expand the project's activities for *Salmonella* and *Campylobacter,* including other food- and waterborne pathogens.

6. Conclusions

A range of networking, capacity building and method modernisation activities were provided to 46 NRLs in PH from 37 countries that participated in the FWD AMR-RefLabCap project, with the purpose to strengthen coordination, support and capacity in national microbiology reference laboratory functions for AMR in *Salmonella* and *Campylobacter*.

The project successfully established a comprehensive laboratory network across European countries. To facilitate interaction between NRLs, physical and online meetings, promoting knowledge exchange through group discussions and country presentations were organised. Participants provided feedback and suggestions, and interacted with key stakeholders such as the ECDC and DG SANTE for updates on EU-level AMR policies. The involvement of EURLs in food and feed control area provided insights into activities related to foodborne pathogens within the One Health context. The project website served as a valuable resource, offering comprehensive information and access to relevant materials, both about the activities in the project, but also protocols, legislative documents, and reports from relevant stakeholders. The project activities conveyed interactions between NRL personnel, led to new collaborations, and provided updated information on pathogens from the project, ECDC, and international colleagues.

The capacity of NRLs to address AMR in *Salmonella* and *Campylobacter* from human infections was significantly improved through a series of targeted activities. Network members received comprehensive hands-on training to enhance their proficiency in high-quality AMR testing using phenotypic and genotypic methods. Their skills in outbreak investigations were also improved through multidisciplinary training involving WGS and collaboration with epidemiologists. These sessions provided practical experience in best practices for AMR testing, introduced bioinformatics tools for resistance prediction, and included simulated outbreak exercises. Group discussions facilitated experience sharing and addressing challenges. The project team provided technical guidance through presentations and protocols, enhancing participants' understanding of the techniques. As a result, countries gained valuable knowledge and experience in AMR surveillance and outbreak detection and reporting, both nationally and internationally. The project also focused on capacity building in countries with significant gaps in AMR testing skills. Tailored support strengthened laboratories' roles in reference diagnostics, monitoring, alert, and response. New reference laboratories for *Campylobacter* were established, and many laboratories implemented WGS for AMR characterisation and outbreak investigations. Activities also supported national surveillance and local capacity building, leading to the establishment of national or sentinel laboratory networks for AMR surveillance. This improved collaboration, sample/isolate referral to NRLs, and ultimately strengthened

national AMR surveillance systems for *Salmonella* and *Campylobacter*, enhancing monitoring at both national and European levels.

To modernise diagnostic and molecular typing tests using WGS, the project developed several key documents and protocols. These provided guidance on:

i) standard procedures for harmonised phenotypic AMR testing;
ii) implementation of WGS for detecting resistant clones;
iii) standardised and recommended methods for AMR detection, including routine QC processes;
iv) establishing or improving national surveillance systems for both pathogens, with suggestions for setting up representative national sampling schemes.

These documents were invaluable additions to the project's training and capacity building activities and will serve as a foundation for maintaining and enhancing capacities across NRLs and national laboratory networks in the future. Annual EQAs and ring trials were organised to evaluate participants' ability to produce high-quality sequence data for genomic characterisation of AMR, and annual ring trials to assess their proficiency in identifying AMR determinants using their own bioinformatics workflows. These activities were highly valuable for participants, enabling them to evaluate their performance and make improvements in their laboratory procedures and bioinformatics workflows. These activities were also instrumental for the project team, providing insights for enhancing future EQA and ring trial organisation. Overall, these initiatives have contributed to more updated and harmonised methods for testing and characterisation of AMR across Europe.

Overall, through dedicated efforts, the NRLs capabilities and capacities for *Salmonella* and *Campylobacter* were improved, leading to more effective monitoring and management of AMR and outbreaks at local, regional, national, and European levels. The impact of these improvements is significant, as they will contribute to better PH outcomes by ensuring timely and accurate detection and reporting of resistant strains nationally and to the European level.

7. Annexes

7.1. Annex 1: FWD AMR – RefLabCap laboratory network

Table S1 - List of laboratories in FWD AMR-RefLabCap network

Country	Affiliation
Albania	Institute of Public Health, Laboratory of Enterobacteriology
Austria	Austrian Agency for Health and Food Safety, Institute for Medical Microbiology and Hygiene Graz, National Reference Centre for Salmonella
	Austrian Agency for Health and Food Safety, Institute for Medical Microbiology & Hygiene, Centre for Foodborne Infectious Diseases, National Reference Centre for Campylobacter
Belgium	Sciensano, NRC Salmonella
	NRC Campylobacter, University Laboratory Brussels
Bosnia and Herzegovina	Public Health Institute of The Republic of Srpska, Department of Microbiology
Bulgaria	National Center for Infectious and Parasitic Diseases, NRL of Enteric Infections, Pathogenic cocci and Diphtheria
Croatia	Croatian Institute of Public Health, NRL for Salmonella
Cyprus	Nicosia General Hospital, National Reference Laboratory for Salmonella and other Enteric Pathogens, Microbiology Department
Czechia	National Institute of Public Health, National reference laboratory for antibiotics
Denmark	Statens Serum Institut, Section for Foodborne Infections Dept. Bacteria, Parasites and Fungi
Estonia	Health Board, The Laboratory of Communicable Diseases
Finland	Finnish Institute for health and Welfare, Expert microbiology
	Clinical Microbiology Laboratory, Turku University Hospital
France	Institut Pasteur, National Reference Centre for E. coli, Shigella & Salmonella
	CHU de Bordeaux, CNR Campylobacters et Hélicobacters
Germany	Robert Koch Institute, NRC Salmonella
Greece	University of West Attica, National Reference Laboratory for Salmonella and Shigella
	National Public Health Organization, Central Public Health Laboratory
Hungary	National Public Health Center, FWD National Reference Laboratory
Iceland	Landspítali University Hospital, Dept. of Clinical Microbiology
Ireland	University Hospital, Galway, National Salmonella Shigella and Listeria Reference Laboratory, University Hospital, Galway

	Cherry Orchard Hospital, HSE-Public Health Laboratory-Dublin Mid Leinster
Italy	Istituto Superiore di Sanità, Infectious diseases department
Kosovo	National Institute of Public Health of Kosovo, National Reference Laboratory for Antimicrobial Resistance
Latvia	Riga East University Hospital, Infectology Centre of Latvia, National Microbiology Reference laboratory
Lithuania	National Public Health Surveillance Laboratory
Luxembourg	Laboratoire National de Santé, Bactériologie-Mycologie-Antibioresistance-Hygiène Hospitalière
Malta	Mater Dei Hospital, Bacteriology laboratory
Moldova	National Agency for Public Health, Microbiological laboratory
Montenegro	Institute of Public Health of Montenegro, Reference laboratory for monitoring resistance to antimicrobial drugs
North Macedonia	Institute of Public Health, Department for bacteriology
Norway	Norwegian Institute of Public Health, National Reference Laboratory for Enteropathogenic Bacteria
Poland	National Institute of Public Health - National Institute of Hygiene, Department of Bacteriology and Decontamination Control
Portugal	National Institute of Health Dr. Ricardo Jorge, NRL of Gastrointestinal infections - NRL of Salmonella and NRL of Campylobater
Romania	National Institute of Medico-Military Research and Development Cantacuzino, Bacterial Enteric Infections Laboratory
Serbia	Institute of Public Health of Serbia, Reference Laboratory of Salmonella
	Institute for Public Health, Reference Laboratory for Campylobacter and Helicobacter
Slovak Republic	Public Health Authority of the Slovak Republic, NRC for Salmonelloses and NRC for ATB resistance monitoring
	Public Health Authority of the Slovak Republic, NRC for Campylobacter
Slovenia	Centre for Medical Microbiology, National laboratory of health, environment and food, Department for Public Health Microbiology Ljubljana
Spain	Instituto de Salud Carlos III, Centro Nacional de Microbiología, Laboratorio de Referencia e Investigación en Enfermedades Bacterianas Transmitidas por Agua y Alimentos
Sweden	Folkhälsomyndigheten, Dept of Microbiology, MI-LB
The Netherlands	Wageningen University and Research Centre, Wageningen Bioveterinary Research (WBVR)
	National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control
Turkey	Ministry of Health, General Directorate of Public Health, National Reference Laboratory for Enteric Pathogens

7.2. Annex 2: List of deliverables

Table S2 - General deliverable list

Deliverable	Description	Date (month)
G1.1	**Kick off meeting** Luxembourg (agenda, presentations, minutes of the meeting and other relevant documents)	01-2021 (M1)
G1.2	Draft inception report: Description of work, timetable, risk analysis	01-2021 (M1)
G1.3	**Final inception report**	03-2021 (M3)
G2.1	Progress report 1: progress, achieved results; plan for next 6 months	06-2021 (M6)
G2.2	Progress report 2: progress, achieved results; plan for next 6 months	12-2021 (M12)
G2.3	Progress report 3: progress, achieved results; plan for next 6 months	06-2022 (M18)
G2.4	Progress report 4: progress, achieved results; plan for next 6 months	12-2022 (M24)
G3.1	**Interim report** Description of work first 24 months, main outcomes and results, proposals for further work. Evaluation of the work	12-2022 (M24)
G3.2	**Mid-term meeting**	01-2023 (M25)
G2.5	Progress report 5: progress, achieved results; plan for next 6 months	06-2023 (M30)
G2.6	Progress report 6: progress, achieved results; plan for next 6 months	12-2023 (M36)
G2.7	Progress report 7: progress, achieved results; plan for next 6 months	06-2024 (M42)
G4.1	Draft final report	10-2024 (M46)
G4.2	**Final report** Description of work carried out under all tasks, main outcomes and results, lessons learns, proposals for further work. Evaluation of the work	12-2024 (M48)

Table S3 - Deliverables linked to Task 1

Deliverable	Activity	Responsible team	Description	Date (month)
T1.1	1.a	Network	Establish NRL network, directory of contact details of labs and focal points	04-2021 (M4)
T1.2	1.b	Network	Website live	04-2021 (M4)
T1.3	1.c	Network	Two 1-day meetings: EURLs, ECDC, EFSA, DG SANTE. Meeting report	03-2021 (M3) 01-2023 (M25)
T1.4	1.d	Network	Recommendations of requirements in PH NRL, AMR surveillance at national level (→T2.4)	06-2021 (M6)
T1.5	1.e	Network	Summary report and questionnaire	08-2021 (M8)
T1.6	1.f	Network	Report on gaps and priority countries	10-2021 (M10)
T1.7	1.g	Training + Network	Work plan for technical support activities	11-2021 (M11)
T1.8	1.h	Network	Short evaluations reports, lessons learned and further work	01-2022 (M25) 06-2024 (M42)
T1.9	1.i	Training	Action plans for priority countries	12-2022 (M24)
T1.10	1.j	Network	3 network meetings, meeting reports with concluding statements	10-2021 (≤M12) 04-2023 (≤M36) 10-2024 (≤M48)
T1.11	1.k	Training	Two 2-day training courses, phenotypic testing	11-2022 (≤M24) 11-2022 (≤M24)
T1.12	1.l	Methods	Methodological guidance document, incl. resistance genes for detection/tracing epidemic clones	02-2022 (M14)
T1.13	1.m	Methods + Network	Report with agreed common WGS-based genome analysis methods and standard protocols	04-2022 (M16)
T1.14	1.n	Methods	Proposal for updated EU protocol for AMR monitoring Revised version	06-2022 (M18) 08-2022 (M20)
T1.15	1.o	Methods + Training	Training plan, multidisciplinary	06-2022 (M18)
T1.16	1.p	Methods	Proposed WGS-based EQAs; Report from 1st EQA exercise (10-2022) Report from 2nd EQA exercise (10-2023) Report from 3rd EQA exercise (10-2024)	08-2022 (M18) 12-2022 (≤M24) 12-2023 (≤M36) 12-2024 (≤M48)
T1.17	1.q	Methods	Report 1st ring trial (08-2022) Report 2nd ring trial (08-2023) Report 3rd ring trial (08-2024)	12-2022 (M24) 12-2023 (M36) 12-2024 (M48)

Table S4 - Deliverables linked to Task 2

Deliverable	Activity	Responsible team	Description	Date (month)
T2.1	2.a	Network + Training	State-of-play reports for minimum 16 countries + overview report	04-2023 (M28)
T2.2	2.b	Training	Plan for support to NRLs to develop national capacity building, incl. proposed activities. Implementation to be reported in progress reports (General tasks)	06-2022 (M18)
T2.3	2.c	Network + Training	Short report on planned support to countries for implementation of national lab networks Implementation to be reported in progress reports (General tasks)	08-2022 (M20)
T2.4	2.d	Network	Draft model national protocol for surveillance of AMR Revision of protocol	08-2022 (M20) 10-2022 (M22)
T2.5	2.e	Methods	Guidance document on internal quality control schemes	12-2022 (M24)

7.3. Annex 3: Evaluation of the capacity building activities

In the tables, average score represents average rating given by the respondents of the surveys carried out during the project. Where indicated, the detailed description of the results is provided in **Deliverables T1.8.1-2**, **T1.10.1-3** and **T1.11.**

Table S5 - Average score for project activities as collected from project participants through a mid-term survey and three activities-specific surveys for the period of M1-M24. Note scoring scale either 1-5 or 1-6 as indicated in the table.

Activity	Description	No. of respondents	Average score
Mid-term survey (scale 1-5) [A]			
1.f	NRL capacity survey for *Salmonella* and *Campylobacter*	25	3.6
1.i	Activities dedicated to the priority countries	10	4.0
1.m	WGS Protocol	24	4.1
1.n	Updated EU protocol for AMR	25	3.8
1.p	*In vitro* EQA: EQA1-WGS-AMR	21	3.8
1.q	*In silico* inter-laboratory ring trial: RingTrial1-WGS-AMR	19	3.6
2.a	Support to NRLs for capacity building in local/regional laboratories	25	3.7
2.a/2.c	Mini-survey	25	3.8
2.d	Model protocol for national surveillance of AMR	25	3.8
	Effectiveness of communication	25	3.7
First network meeting evaluation (scale 1-6) [B]			
1.j	1st Network meeting	32	5.4
Hands-on training evaluation (scale 1-5) [C]			
1.k	*Salmonella* training course	14	4.4
1.k	*Campylobacter* training course	23	4.6
1st Multidisciplinary training evaluation (scale 1-5) [A]			
1.o	1st multidisciplinary training workshop	16	4.2

[A] Detailed result described in **Deliverable T1.8.1**
[D] Detailed result described in **Deliverable T1.10.1**
[C] Detailed result described in **Deliverable T1.11**

Table S6 - Average score for project activities as collected from project participants through three activity-specific surveys for a period of M25-M39

Activities evaluated in activities-specific surveys	No. of respon-dents	Average score
1.j: Mid-term network meeting (scale 1-6)[A]		
Organisation and logistics of the meeting	36	5.1
Sessions 1-6	33	5.6
How well did the meeting fulfill your expectations?	31	5.5
1.o: 2nd Multidisciplinary training workshop (scale 1-5)		
Day 1-23 Oct. : Presentations	43	4.1
Day 2: 24 Oct: Data analysis – Epidemiologist and microbiologist	43	4.1
Day 3 and 4-25 and 26 Oct: Group discussions on the exercise's outcome	43	4.1
Day 5-27 Oct: Plenum discussion	43	3.9
Day 5-27 Oct. : Presentations	43	4.1
Relevance of the workshop in relation to your job	43	3.9
Knowledge gained during the workshop	43	3.9
2.b: Train the trainers workshop (scale 1-10)		
Networking exercise on the activities for the national laboratory network support	20	8.2
How to prepare and share reference documents, materials and data	17	7.6
Practical strategies and examples for courses, exercises and workshops	21	8.7
Real-life example of building a sentinel surveillance network in Denmark	22	8.1
Countries experience on the mapping exercise results and outcomes	20	8.4
Data management and ISO standards	21	6.7
Presentations from countries on their pilot studies and discussion	21	8.6
Exercise on designing pilot projects	21	8.8
Presentation on pedagogical methods	19	7.8
Relevance of the workshop in relation to your job	21	8.5
Knowledge gained during the workshop	22	7.9
Duration of the sessions of the workshop	18	7.1
Opportunity for discussions and networking	15	7.5
Efficiency of the organisation prior to the workshop	19	8.9
The workshop location and facilities were adequate	19	8.7
The refreshments, meals and transportations provided were satisfactory	19	8.6

[A] Detailed result described in **Deliverable T1.10.2**

Table S7 - Average score for project activities as collected from project participants through final project evaluation survey for a period of M25-M39

Scale 1-5 was used in this survey

Activities evaluated in a final project evaluation survey	Priority countries, (n=14)	Other countries, (n=17)	Candidate/potential candidate countries, n=5	All NRLs
2.e: Guidance on internal quality control schemes	4.1	3.7	4.5	3.9
1.p: EQA2-WGS-AMR	4.2	4.4	-	4.3
1.q: RingTrial2-WGS-AMR exercise	4.2	4.6	-	4.4
1.p/1.q: Highlights from EQA2-WGS-AMR and RingTrial2-WGS-AMR	4.1	4.3	-	4.2
2.a: Support to NRLs for capacity building in local/regional laboratories	3.9	3.6	3.8	3.8
1.i: Activities dedicated to the priority countries	4.1	-	-	-
Effectiveness of communication	4.1	3.9	3.9	4.0

[A] Detailed result described in **Deliverable T1.8.2**

Table S8 - Average score for project activities as collected from project participants through activity-specific surveys for a period of M40-M46

Scale 1-5 was used in these survey

Activities evaluated in activities-specific surveys	No. of respondents	Average score
1.j: Final network meeting [A]		
Organisation and logistics of the meeting	11-15	4.5
Sessions 1-5	14-15	4.5
Overall evaluation	14	4.6
1.o: 3rd Multidisciplinary training		
Day 2: Data analysis – Epidemiologist and microbiologist	18	3.9
Day 3 and 4: Group discussions on the exercises outcome	20	3.9
Day 5: Plenum discussion	19	4.1
Day: Presentations	19	4.2
Relevance of the workshop in relation to your job	20	4.1
Knowledge gained during the workshop	20	4.0
1.p: EQA3-WGS-AMR		
Instructions for participation	14	4.5
The content	14	4.6
Web-tool for submitting results	14	4.4
Support provided during participation	13	4.7
Report	13	4.6
1.q: RingTrial3-WGS-AMR		
Instructions for participation	14	4.8
The content	14	4.7
Web-tool for submitting results	14	4.5
Support provided during participation	13	4.8
Report	13	4.8
2.b: Webinar on sample size for surveillance		
Duration	16	4.4
Relevance of the presentations	16	4.4

[A] Detailed result described in **Deliverable T1.10.3**

7.4. Annex 4: Remaining challenges and needs for future support in priority countries

The content of the annex is not publicly available.

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