D6.15: Report on outputs of the short-term missions

Work Package 6

Responsible Partner: UoS, UK (P23)

GENERAL INFORMATION

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REPORT ON SHORT TERM MISSIONS OUTPUTS

Overview of the One Health EJP Short Term Missions

The One Health EJP (OHEJP) Short Term Missions (STMs) were small co-funded travel grants which facilitated the exchange of scientific expertise, methodologies, equipment, and facilities to our consortium members. The aim of these missions was to harmonise the existing approaches and methodologies within the OHEJP.

These STMs drove research forward in a collaborative and non-duplicative fashion to strengthen the scientific capacity within the OHEJP, and to also contribute to the future prevention, preparedness, detection, and response of the EU to foodborne and other emerging threats across human-animal-environmental sectors.

The OHEJP co-funded a total of 19 STMs (each funded 44% by the EU), which involved 22 researchers and a total of 27 institutes within the OHEJP consortium. The Education and Training activities team from Work Package 6 (WP6) was responsible for co-ordinating the calls, selection of applicants for funding and reporting on the short term missions funded each year.

Table 1: All OHEJP short-term missions conducted between 2019 and 2023, with their titles, home and hosting institutes.

STM title	Home institute	Hosting institute
Training on the application of source attribution models.	IZLER, Italy	RIVM, The Netherlands
Application of advanced epidemiological analytical methods for antimicrobial resistance data in *Salmonella* in pigs.	VISAVET-UCM, Spain	Hasselt University, Belgium
Training in bioinformatics to study the dynamics of *E. coli* in laying hens.	VISAVET-UCM, Spain	DTU, Denmark
Skills development focused on development of a framework for reporting outbreak investigations using consumer purchase data.	FHI, Norway	SSI, Denmark
Study of the interactions between STEC and human gut microbiota in the ARCOL model.	ISS, Italy	UCA, France
Cross-domain and cross-country collaboration to develop multivariate syndromic surveillance.	SVA, Sweden	NIPH, Norway
Startup of an efficient sequencing facility.	NVI, Norway	SSI, Denmark
CarbaPlasmid – Tracking endemic carbapenemase plasmids in human, animal and environmental isolates.	NUI Galway, Ireland	VISAVET-UCM, Spain
Zoonotic pathogen detection in rats.	RIVM, The Netherlands	FLI, Germany
Surveillance and source-attribution of AMR based on metagenomic analysis.	INIAV, Portugal	DTU Food, Denmark

Validation and exchange of modelling tools to assess the risk of human salmonellosis based on environmental factors using multiple sources of data.	UoS, UK	RIVM, The Netherlands
Application of spatial models to identify new environmental surveillance indicators of *Salmonella* and *Campylobacter* in pig and poultry.	INIA, Spain	RIVM, The Netherlands
Construction of double-labelled *E. coli* strains to study the effect of antibiotics and interventions on horizontal ESBL genes transfer in the chicken's caecal microbiome.	WUR, The Netherlands	University of Copenhagen, Denmark
Microbiological and molecular techniques for *Brucella* identification from strains.	NDVRI, Bulgaria	ANSES, France
Tolerance of biofilm forming bacteria to disinfectants after repeated disinfectant exposure.	APHA, UK	NVI, Norway
Metagenomic Tools for the Study of SARS-CoV-2 in Animals.	INIA, Spain	INSA, Portugal
Genotypic characterisation of antimicrobial susceptibility and isolation of *Aeromonas* and *Vibrio* phages from water samples.	ANSES, France	BfR, Germany
Stabile Intra-specific genomic bacterial classification systems.	NVI, Norway	Pasteur Institute, France
Application of Single-Cell Genomics for the study of the bacterial reservoirs of plazomicin resistance determinants.	UCM, Spain	KIT, Germany

Short Term Missions calls and selection

During the five years of the OHEJP, five STM calls were launched: one for completion of the STMs in 2019, one for 2020, one for 2021, and two for 2022. For all calls, a promotional email marketing strategy was used to disseminate the call information to the Scientific Steering Board (SSB), Project Management Team (PMT), Progamme Managers Committee (PMC), Project Leaders, PhD teams, and Communication Contact Persons. The branded and visually attractive emails contained instructions on how to access the guidelines, application form and templates. The calls were also disseminated in the Education and Training activities monthly bulletins, the OHEJP consortium newsletters, and social media channels, Twitter and Linkedin.

The WP6 Team co-ordinated the calls and selection of the STMs following the validated procedure. STMs application forms, work plans, financial budget templates as well as letters of recommendation from the home and hosting institute and the applicants' *Curriculum Vitae* were sent to three independent reviewers. The reviewers were nominated by the SSB.

The WP6 Team compiled the scores and validated the selection with the Project Management Team (PMT), and the final decision was then communicated to the SSB and applicants. A total of 25 applications were received: five for 2019, five for 2020, four for 2021, and eleven for 2022

(six from the first 2022 call and five from the additional call) and 23 were approved for funding: four for 2019, five for 2020, four for 2021, and ten for 2022 (six from the first 2022 call and four from the additional 2022 call).

Impact of COVID-19

The COVID-19 pandemic affected the success of STMs scheduled in 2020 and 2021 due to the disruption to travel plans and local restrictions. This resulted in the cancellation of four STMs: three in 2020 and one in 2021. One of the four STMs funded for 2021 was post-poned for travelling reasons and took place early in 2022. Therefore, due to the COVID-19 pandemic, only 19 of the 23 awarded STMs took place: four in 2019, two in 2020, two in 2021, and eleven in 2022.

Short Term Missions – Call 2019

Four STMs were funded for 2019, they all took place as scheduled.

1. Short Term Mission 1

STM Researcher: Virginia Filipello
Title of the STM: Training on the application of source attribution models
Home Institute: IZLER, Italy
Mission Hosting Institute: RIVM, the Netherlands
Duration of mission: 1 week

Aim of the mission: The aim of this mission was to enhance source attribution skills through an ad hoc structured and supervised training, which included exercises with real data and validation of learnt competences.

Case study:

Figure 1: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2019.

2. Short Term Mission 2

STM Researchers: Kendy Tzu-Yun Teng
Title of the STM: Application of advanced epidemiological analytical methods for antimicrobial resistance data of *Salmonella* in pigs.
Home Institute: VISAVET-UCM, Spain.
Mission Hosting Institute: Centre for Statistics, Hasselt University, Belgium.
Duration of mission: 7 weeks.

Aim of the mission: The aim of this mission was to develop skills in analysing data on phenotypic antimicrobial resistance (AMR), to identify patterns among the occurrences of resistance of different antimicrobials and to better understand the interactions and dynamics of *Salmonella* in pigs.

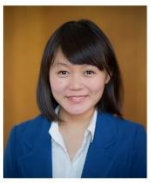
Case study:

SHORT TERM MISSIONS

Short Term Missions (STMs) are small travel grants with the aim of:

- Sharing scientific expertise, methodologies, equipment and facilities to harmonise the existing approaches and methodologies within the large OHEJP European network
- Driving the research forward in a collaborative and non-duplicative fashion to strengthen both the scientific capacity within the OHEJP
- Contributing to future prevention, preparedness, detection and response of the EU to foodborne and other emerging threats across human-animal-environmental sectors.

Application of advanced epidemiological analytical methods for antimicrobial resistance data of Salmonella in pigs



Theme: Skills Development Missions
Home Institute: VISAVET-UCM, Spain
Mission Hosting Institute: Centre for Statistics, Hasselt University
Duration of mission: 7 weeks

...This short-term mission allowed us to extend connection to institutes external to OHEJP and to form new collaboration. Besides the benefits at the project and institute level, I as a researcher also benefited greatly... Thanks to OHJEP for giving me this opportunity!"
Kendy Tzu-Yun Teng, VISAVET-UCM

The aim of this mission was to develop skills in analysing data on phenotypic antimicrobial resistance (AMR) to identify patterns among the occurrences of resistance of different antimicrobials to better understand the interactions and dynamics in Salmonella in pigs.

During this STM, analysis of AMR data for Salmonella isolates collected between 2001-2013 through the Spanish Veterinary Antimicrobial Resistance Surveillance Network programme was performed. The antimicrobials included Cefotaxime, Chloramphenicol, Ciprofloxacin, Florfenicol, Gentamicin, Nalidixic acid and Tetracycline.

Multiple techniques, including principal component analysis, multiple correspondence analysis, hierarchical clustering, and latent class analysis, were performed to detect potential patterns and clusters among the AMR data. Generalised estimating equations were conducted to examine the evolution of the proportion of the resistant strains of each of the seven antimicrobials. Additionally, the structures of relationships among the antimicrobials were examined by Bayesian network analysis. Efforts to develop new approaches to Bayesian network analysis in Stan also took place.

The work performed during this STM demonstrated useful analytical techniques to explore the interactions among AMR and associations between AMR and Salmonella serotypes. On the base of this action, development to explore AMR phenotypes continues to be carried on.

One Health EJP has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773830.

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Figure 2: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2019.

3. Short Term Mission 3

STM Researcher: Irene Aldea Ramos
Title of the STM: Training in bioinformatics to study the dynamics of *E. coli* in laying hens: Training on the application of source attribution models.
Home Institute: VISAVET-UCM, Spain
Mission Hosting Institute: DTU, Denmark
Duration of mission: 4 weeks.

Aim of the mission: The aim of this mission was to improve the participants' existing bioinformatics knowledge. The National Food Institute of DTU is part of the Centre of Genomic Epidemiology which has several web services for the analysis of whole genome sequences.

Case study:

Figure 3: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2019.

4. Short Term Mission 4

STM Researcher: Solveig Jore
Title of the STM: Skills development focused on development of a framework for reporting outbreak investigations using consumer purchase data.
Home Institute: FHI, Norway.
Mission Hosting Institute: SSI, Denmark.
Duration of mission: 1 week.

Aim of the mission: The aim of this mission was to work towards developing a framework for reporting outbreak investigations using consumer purchase data. During this mission, the participant also reviewed the current and previous use of purchase data and the potential of its future use.

Dissemination and communication activities: The researcher published results obtained using the framework developed during the STM, in two research articles:

- Jore, S., Braae, U. C., Møller, F. T., Friesema, I., Paranthaman, K., Jalava, K., Jourdan-DaSilva, N., Löf, E., Rehn M. & Ethelberg S. (2022). A common framework for using and reporting consumer purchase data (CPD) in foodborne outbreak investigations in Europe, Infection Ecology & Epidemiology, 12:1.
  DOI: https://doi.org/10.1080/20008686.2021.2007828

- Dougherty, P.E., Møller, F.T., Ethelberg, S., Isaksson Rø G. Ø. & Jore S. (2022). Simulation and identification of foodborne outbreaks in a large supermarket consumer purchase dataset. Sci Rep 12, 11491.
  DOI: https://doi.org/10.1038/s41598-022-15584-x

Case study:

Figure 4: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2019.

Short Term Missions – Call 2020

Five STMs were awarded for 2020 but the onset of the COVID-19 pandemic affected the success of these missions considerably due to the disruption to travel plans. Two of the five STMs took place before travel was disrupted in March 2020. Unfortunately, the remaining three STMs were cancelled.

1. Short Term Mission 1

STM Researcher: Paola Chiani
Title of the STM: Study of the interactions between STEC and human gut microbiota in the ARtificial COLon (ARCOL) model.
Theme: Skills Development Missions
Home Institute: Istituto Superiore di Sanità (ISS), Italy
Mission Hosting Institute: Université d'Auvergne Clermont, France
Duration of mission: 5 weeks

Aim of the mission: The aim of this study was to investigate the interactions between Shiga toxin-producing *E. coli* (STEC) and human intestinal microbiota, using the ARtificial COLon model (ARCOL), which simulates the human large intestine functionality. In details, the aim of the STM was to perform *in vitro* infection experiments with STEC in presence of a normal human intestinal microbiota using this innovative model to understand the role played by the human intestinal microflora upon STEC infection, and to investigate changes occurring in the gut microbiota composition in presence of infecting STEC strains.

Case study:

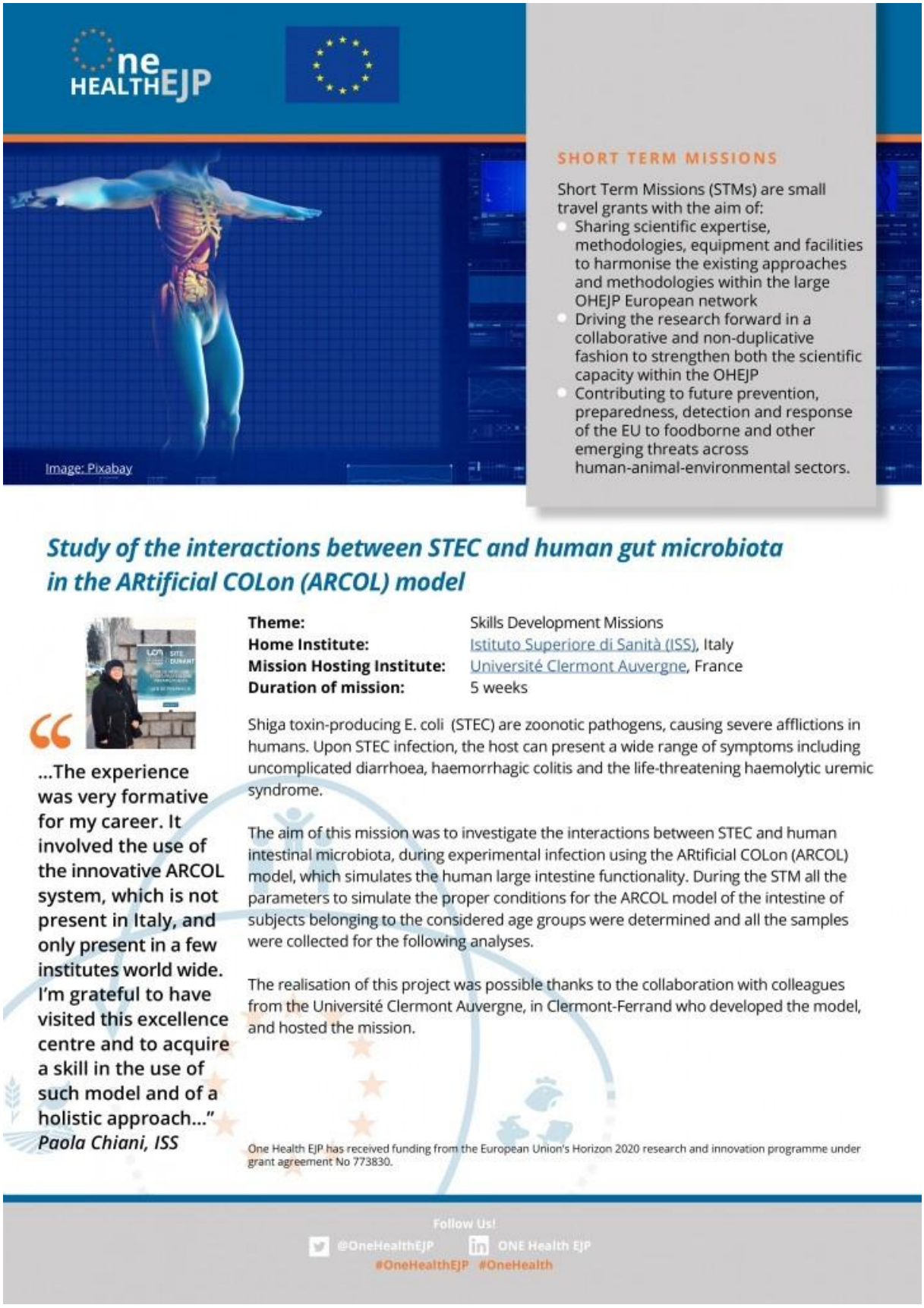


Figure 5: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2020

2. Short Term Mission 2

STM Researcher: Wiktor Gustafsson
Title of the STM: Cross-domain and cross-country collaboration to develop multivariate syndromic surveillance
Theme: Skills Development Missions
Home Institute: Swedish National Veterinary Institute (SVA), Sweden Mission
Hosting Institute: Norwegian Institute of Public Health (NIPH), Norway
Duration of mission: 2 weeks

Aim of the mission: The aim of the STM was collaboration and knowledge exchange between Norway and Sweden in the development of One Health systems for multivariate syndromic surveillance of veterinary and public health data combined. Two separate systems are in development in Sweden at the SVA and Norway at the NIPH. The Swedish system is explanatory, aiming to combine several data sources to explain outbreaks and improve the accuracy of detection. Norway, on the other hand, is developing a predictive system, which aims to use some source(s) of data to predict the outcome of others.

Dissemination and communication activities: The results obtained during the STM contributed to the OHEJP NOVA project's work on the development of a multivariate syndromic surveillance engine, and a dashboard showcasing the surveillance results from the *Campylobacter* test case. This has been presented as a poster at the OHEJP Annual Scientific Meeting 2020.

Case study:

Figure 6: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2020

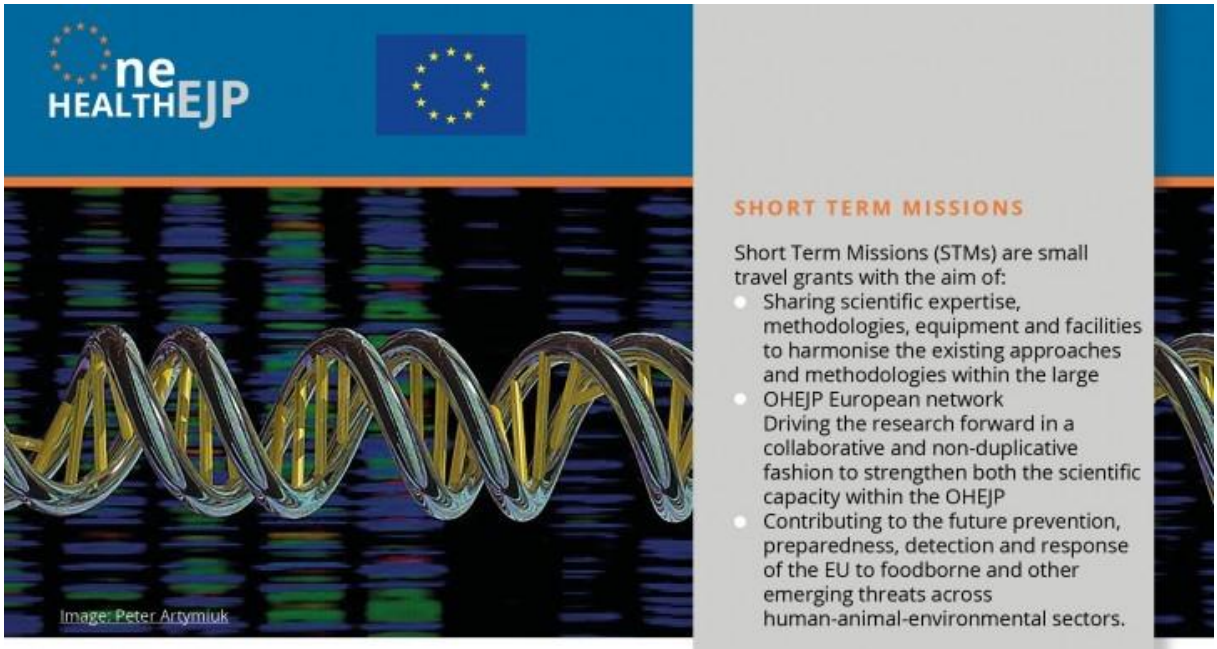
Short Term Missions – Call 2021

Four STMs were awarded for 2021 but the COVID-19 pandemic continued to affect the success of these missions due to the disruption to travel plans. Two of the four STMs took place, one was post-poned to 2022 and one was cancelled.

1. Short Term Mission 1

STM Researcher: Catherine Arnason Boe
Title of the STM: Start-up of an efficient sequencing facility
Theme: Skills Development Missions
Home Institute: The Norwegian Veterinary Institute, Norway
Mission Hosting Institute: Statens Serum Institut (SSI), Denmark
Duration of mission: 1 week

Aim of the mission: The project SEQ-TECH at The Norwegian Veterinary Institute (NVI) has acquired a platform of sequencing machines, pipetting robots and other equipment through 2019-2021 and now set-up for an automated flow-through of several hundred high throughput sequencing samples per week. Although the equipment was there, the routine workflow was not fully established, and the potential of the different machines not exploited. The aim of this mission was to develop skills on the management of a sequencing facility using an automated workflow.



SHORT TERM MISSIONS

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- Sharing scientific expertise, methodologies, equipment and facilities to harmonise the existing approaches and methodologies within the large OHEJP European network Driving the research forward in a collaborative and non-duplicative fashion to strengthen both the scientific capacity within the OHEJP
- Contributing to the future prevention, preparedness, detection and response of the EU to foodborne and other emerging threats across human-animal-environmental sectors.

Start-up of an efficient sequencing facility

Theme: Foodborne Zoonoses, AMR and Emerging threats
Home Institute: Norwegian Veterinary Institute, Norway
Mission Hosting Institute: SSI, Denmark
Duration of Mission: 1 week

The aim of this mission was to develop skills on the management of a sequencing facility using an automated workflow. The project SEQ-TECH at the Norwegian Veterinary Institute (NVI) has recently acquired equipment to set-up an automated high throughput sequencing platform. However, the routine workflow is not yet entirely established, and the potential of the different machines not fully exploited. By visiting the SSI and following their weekly routine for library preparation and high-throughput sequencing (HTS), the participant aimed to learn how to improve the NVI workflow.

During this mission, routine whole genome sequencing (WGS) of bacteria and virus (SARS-CoV-2) were performed, the data quality control process was described and one-on-one meetings with key personnel were held. Inspiration and several ideas of how to improve routine sequencing and start novel sequencing tasks was gained by observing the SSI workflow and quality control pipeline. In addition, sharing of experiences regarding Nanopore sequencing and WGS will be valuable going forward.

The STM was a revelation for all the possibilities available at NVI, using the new equipment, to implement higher throughput sequencing for surveillance, preparedness, diagnostics and research in the near future.

"I will definitely use the new knowledge to advance and streamline several steps of our ongoing sequencing projects and routine workflows. The connections made with researchers and technical staff will be valuable for the future. I am very grateful to the OHEJP for granting this opportunity. I would also like to express gratitude to SSI and the kind staff for allowing me into their labs."

Cathrine Arnason Bøe, Norwegian Veterinary Institute

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Figure 7: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2021

2. Short Term Mission 2

STM Researcher: Liam Burke
Title of the STM: CarbaPlasmid – Tracking endemic carbapenemase plasmids in human, animal and environmental isolates
Theme: One Health, AMR
Home Institute: NUI Galway, Ireland
Mission Hosting Institute: VISAVET, Universidad Complutense Madrid, Spain
Duration of mission: 1 month

Aim of the mission: The aim of this mission was to develop skills in nanopore sequencing and hybrid sequence analysis, to characterise antimicrobial resistance plasmids for One Health epidemiological investigations.

Case study:

SHORT TERM MISSIONS

Short Term Missions (STMs) are small travel grants with the aim of:

- Sharing scientific expertise, methodologies, equipment and facilities to harmonise the existing approaches and methodologies within the large OHEJP European network
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CarbaPlasmid – Tracking endemic carbapenemase plasmids in human, animal and environmental isolates

Theme: One Health, AMR
Home Institute: NUI Galway, Ireland
Mission Hosting Institute: VISAVET, Universidad Complutense Madrid, Spain
Duration of Mission: 1 month

"This STM allowed me to learn cutting edge techniques in bacterial sequence analysis, which will greatly benefit researchers within my lab and our collaborators. I had an amazing experience in Madrid: everyone at the ARU made me feel like one of the team! This mission has paved the way for further scientific collaboration and intercultural opportunities..."

Liam Burke, NUI Galway

The aim of this mission was to develop skills in nanopore sequencing and hybrid sequence analysis in order to characterise antimicrobial resistance plasmids for One Health epidemiological investigations.

In this project, Carbapenemase-producing Enterobacterales (CPE) isolated from the natural environment, hospital wastewater, the hospital environment and hospital patients in Galway, Ireland, were analysed by hybrid sequencing analysis. Several techniques and applications for analysis of hybrid bacterial sequence data were learned. A harmonised hybrid sequence analysis pipeline was successfully transferred between One Health EJP partners Universidad Complutense Madrid (UCA) and NUI Galway.

This short term mission developed capacity for long-read sequencing and hybrid sequence assembly in Irish OHEJP partner NUI Galway, which will also be used to support the surveillance function of the Irish National CPE Reference Laboratory. This expertise will be fundamental in ongoing and future collaborative AMR research projects with OHEJP partners and in the surveillance of endemic carbapenemase plasmids in a One-Health context in Ireland.

The data will contribute to bridging the knowledge gaps that exist in our understanding of the role of the environment in the persistence, evolution and transmission of AMR plasmids and the propensity for carbapenemase plasmid transfer between different bacterial species and strains in distinct ecological niches. It may also identify emerging AMR threats from a One Health perspective.

The training and harmonisation elements of the STM were successful, resulting in successful training of Dr Burke in nanopore sequencing and hybrid sequence analysis methodology and transfer of the protocol to his home Institution. The research outcome of the STM was also highly successful, resulting in 39 complete hybrid-assembled CPE genomes including 38 fully circularised CPE plasmids. Further analysis will result in a high quality scientific publication. Furthermore, the STM has strengthened links between NUI Galway's ARME lab and UCM's ARU lab, paving the way for further One Health collaborative research on antimicrobial resistance.

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Figure 8: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2021

Short Term Missions – Call 2022

Ten STMs were awarded for 2022, six from the first call and four from the additional call. One of the STMs awarded in the 2021 call also took place early 2022. A total of eleven STMs were conducted in 2022.

1. Short Term Mission 1

STM Researcher: Ana Christina Ferreira
Title of the STM: Surveillance and source-attribution of AMR based on metagenomic analysis.
Theme: Skills Development Missions.
Home Institute: National Institute for Agrarian and Veterinary Research (INIAV), Portugal.
Mission Hosting Institute: Technical University of Denmark (DTU Food), Denmark.
Duration of mission: 2 weeks.

Aim of the mission: The aim of this STM was to learn novel approaches and models based on metagenomic data for surveillance and to infer source attribution of AMR determinants. INIAV is the National Reference Laboratory for AMR in animals and food of animal origin and is in need of scientists with the skills to analyse and interpret metagenomics data.

Dissemination and communication activities:
The following conference talk was presented: Nogueira, T., Ferreira, A.C., Botelho, A. (2021). Comparative analysis of the resistome and the mobilome of estuarine aquacultures and other aquatic environments. Oral presentation, One Health EJP Annual Scientific Meeting 2021 (Online Event).
A research publication is also being prepared by the STM researcher, in collaboration with DTU colleagues.

Case study:

SHORT TERM MISSIONS

Short Term Missions (STMs) are small travel grants with the aim of:

- Sharing scientific expertise, methodologies, equipment and facilities to harmonise the existing approaches and methodologies within the large OHEJP European network
- Driving the research forward in a collaborative and non-duplicative fashion to strengthen both the scientific capacity within the OHEJP
- Contributing to the future prevention, preparedness, detection and response of the EU to foodborne and other emerging threats across human-animal-environmental sectors.

Surveillance and source-attribution of AMR based on metagenomic analysis

Theme:	One Health, AMR
Home Institute:	National Institute for Agrarian and Veterinary Research (INIAV), Portugal
Mission Hosting Institute:	Technical University of Denmark (DTU Food), Denmark
Duration of Mission:	2 weeks

...it was very useful to become part of the DTU group and work together for two weeks. This collaboration allowed me to increase my knowledge and to improve skills. The information and tools acquired will be applied to the analysis of metagenomic data for source tracking in aquaculture environments and in future collaborative projects in this area....”

Ana Cristina Ferreira, INIAV, Portugal

The aim of this STM was to learn novel approaches and models based on metagenomic data for surveillance and to infer source attribution of AMR determinants.

INIAV is the National Reference Laboratory for AMR in animals and food of animal origin, and is in need of scientists with the skills to analyse and interpret metagenomics data.

The STM gave Ana the opportunity to improve her skills for analysing metagenomics data for the purpose of surveillance and source attribution of AMR. Ana was engaged in the whole process from when the samples arrived in the lab to the final epidemiological data analyses, although the main focus was on the bioinformatic analyses and epidemiologically modelling.

Moreover, this mission will contribute to the development of skills in metagenomics, bioinformatics, and AMR source-attribution approaches, needed in the frame of the OHEJP consortium and in future collaborations. Use of data from omics technologies (WGS and metagenomics) for source-attribution WGS and metagenomics based surveillance allows the characterisation of antimicrobial resistance determinants, making it possible to identify the potential for their dissemination.

The training will improve the participation of INIAV in the OHEJP DISCOVER project methods: assessment/improvement- related with source attribution of AMR based on metagenomics.

Sharing of scientific expertise and methodologies will allow the harmonisation of approaches within the OHEJP network. Furthermore, it will strengthen the collaboration and scientific capacity of partners contributing to future preparedness in the surveillance, detection and response of the EU to foodborne, AMR and emerging threats.

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Figure 9: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2022.

2. Short Term Mission 2

STM Researcher: Marieke de Cock
Title of the STM: Zoonotic pathogen detection in rats (funded through the 2021 STM call, postponed to 2022 due to COVID-19).
Theme: Emerging threats.
Home Institute: National Institute of Public Health and the Environment (RIVM).
Mission Hosting Institute: Friedrich-Loeffler-Institut (FLI).
Duration of mission: 1month.

Aim of the mission: The aim of the mission was for the PhD student to test rat and mouse samples for different zoonotic pathogens. The samples were tested for Cowpox virus, rat Hepatitis E virus (rat HEV), Lymphocytic choriomeningitis virus (LCMV) and *Streptobacillus moniliformis*.

Dissemination and communication activities: The STM led to the publication of a joint research article between the institutes: Heuser, E., Drewes, S., Trimpert, J., Kunec, D., Mehl, C., de Cock, M.P., de Vries, A., Klier, C., Oskamp, M., Tenhaken, P., et al. (2023) Pet Rats as the Likely Reservoir for Human Seoul Orthohantavirus Infection. Viruses. 15(2):467. DOI: https://doi.org/10.3390/v15020467

Case study:

Figure 10: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2021.

3. Short Term Mission 3

STM Researcher: Laura Gonzalez Villeta
Title of the STM: Validation and exchange of modelling tools to assess the risk of human Salmonellosis based on environmental factors using multiple sources of data.
Theme: One Health, skills development.
Home Institute: University of Surrey, UK.
Mission Hosting Institute: Dutch National Institute for Health and Environment (RIVM).
Duration of mission: 1 month.

Aim of the mission: The aim of this mission was to investigate whether the effect of weather on human salmonellosis cases is similar regardless of the country under study, using a novel statistical modelling approach developed at the University of Surrey, UK.

Dissemination and communication activities: The PhD student drafted a research article that includes results obtained during the STM. This work should be published soon.

Case study:

Figure 11: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2022.

4. Short Term Mission 4

STM Researcher: Antonio Rodriguez
Title of the STM: Application of spatial models to identify new environmental surveillance indicators of *Salmonella* and *Campylobacter* in pig and poultry.
Theme: One Health, skills development.
Home Institute: The National Institute for Agricultural and Food Research and Technology (INIA), Spain.
Mission Hosting Institute: National Institute for Public Health and the Environment (RIMV), The Netherlands.
Duration of mission: 1 month.

Aim of the mission: The aim of this mission was to develop a procedure to model surveillance data of zoonotic diseases using machine learning methods. The model was tested on the Wild Boar Surveillance Plan of Andalucía (Spain) data, with the aim to determine the most important risk factors that shape wild boar diseases in Andalucía and study their interaction between each other.

Dissemination and communication activities: The STM researcher trained their team members on the methods learnt during the STM. They also organised a teleconference with people in charge of the Wildlife Epidemiological Surveillance Program of Andalusia (Spain) at the Water and Environment Agency from Andalusia and the Animal Health Research Centre, to give them feedback about the data in order to strengthen the surveillance program.

Case study:

SHORT TERM MISSIONS

Short Term Missions (STMs) are small travel grants with the aim of:

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- Contributing to the future prevention, preparedness, detection and response of the EU to foodborne and other emerging threats across human-animal-environmental sectors.

Application of spatial models to identify new environmental surveillance indicators of Salmonella and Campylobacter in pig and poultry

I had the chance to meet a new research centre and to collaborate with and take advice from experts on the field. We obtained results that we could not have obtained working independently. I carried out an important advance in my research project and acquired new skills that we will use in future projects. I would like to highlight that thanks to my short-term mission I pushed my data analysis skills to an upper level."

Antonio Rodríguez
The National Institute for Agricultural and Food Research and Technology
Spain

Theme: One Health Missions - Skills development missions
Home Institute: The National Institute for Agricultural and Food Research and Technology (INIA), Spain
Mission Hosting Institute: National Institute for Public Health and the Environment (RIMV), The Netherlands
Duration of Mission: 1 month

The aim of this mission was to develop a procedure to model surveillance data of zoonotic diseases using machine learning methods. The model was tested on the Wild Boar Surveillance Plan of Andalucía (Spain) data, with the aim to determine the most important risk factors that shape wild boar diseases in Andalucía and study their interaction between each other.

During this mission, the model validation was performed in two main steps. First, a single response variable was selected for the study: the *Salmonella* serology of hunted wild boars. In the first week of the STM, the data was organised in an appropriate way for training machine learning algorithms. Additionally, climate and wild boar distribution information from published maps were collected, to include them as potential drivers of the pathogens. The results being conclusive, a modelling procedure valid for all the wild boar diseases in the dataset was designed. This includes the processing of the dataset before the modelling (pre-processing step), the model application itself and its validation, and the study of the model output. The best way to compensate the unbalance of the positives and negatives in the dataset was explored, to avoid reducing the sensitivity or specificity of the model. A script was prepared to provide several validation statistics based on the comparison between the predictions on the test data and the real positive and negative values. The variable importance was assessed on the training and test data, partial dependence plots based on these data were created and detection of interactions between predictors on the test data was conducted. The developed procedure successfully determined the most important risk factors that shape wild boar diseases in Andalucía and studied their interaction between each other. Using the data from previous hunting seasons, it also allowed to identify areas with higher and lower probabilities of boar disease and to represent the predictions in a map.

The STM has improved on the relationship between two existing One Health EJP partners and enhanced both current and future collaborations between the partner institutes. Additionally, this procedure has been automatised and can be easily applied to the data from the *Salmonella* and *Campylobacter* surveillance in the food chain by the Spanish Agency of Food Security and Nutrition (AESAN) and the *Salmonella* and *Campylobacter* Spanish surveillance plan in poultry farm.

One Health EJP has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773830.

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Figure 12: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2022.

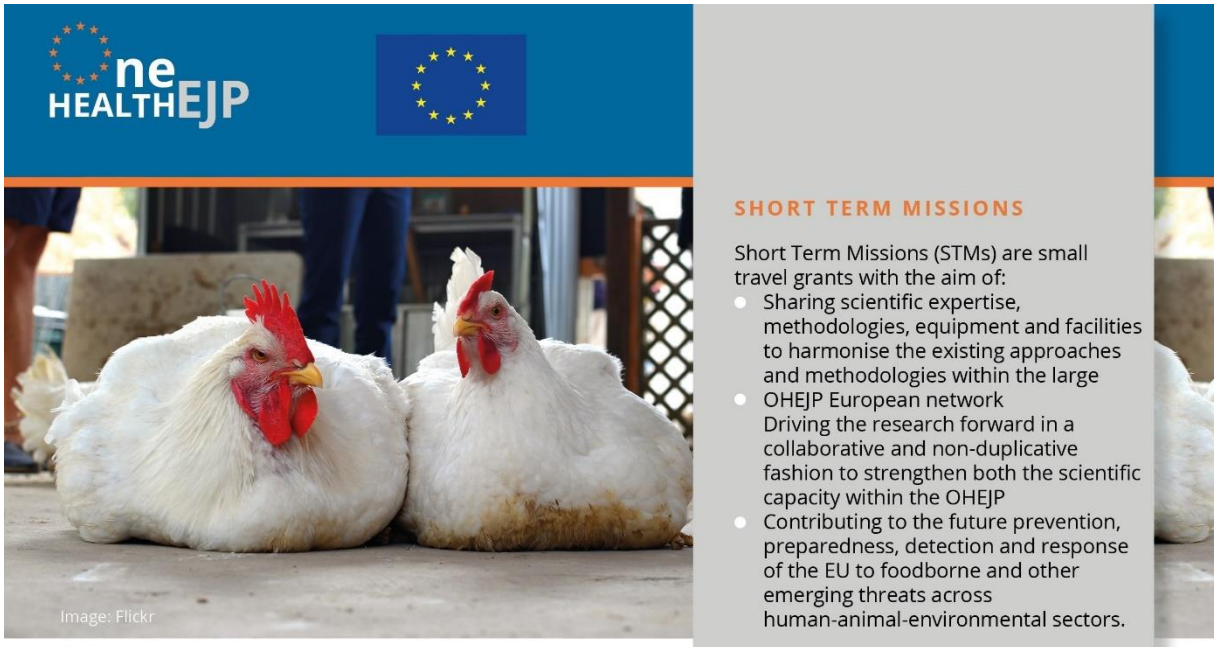
5. Short Term Mission 5

STM Researcher: Ingrid Cardenas Rey
Title of the STM: Construction of dual labelled *E. coli* strains to study the effect of antibiotics and microbiota interventions on the horizontal transfer of ESBL genes in the *in vitro* chicken caecal microbiota.
Theme: One Health Missions - Antimicrobial Resistance (AMR).
Home Institute: Wageningen Bioveterinary Research (WUR), The Netherlands.
Mission Hosting Institute: University of Copenhagen, Denmark.
Duration of mission: 2 months.

Aim of the mission: The aim of this mission was for the PhD student to learn bacterial cloning methods using fluorescent reporter proteins, to produce dual labelled *E. coli* strains. Dual labelling (fluorescent tagging of the chromosome and AMR-gene carrying plasmids) of bacteria is a powerful tool to study plasmid-mediated antimicrobial resistance among complex *in vitro* microbial communities simulated on *in vitro* gut systems like the chicken caeca.

Dissemination and communication activities: The PhD student presented the results obtained thanks to the STM at a poster carousel of the microbial ecology group in June 2022 at a workshop in Nunspet (The Netherlands) and at the OHEJP side event "Lessons Learnt from a European Multidisciplinary Initiative" at the ONE2022 Conference in June 2022 in Brussels (Belgium).

Case study:



SHORT TERM MISSIONS

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- Driving the research forward in a collaborative and non-duplicative fashion to strengthen both the scientific capacity within the OHEJP
- Contributing to the future prevention, preparedness, detection and response of the EU to foodborne and other emerging threats across human-animal-environmental sectors.

Construction of dual labelled E. coli strains to study the effect of antibiotics and microbiota interventions on the horizontal transfer of ESBL genes in the in vitro chicken caecal microbiota

Theme: One Health Missions - Antimicrobial Resistance (AMR)
Home Institute: Wageningen Bioveterinary Research (WUR), The Netherlands
Mission Hosting Institute: University of Copenhagen, Denmark
Duration of Mission: 2 months

A short term mission should be a must-do for all young researchers! It was a priceless experience that enriched my knowledge and skillsets and expanded my network. I built up and exercised my lab skills, critical thinking and cooperation in a multidisciplinary group. I was challenged daily with new knowledge and experimental work but also strongly supported by a wonderful work team!"

Ingrid Cardenas Rey
Wageningen Bioveterinary Research, The Netherlands

The aim of this mission was for the PhD student to learn bacterial cloning methods using fluorescent reporter proteins, to produce dual labelled *E. coli* strains. Dual labelling (fluorescent tagging of the chromosome and AMR-gene carrying plasmids) of bacteria is a powerful tool to study plasmid -mediated antimicrobial resistance among complex *in vitro* microbial communities simulated on *in vitro* gut systems like the chicken caeca. This STM enabled the PhD student to reach the objectives proposed in the OHEJP PhD project VIMOGUT, which studies the chicken gut microbiota and microbiota interventions to reduce horizontal transmission of Extended Spectrum ß-Lactam (ESBL) genes.

During this mission, five commensal ESBL *E. coli* strains that originated from chicken broiler caeca and belonged to the collection of The Dutch National Reference Lab were used for bacterial cloning experiments. All strains carried an ESBL (blaCTXM-1 and blaSHV-12) or AmpC β-lactamase (blaCMY-2) gene on plasmids highly prevalent in the broilers production and were susceptible to three antimicrobials. Two fluorescent reporter proteins were used for chromosome and plasmid tagging, namely, mCherry and Green fluorescent protein (GFP). Bacterial cloning is a challenging research area that requires time and repeatability of the experimental work. Technical issues and challenges were encountered during this multi-step process. However, the PhD student learned the bacterial cloning techniques needed for the completion of future *in vitro* experiments and developed and strengthened her lab skills.

The STM opened significant cooperation channels between the AMR group at Wageningen Bioveterinary Research and the One Health Antimicrobial Resistance (OHAR) research group at the University of Copenhagen. The output of the work performed during the STM, and upcoming in vitro experiments are expected to be published as a collaborative research article in a scientific journal.

One Health EJP has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773830.

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Figure 13: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2022.

6. Short Term Mission 6

STM Researchers: Albena Dimitrova Angelova, Gergana Mateva et Mihail Vladimirov Milanov
Title of the STM: Microbiological and molecular techniques for *Brucella* identification.
Theme: One Health Missions.
Home Institute: NDVRI, Bulgaria.
Mission Hosting Institute: Anses, France.
Duration of mission: 1 week.

Aim of the mission: The aim of this mission was for the three researchers to learn standardised microbiological and molecular techniques, including DNA extraction and real-time PCR, for the identification of *Brucella spp.* As Brucellosis is the among the leading lab-acquired infections, learning effective biosafety/ biosecurity whilst working with the causative agent is paramount. This STM enabled the researchers to complete objectives in the OHEJP IDEMBRU project, which studies the zoonotic potential, virulence, and persistence markers in isolated strains of *Brucella spp*.

Case study:

Microbiological and molecular techniques for Brucella identification

Theme:	One Health missions
Home Institute:	NDVRI, Bulgaria
Mission Hosting Institute:	ANSES, France
Duration of Mission:	1 week
STM Researchers:	Albena Dimitrova Angelova Gergana Mateva Mihail Vladimirov Milanov

"The STM was an amazing experience and has provided newfound knowledge regarding the skill and requirements for microbiological manipulation and molecular diagnostics, especially when working with potentially infectious bacteria, such as Brucella. Disseminating this knowledge to other colleagues will further help and enhance everyday tasks in our laboratories"

Albena Dimitrova Angelova, NDVRI, Bulgaria

The aim of this mission was for the three researchers to learn standardised microbiological and molecular techniques, including DNA extraction and real-time PCR, for the identification of *Brucella spp*. As Brucellosis is the among the leading lab-acquired infections, learning effective biosafety/ biosecurity whilst working with the causative agent is paramount. This STM enabled the researchers to complete objectives in the OHEJP IDEMBRU project, which studies the zoonotic potential, virulence and persistence markers in isolated strains of *Brucella spp*.

During this mission, theoretical training was provided by the host institute on the critical steps for *Brucella* culture followed by principles of molecular approaches. Prior to the researchers undertaking any laboratory activities, practical demonstrations on bacterial isolation, culture and typing were provided, followed by demonstrations of molecular techniques including DNA extraction, real-time PCR protocols, gel electrophoresis and High-Resolution Melting PCR. Each day, round table discussions provided additional insight into each step of the training. A final presentation on molecular epidemiology afforded further knowledge on applications, perspectives and interpretation of data. The researchers strengthened existing and developed new laboratory skills that will assist their future research.

The STM provided valuable training, allowing for the isolation and identification of *Brucella spp.*; further experimentation will be performed on *Brucella spp.* strains, under rigorous biosafety standards, at the NDVRI laboratories in Bulgaria.

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Figure 14: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2022.

7. Short Term Mission 7

STM Researcher: Emma Brook
Title of the STM: Tolerance of biofilm forming bacteria to disinfectants after repeated disinfectant exposure.
Theme: One Health Missions- Veterinary, Food, Medical and or Environmental research.
Home Institute: Animal and Plant Health Agency (APHA), UK.
Mission Hosting Institute: Norwegian Veterinary Institute (NVI), Norway.
Duration of mission: 3 weeks.

Aim of the mission: The aim of this mission was to test methods for measuring disinfectants efficacy, after repeated use on bacteria that survived treatment due to the formation of biofilms. Methods for disinfectant efficacy testing against planktonic foodborne bacteria are well established, yet methods for efficacy of disinfectants for biofilms are still novel, particularly the effect of repeated exposure to disinfectants on the survivability of bacteria in biofilms. This STM aimed to promote harmonisation of current test methods and models used in biofilm and disinfectant testing.

Case study:

Tolerance of biofilm forming bacteria to disinfectants after repeated disinfectant exposure

Theme: One Health Missions- Veterinary, Food, Medical and/or Environmental research
Home Institute: Animal and Plant Health Agency (APHA), UK
Mission Hosting Institute: Norwegian Veterinary Institute (NVI), Norway
Duration of Mission: 3 weeks

The aim of this mission was to test methods for measuring disinfectants efficacy, after repeated use on bacteria that survived treatment due to the formation of biofilms. Methods for disinfectant efficacy testing against planktonic foodborne bacteria are well established, yet methods for efficacy of disinfectants for biofilms are still novel, particularly the effect of repeated exposure to disinfectants on the survivability of bacteria in biofilms. This STM aimed to promote harmonisation of current test methods and models used in biofilm and disinfectant testing.

During this mission, biofilms of Staphylococcus aureus were exposed to different concentrations of Benzalkonium chloride disinfectant. Following neutralisation of the disinfectant, biofilm was removed via mechanical action. Persisting bacteria were enumerated and isolated to prepare next-generation biofilms. These were then exposed to further disinfection using the same concentration to which they were previously exposed. This was repeated over nine consecutive experiments. The results have shown that increased tolerance to biocides by biofilm forming bacteria can occur. However, conflicting statistical results suggest the need for further investigation. Future studies should expand on the data collected in this study, to help develop knowledge on the role of biofilm producing bacteria in the dissemination of biocide resistance and factors affecting the variability in biofilm behaviour.

The STM has improved on the relationship between two existing One Health EJP partners and enhanced both current and future collaborations between the partner institutes. The STM has supported collaborative research in the current project (BIOPIGEE) through improvements of working arrangements and understanding of the used methods.

My personal and professional confidence grew during the STM. The information shared has been invaluable to my learning and development. I have gained experience in a wider range of methods used in biofilm and disinfectant research, as well as with a species of bacteria. We exchanged skills and to allow harmonisation of approaches. The methods and techniques learnt will be applied to ongoing and future projects back at my home institute...."

Emma Brook
APHA, UK

One Health EJP has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773830.

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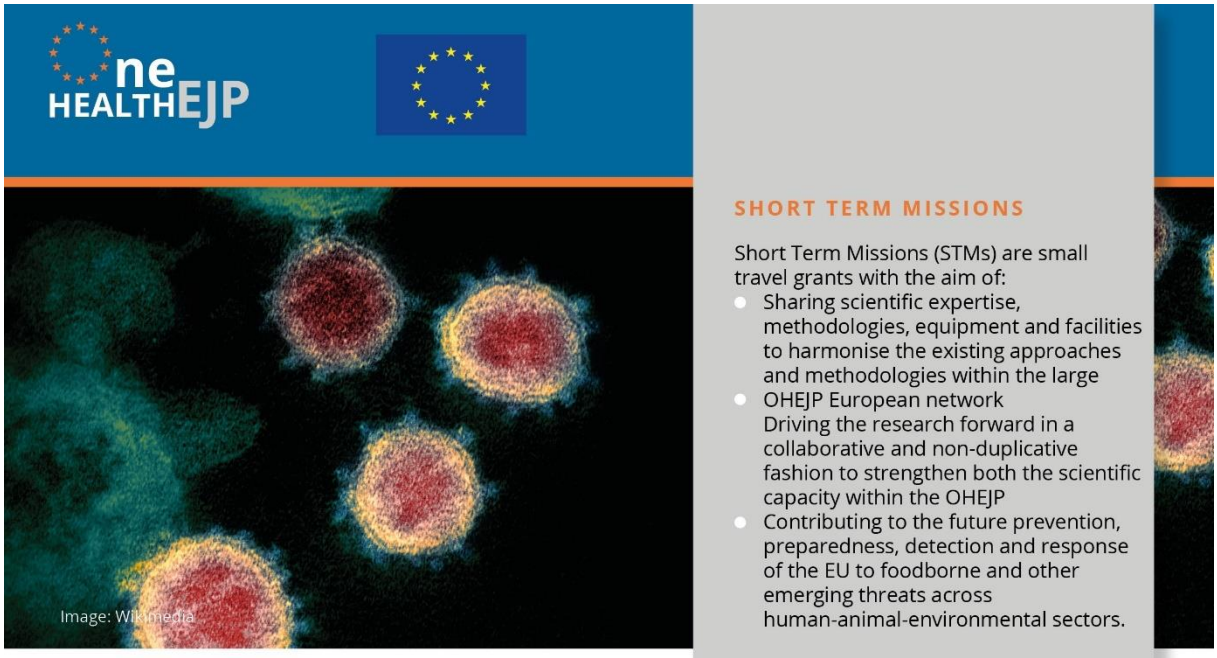
Figure 15: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2022.

8. Short Term Mission 8

STM Researcher: Carlos Sacristán Yagüe
Title of the STM: Metagenomics tools for the study of SARS-COV-2 in animals.
Theme: One Health, Skills Development Missions.
Home Institute: INIA, Spain.
Mission Hosting Institute: INSA, Portugal.
Duration of mission: 2 weeks.

Aim of the mission: The aim of this mission was to expand the researcher's training to a higher and more practical level by learning the application of metagenomics and completing sequencing within a One Health context. These tools are essential for modern surveillance and diagnostics of infectious diseases and are key to shed light on the epidemiology of novel viruses.

Case study:



SHORT TERM MISSIONS

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- Contributing to the future prevention, preparedness, detection and response of the EU to foodborne and other emerging threats across human-animal-environmental sectors.

Metagenomic Tools for the Study of SARS-CoV-2 in Animals

...a great personal and professional experience. I closely followed the group's daily activities, and performed specific training activities designed to maximize my learning experience. I had the opportunity of getting acquainted with the National Health System of Portugal, and participated in several cultural and scientific activities carried out at INSA during my stay. I gained valuable skills, that will allow me to use metagenomics in my future research.

Carlos Sacristán Yagüe
INIA, Spain

Theme:	One Health, Skills Development Missions
Home Institute:	INIA, Spain
Mission Hosting Institute:	INSA, Portugal
Duration of Mission:	2 weeks

The aim of this mission was to expand the Carlos' training to a higher and more practical level by learning the application of metagenomics and completing sequencing within a One Health context. These tools are essential for modern surveillance and diagnostics of infectious diseases and are key to shed light on the epidemiology of novel viruses.

During the mission, Carlos had the opportunity to learn how to use the INSaFLUTELE-VIR suite: an easy-to-use open web-based bioinformatics suite that was designed for the genomic surveillance of human seasonal influenza and SARS-CoV-2, and recently adapted to monkey pox virus (now renamed Mpox). This platform was proven to be an excellent surveillance tool during the SARS-CoV-2 pandemic and a new module has been designed by researchers in the mission hosting institute to simplify metagenomic analyses and identify new viruses. Carlos also learned about the ReporTree tool, a surveillance-oriented tool to strengthen the linkage between pathogen genetic clusters and epidemiological data and was trained on the use of nanopore sequencing with MinION. The MinION was used during the mission for the complete sequencing of SARS-CoV-2, the diagnostics of MPox virus and sequencing of a poliovirus. The "Young Researcher Day" and "INSA Day" took place during the mission, showcasing scientific and cultural activities. It allowed Carlos to learn about the different research lines carried out in the three INSA headquarters in Portugal and to expand his professional network.

The mission provided Carlos the training to diagnose and characterize future SARS-CoV-2 cases in animals using metagenomics, which is key to determine whether a virus has changed/mutated during a spill over event. One of the outputs of the project will be the adaptation of modern metagenomics and bioinformatics tools in the COVRIN project. The mission also strengthened further the collaboration between the institutes, with a new collaboration established to research on hepatitis E virus in wildlife.

One Health EJP has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773830.

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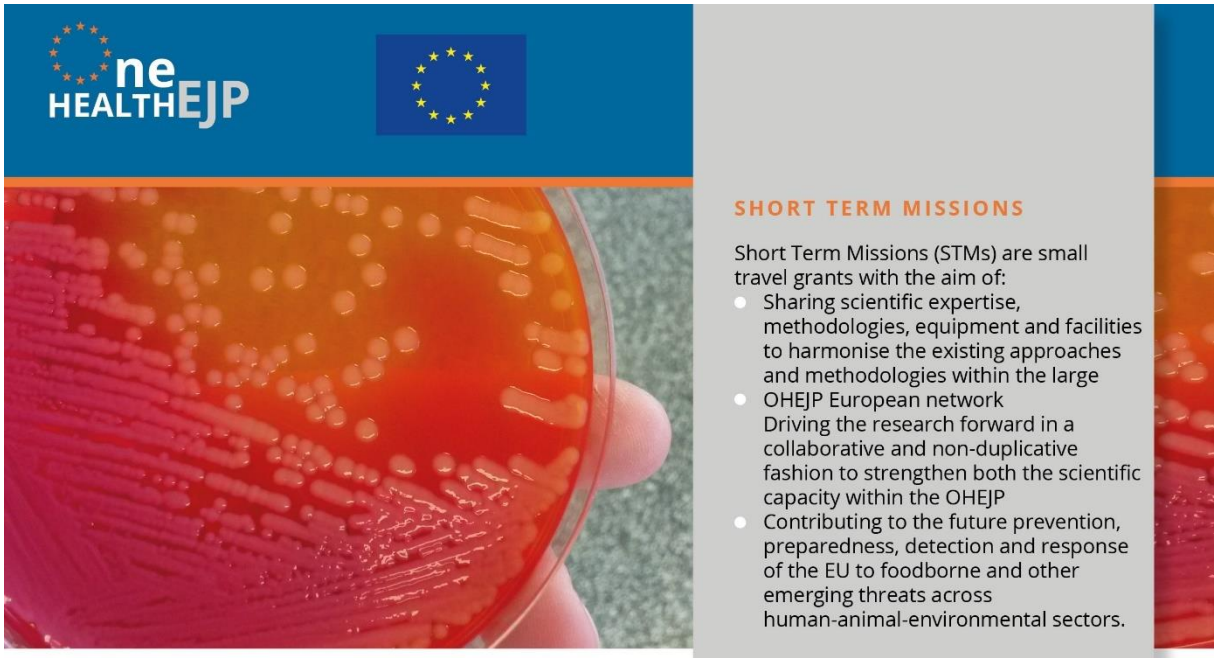
Figure 16: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2022.

9. Short Term Mission 9

STM Researchers: Sandrine Baron and Laetitia Le Devendec
Title of the STM: Genotypic characterisation of antimicrobial susceptibility and isolation of *Aeromonas* and *Vibrio* phages from water samples.
Theme: One Health, Skills Development Missions.
Home Institute: Anses, France.
Mission Hosting Institute: BfR, Germany.
Duration of mission: 4 weeks.

Aim of the mission: The objective of the mission was to share technical knowledge on the detection and characterisation of *Aeromonas* and *Vibrio* genus and to establish a collaboration between the institutes, to study of the dissemination of antibiotic resistance in aquatic environments.

Case study:



SHORT TERM MISSIONS

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- Contributing to the future prevention, preparedness, detection and response of the EU to foodborne and other emerging threats across human-animal-environmental sectors.

Genotypic characterisation of antimicrobial susceptibility and isolation of Aeromonas and Vibrio phages from water samples

Theme: One Health, Skills Development Missions
Home Institute: ANSES, France
Mission Hosting Institute: BfR, Germany
Duration of Mission: 4 weeks

"This mission was a great opportunity to discuss the dissemination of antibiotic resistance in the aquatic environment in connection with aquaculture activities. In addition to the improvement of the technical skills of both teams, the diversity of our fields of study (water, fish farming vs food) opened up perspectives for new collaborations, using a One Health approach."

Sandrine Baron and Laetitia Le Devendec ANSES, France

The objective of the mission was to share technical knowledge on the detection and characterisation of *Aeromonas* and *Vibrio* genus and to establish a collaboration between the institutes, to study of the dissemination of antibiotic resistance in aquatic environments.

During this mission, Sandrine and Laetitia were trained on phages' cultivation, enumeration and conservation methods. They now have in hands the detailed protocols, as well as phages and competent bacteria cultures, provided by the BfR team. They will be able to put the methods into practice in Anses. Sandrine and Laetitia travelled to BfR with culture media for the detection of *Aeromonas sp.*, from water and fish samples. Water samples have been collected nearby the BfR laboratory and the German team performed the entire handling: filtration, cultivation and presomptive identification. The partner teams established common protocols for the study of the *Aeromonas* and *Vibrio* genera and initiated the sharing of the Maldi-Tof databases, for the improvement of the species identification of bacteria from the *Aeromonas* and *Vibrio* genera. The two institutes are also now both involved in a research group, which aims to improve the methodology of antimicrobial susceptibility testing in aquatic bacteria and determine Epidemiological cut off values (Ecoff). Meetings and video conferences with French and Canadian researchers took place, to discuss research ideas. At the end of this STM, two research project proposals were elaborated.

This short-term mission strengthened the emerging collaboration between the two teams from the One Health EJP consortium. It has also led to a reflection on the use of phages to improve and reduce the use of antibiotics in aquaculture, a farming sector from which a One Health approach is absolutely needed.

One Health EJP has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773830.

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Figure 17: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2022.

10. Short Term Mission 10

STM Researcher: Eve Zeyl Fiskebeck
Title of the STM: Stable Intra-specific genomic bacterial classification systems.
Theme: One Health Missions.
Home Institute: Norwegian Veterinary Institute (NVI), Norway.
Mission Hosting Institute: Institut Pasteur, France.
Duration of mission: 4 days.

Aim of the mission: The aim of this mission was for the researcher to gain knowledge on the usage and design of a stable classification system and nomenclature approach for bacterial strains and to understand advantages and limitations of its use in genomic epidemiology. Previous isolate nomenclature/classification systems including serotyping and MLST typing do not always satisfy two major criteria: long-term stability and congruence with phylogenetic relationships, which are essential for routine epidemiological surveillance.

Case study:

SHORT TERM MISSIONS

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- Contributing to the future prevention, preparedness, detection and response of the EU to foodborne and other emerging threats across human-animal-environmental sectors.

Stable Intra-specific genomic bacterial classification systems

Theme:	One Health
Home Institute:	Norwegian Veterinary Institute (NVI), Norway
Mission Hosting Institute:	Institut Pasteur, France
Duration of Mission:	5 days

The aim of this mission was for the researcher to gain knowledge on the usage and design of a stable classification system and nomenclature approach for bacterial strains and to understand advantages and limitations of its use in genomic epidemiology.

Previous isolate nomenclature/classification systems including serotyping and MLST typing do not always satisfy two major criteria: long-term stability and congruence with phylogenetic relationships, which are essential for routine epidemiological surveillance. During this mission, theoretical training was provided by the host institute, to gain deep understanding and practical experience of a novel classification system and nomenclature approach for bacterial strains (cgLIN codes). Training also allowed further understanding of the advantages and limitations of its use in genomic epidemiology.

The STM provided valuable training, allowed knowledge transfer and strengthened the collaboration between One Health partners. Further funding will be sought to continue this valuable collaboration, in order to further develop and expand the nomenclature system for additional bacterial pathogens.

"The STM allowed me to understand and practice applying the newly developed cgLIN bacterial nomenclature system. I now have a better understanding on how the classification system can be used for genomic epidemiology of zoonotic and infectious diseases and able to disseminate this knowledge to other scientists. This will strengthen the activities in One Health to address existing and emerging threats of foodborne and zoonotic agents as well as animal pathogens"

Eve Zeyl Fiskebeck
NVI, Norway

One Health EJP has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773830.

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Figure 18: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2022.

11. Short Term Mission 11

STM Researcher: Bosco Rodríguez Matamoros
Title of the STM: Application of 'Single-Cell Genomics' for the study of the bacterial reservoirs of plazomicin resistance determinants.
Home Institute: Universidad Complutense de Madrid, Spain.
Mission Hosting Institute: Karlsruhe Institute of Technology, Germany.
Duration of mission: 3 months.

Aim of the mission: The aim of this mission was the development of a single cell workflow, for the detection of low abundant reservoirs of next-generation aminoglycoside resistance mechanisms. The main objective was to label single cells from complex environments with fluorescence markers specific for the genes of interest. This Short-Term Mission was framed within the OHEJP PhD METAPRO project and was expected to produce results complementing the ones already produced with the metagenomic analyses.

Case study:

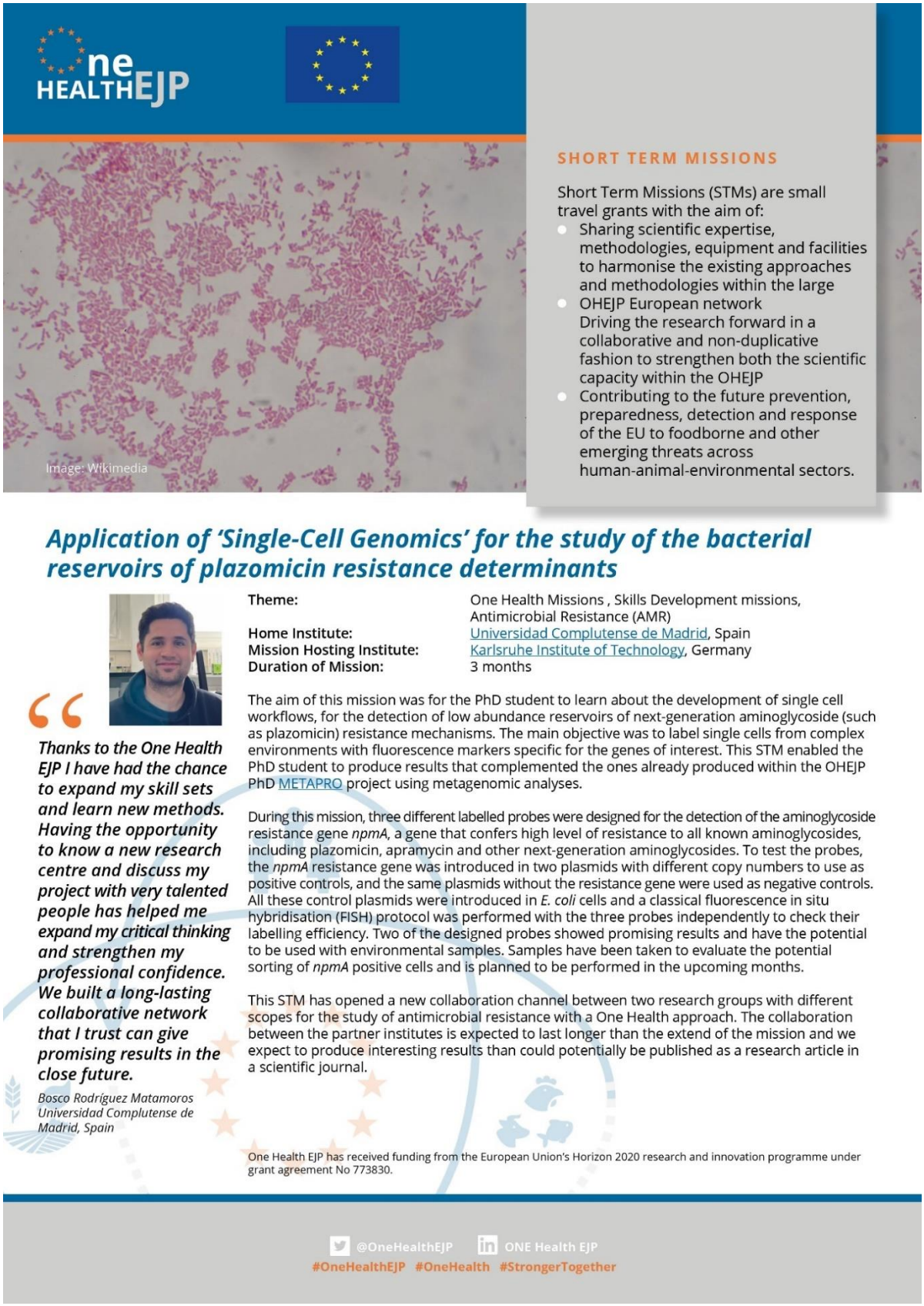

Figure 19: This case study can be found on the OHEJP website: https://onehealthejp.eu/community/education-and-training/short-term-missions-2022.