

## FINAL REPORT

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European Public Health Microbiology Training Programme (EUPHEM), Cohort 2021  
NATIONAL INSTITUTE OF HEALTH DOUTOR RICARDO JORGE, Portugal

## Background

The ECDC Fellowship Programme is a two-year competency-based training with two paths: the field epidemiology path (EPIET) and the public health microbiology path (EUPHEM). After the two-year training, EPIET and EUPHEM graduates are considered experts in applying epidemiological or microbiological methods to provide evidence to guide public health interventions for communicable disease prevention and control.

Both curriculum paths provide training and practical experience using the 'learning by doing' approach at acknowledged training sites across European Union (EU) and European Economic Area (EEA) Member States.

According to Article 9 (6), Article 5 (8) and Article 11a (1) of Regulation (EU) 2022/2370 of the European Parliament and of the Council of 23 November 2022 amending Regulation (EC) No 851/2004 establishing a European centre for disease prevention and control (the ECDC Founding Regulation):

Article 9 (6) 'The Centre shall, as appropriate, support and coordinate training programmes, in particular in relation to epidemiological surveillance, field investigations, preparedness and prevention, response to public health emergencies, public health research and risk communication. Those programmes shall take into consideration the need for training to be kept up-to-date, take into account the training needs of Member States and shall respect the principle of proportionality.'

Article 5 (8) 'By encouraging cooperation between experts and reference laboratories, the Centre shall foster the development of sufficient capacity within the Union for the diagnosis, detection, identification and characterisation of infectious agents that have the potential to pose a threat to public health. The Centre shall maintain and extend such cooperation and support the implementation of quality assurance schemes'.

Article 11a (1) 'The Centre shall establish an EU Health Task Force and ensure that there is a permanent capacity and an enhanced emergency capacity to mobilise and use it. The EU Health Task Force shall provide assistance with regard to requests for prevention, preparedness and response planning, local responses to outbreaks of communicable diseases and after-action reviews in Member States and in third countries, in cooperation with the WHO. The EU Health Task Force shall include the Centre's staff and experts from Member States, fellowship programmes and international and non-profit organisations'.

Moreover, Article 47 of the Lisbon Treaty states that 'Member States shall, within the framework of a joint programme, encourage the exchange of young workers.' Therefore, ECDC initiated the two-year EUPHEM training programme in 2008. EUPHEM is closely linked to the European Programme for Intervention Epidemiology Training (EPIET). Both EUPHEM and EPIET are considered 'specialist pathways' of the two-year ECDC fellowship programme for applied disease prevention and control.

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Stockholm, November 2023

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This final report describes the output of the fellow and the competencies they acquired by working on various projects, activities, theoretical fellowship training modules, other modules or trainings and international assignments or exchanges during the fellowship.

## Pre-fellowship short biography

Corrado Minetti is a biologist specialised in medical parasitology and entomology. He graduated in Biological Sciences and Zoology from the University of Turin, Italy, before moving to the United Kingdom for a Masters in Medical Parasitology and Entomology at the Liverpool School of Tropical Medicine. He then completed his PhD at the University of Liverpool, sponsored by Public Health England, with a thesis entitled 'The epidemiology and molecular epidemiology of giardiasis in North West England (2014)'. Before starting the Fellowship, Corrado worked in the Vector Biology Department of the Liverpool School of Tropical Medicine as a postdoctoral researcher (2015-2020). His work focused on applying molecular xenomonitoring approaches to the surveillance and epidemiology of vector-borne infections (filariasis, malaria, and tropical arboviruses), and included both laboratory work and fieldwork in Ghana, Africa.

## Results

The objectives of these core competency domains were achieved partly through project and activity work and partly by participating in the training modules. Results are presented in accordance with the EPIET core competencies, as set out in the ECDC Fellowship Manual<sup>1</sup>.

### 1. Epidemiological investigations

#### 1.1. Outbreak investigations

##### ***1.1.1 Epidemiological investigation of a Norovirus GII.4 Sydney [P16] variant outbreak in a long-term care facility, 2022***

Supervisor: Rita de Sousa (INSA)

Category: Food and waterborne diseases

On the 29 April 2022, the local Public Health Unit Moinhos (PHUM) was notified of 12 cases of acute gastroenteritis in residents and staff of a long-term care facility in the parish of Ramalhal, municipality of Torres Vedras. The unit contacted the viral gastrointestinal laboratory at INSA to organise the collection and microbiological testing of biological (stools and vomit) and environmental specimens, and a joint outbreak investigation team between PHUM and INSA was established to characterise the outbreak, confirm the identity of the responsible pathogen, and implement infection control measures. Out of 60 residents and 48 staff members of the facility, 43 people met the probable case definition (diarrhoea, vomiting, or nausea between 25 April – 14 May, 2022), and eight were laboratory-confirmed. The overall attack rate was 41.4% (45.5% among staff and 38.3% among residents). The highest attack rates were observed in male residents (50.0%), and in staff that did participate in personal hygiene tasks (50.0%). Out of 11 symptomatic individuals for whom stool specimens were tested, six residents and two staff members were positive for Norovirus (NoV) GII, identified further as NoV GII.4 Sydney [P16] variant by genotyping. Norovirus was also found in two of the six surface swabs taken from the toilet faucet of residents' living rooms, whereas all the 15 food samples collected from the kitchen were negative. Although the source of NoV introduction in the LTCF could not be identified, the virus spread was likely facilitated by deficiencies in hygiene conditions. No more cases were detected after the 7th of May and the outbreak was considered over on the 14th of May. The outbreak was controlled by ensuring that proper disinfection procedures were in place and guidelines for the management of norovirus outbreaks were followed.

Role: The fellow was a co-investigator. He joined the PHUM investigation team and visited the LTCF to help with administering the trawling questionnaire to probable cases and entering data for line listing and analysis. He also helped in extracting RNA from the cases' faecal and vomit specimens, and performing the real time RT-PCRs and nested PCRs for the virus genotype identification. The fellow contributed to data analysis and wrote the final version of the outbreak investigation report (Section 7.1.2, item 1). Additionally, the fellow is preparing a paper including the data from the outbreak (Section 7.1.1, item 1).

<sup>1</sup> European Centre for Disease Prevention and Control. European public health training programme. Stockholm: ECDC; 2020. Available from: <https://www.ecdc.europa.eu/en/publications-data/ecdc-fellowship-programme-manual-cohort-2021>

### **1.1.2 Ongoing monkeypox virus outbreak, Portugal, 29 April to 23 May 2022**

Supervisor: Jorge Machado (INSA)

Category: Emerging and re-emerging diseases including vector-borne diseases

Up to the 27<sup>th</sup> May 2022, Portugal detected 96 confirmed cases of Mpox (monkeypox) as part of the multi-country outbreak. The descriptive analysis of 27 confirmed cases revealed they were all males with a median age of 33 years (range: 22–51). The earliest symptom onset date was 29 April 2023. Almost all cases (n = 25) lived in the Lisbon and Tagus Valley health region. Most cases were neither part of identified transmission chains, nor linked to travel or had contact with symptomatic persons or with animals, suggesting the possible previously undetected spread of monkeypox.

Role: The fellow was involved in writing the microbiological results and contributing to the discussion for the published paper (Section 7.1.1, item 2), which he also revised and approved as co-author.

### **1.1.3 Prevention, Protocols, and Lab Capacity – Lessons from a Norovirus Outbreak in the Algarve**

Supervisor: Rita de Sousa (INSA)

Category: Food and waterborne diseases

On the 19 August 2022, local health authorities received an alert from a hotel in Algarve, reporting a suspected outbreak of acute gastrointestinal illness among 80 guests. The occurrence of illness among guests was also confirmed by the local emergency department, which had received several patients with complaints the previous night. An immediate investigation was initiated to determine the extent of the outbreak, identify the causative agent, and implement control measures. The viral gastrointestinal laboratory at INSA was involved in the microbiological testing of stool specimens from probable cases (individuals exhibiting symptoms of acute gastroenteritis and having stayed at the implicated hotel 15 – 26 August 2022). A total of 244 cases were reported, primarily involving Portuguese families with parents aged 40–50 and children aged 0–19. Five cases were hotel employees. Common symptoms included vomiting, nausea, abdominal pain, and diarrhoea. Norovirus (NoV) GI.3 [P3] variant was detected in stool specimens collected from eight probable cases, while food samples tested negative for NoV and other commonly implicated microorganisms. The most likely hypothesis is that the source of the outbreak was in the hotel's common areas, with subsequent person-to-person transmission occurring primarily within one of the buildings.

Role: The fellow was involved in writing the microbiological results and contributing to the discussion for the published paper (Section 7.1.1, item 3), which he also revised and approved as co-author.

#### **Educational outcome**

The fellow collaborated with public health experts in all the steps of the first investigation, including visiting the outbreak premises with the investigation team, and he learnt how to apply the concepts and tools of outbreak investigations by contributing to line listing, descriptive epidemiology, and microbiological testing. The fellow also collaborated with physicians and epidemiologists to summarise, integrate, and interpret epidemiological and microbiological evidence in outbreak contexts.

## **1.2. Surveillance**

### **1.2.1 Molecular surveillance of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and other sexually transmitted infections in a Lisbon community centre for men who have sex with men, and impact of the COVID-19 pandemic**

Supervisor: Maria José Borrego (INSA)

Men who have sex with men (MSM) are at greater risk for bacterial and other sexually transmitted infections (STIs), but the burden is underestimated due to extragenital asymptomatic carriage. Data are lacking for Portugal. Using a molecular diagnostic approach, the project aimed to assess the genital and extragenital prevalence of bacterial and other STIs in MSM presenting to a Lisbon community-based sexual health clinic (GAT-CheckpointLX) to better inform STIs screening strategies. Laboratory results for *Chlamydia trachomatis* (including lymphogranuloma venereum), *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis* from oral, anorectal, and urine specimens of 4 396 MSM at their first visit between June 2017 and December 2021 were analysed retrospectively. Additionally, results for lymphogranuloma venereum, *Treponema pallidum*, and Herpes Simplex Virus 1 and 2 (in ano-oro-genital lesions) and HIV and *T. pallidum* infection (in blood samples) were included whenever tested. The prevalence of *N. gonorrhoeae* was 16.6%, followed by *C. trachomatis* (13.2%), *M. genitalium* (10.3%) and *T. vaginalis* (0.2%). The most frequent occurrence was anorectal infection with *C. trachomatis* and *M. genitalium* and oral with *N. gonorrhoeae*. Lymphogranuloma venereum was detected in 21.8% of chlamydia-positive anorectal swabs. *T. pallidum* infection was detected in 22.6% of the 1 787 tested MSM, while 13.8% of 3 080 tested MSM had HIV. Gonorrhoea and chlamydia were significantly more prevalent in MSM with HIV or syphilis ( $p < 0.001$ ). The number of MSM attending the clinic increased over the years, and no substantial changes were detected in the number of positive specimens and prevalence of chlamydia, gonorrhoea, or syphilis after the start of the pandemic in 2020.

The substantial prevalence of bacterial STIs in MSM extragenital sites and coinfections with HIV and syphilis suggest that routine screening of attendees of sexual health clinics has value in identifying hidden carriage and providing better care to at-risk individuals.

Role: The fellow wrote the study protocol for seeking ethical approval for data retrieval and collation (see Management section), collated and cleaned the laboratory surveillance data, performed the analysis, and wrote the study report (Section 7.1.2, item 2) and a first author paper (Section 7.1.1, item 4).

### **1.2.2 SARS-CoV-2 genomic surveillance system in Portugal**

Since the start of the COVID-19 pandemic in 2020, INSA played a pivotal role in the surveillance and control of the disease in Portugal alongside the Direção-Geral da Saúde (Directorate-General of Health) (DGS). Besides regularly releasing bulletins on the epidemiological situation of the pandemic, the Institute is also involved in the molecular detection and monitoring of the virus variants using whole-genome sequencing (WGS) through the National Reference Laboratory for Influenza and other Respiratory Viruses and the Bioinformatics Unit. The fellow joined this activity and followed the entire process of the genomic surveillance work in detail. On a weekly basis, the involved hospitals and laboratories scattered over Portugal's territory provided the viral RNA extracted from swab samples of confirmed COVID-19 cases. At INSA, the RNA was first processed by the teams of the Infectious Diseases Department and then sent to the Bioinformatics unit for WGS. The viral genome was fully sequenced using a next generation sequencing (NGS) platform. The data were then published in the form of weekly situational reports on the different circulating variants, their prevalence, and geographic distribution in the country.

#### **Educational outcome**

The fellow gained knowledge of STIs diagnostics and surveillance in at-risk populations, laboratory surveillance data cleaning and analysis, and writing abstract proposals and papers for peer-reviewed journals. He also learnt the importance of screening and laboratory surveillance in assessing the burden of infection. He also learnt about the organisation of a genomic surveillance program, its importance in tracking pathogen variants, and the importance of releasing regular epidemiological reports for public health, particularly during a pandemic.

## **2. Applied public health microbiology and laboratory investigations**

### **2.1 Antibiotic susceptibility profile of *Legionella pneumophila* isolates from laboratory surveillance and outbreak investigations in Portugal, 2006-2022**

Supervisor: Paulo Gonçalves (INSA)

The choice of therapeutics for Legionnaire's disease (LD), caused by the bacillus *Legionella pneumophila*, is limited to antibiotics which can penetrate cells such as macrolides or fluoroquinolones. Although antibiotic resistance in *Legionella* has not been considered a major concern so far, there is little information on the antibiotic susceptibility profile of *L. pneumophila* strains circulating in Portugal. Hence, in order to better inform the management of LD and detect the potential emergence of resistance in this country, the study aimed to determine the antibiotic susceptibility to ten antibiotics in 107 *L. pneumophila* serogroup 1 clinical and environmental isolates, collected between 2006 and February 2022 by the National Reference Laboratory for *Legionella* at INSA. The antibiotics' minimum inhibitory concentration (MIC) was determined using Etest strips, and additionally by Cardiff University collaborators with the broth microdilution (BMD) and LASARUS medium methods. Isolates with a MIC above the cut-off values indicated in the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines were considered resistant. Additionally, the presence of the *lpeAB* gene involved in azithromycin resistance was determined by PCR amplification in all isolates. Out of the 107 isolates, nine (8.4%) carried the gene and had a MIC within the BMD EUCAST breakpoint for *lpeAB*-carrying *L. pneumophila*. However, nine isolates (one by Etest and eight by BMD) without the gene had azithromycin MIC values two- to four-fold higher than the breakpoints. As per BMD, seven (6.5%), one (0.9%), and seven (6.5%) isolates had MICs above the cut-off for ciprofloxacin, levofloxacin, and moxifloxacin respectively. Regarding rifampicin, 11 (10.3%) isolates had a MIC above the cut-off as per the Etest. One (0.9%) and 82 (76.6%) isolates had a MIC above the cut-off for doxycycline as per Etest and BMD, respectively, while four (3.7%) had a MIC above the cut-off for tigecycline as per Etest. Although results differed between the three different methods, there was evidence for reduced susceptibility to both azithromycin and fluoroquinolones in Portuguese *L. pneumophila*. There is an urgent need to collect more data on antibiotic resistance in *L. pneumophila* in Portugal, including its molecular determinants, and monitor LD patients for potential treatment failure.

Role: The fellow wrote the study protocol, revived and passaged the stored bacterial isolates, performed the antimicrobial susceptibility testing (Etest), collated the data from the three testing approaches, performed the data analysis, wrote the full study report (Section 7.1.2, item 3) and the first author submitted manuscript (Section 7.1.1, item 5).

## 2.2 Circulation of multi-resistant strains of *Shigella sonnei* and *S. flexneri* in the Iberian Peninsula from 2015 to 2022: a retrospective study

Supervisors: Ângela Pista (INSA), Silvia Herrera-Leon (ISCIII)

Resistance to first-line antibiotics is on the rise globally, which led WHO to include fluoroquinolone-resistance (FQR) in *Shigella* among the serious antimicrobial resistance (AMR) threats. However, little is known on the apparition and historic circulation of multi-resistant (MDR) *Shigella* species in the Iberian Peninsula. In particular, no data are available for Portugal. The aim of this study, done in collaboration between INSA and the Centro Nacional de Microbiología at Instituto de Salud Carlos III (ISCIII), was to characterize the historic circulation and genomic epidemiology of fluoroquinolone-resistant (FQR) strains of *S. sonnei* and *S. flexneri* in Spain and Portugal from 2015 to 2022. The resistance profile for a total of 21 antibiotics was determined by disk diffusion method in 184 *S. flexneri* and 232 *S. sonnei* isolates. Isolates were considered FQR if they showed resistance to one of the following antibiotics: ciprofloxacin, pefloxacin, or nalidixic acid. FQR isolates underwent whole-genome sequencing (WGS) to characterise the molecular determinants of resistance and identify clusters and phylogenetic relationships, using web-based bacterial genomic analysis tools. Results showed a progressive increase in the percentage of FQR isolates from 2015 until 2022, when 38% and 80% of *S. flexneri* and *S. sonnei* were FQR, respectively. Isolates from men were much more likely to be FQR than isolates from women. Two genetic clusters were identified of more than 30 *S. sonnei* isolates showing different profiles in terms of AMR, genetic determinants of resistance, and plasmid replicons, suggesting the existence of two outbreaks of FQR *S. sonnei* (one from 2015 to 2021 and the other from 2020 to 2022) in both countries. The circulation of resistant strains to both first and second line of treatment raises concerns about the clinical management of patients. The spread of extensively resistant *Shigella* infection, unnoticed before 2021, highlights the need to strengthen surveillance of shigellosis in the Iberian Peninsula.

Role: The fellow wrote the study protocol (with the ISCIII fellow), performed the antimicrobial susceptibility testing (disk diffusion), extracted the DNA, and submitted it for WGS for the Portuguese isolates. The fellow also submitted the Portuguese sequences for genome assembly and compiled the results for the molecular determinants of resistance, and collated all the Portuguese isolates data. Once data from both countries were collated, he wrote (with the ISCIII fellow) the co-first author submitted paper (Section 7.1.1, item 6). With the Portuguese isolates data, he also produced a full project report (Section 7.1.2, item 4) and a poster presented at ECCMID 2023 (Section 7.2).

### Educational outcome

The fellow gained knowledge of laboratory methods in medical bacteriology, antibiotic resistance and susceptibility testing, and genomic data analysis using web-based tools. He learnt how to integrate and interpret phenotypic and molecular (genomic) data for enhanced pathogen surveillance and to infer potential transmission patterns over time. He also learnt how to frame research questions with a clear public health microbiology focus, and to develop a full microbiology study protocol detailing all its steps. Additionally, by working with another fellow, he practiced developing a study and analysis plan together and sharing data and results.

## 3. Biorisk management

### 3.1 Biosafety management module homework: risk assessment using the BioRAM tool

The aim of this group exercise, part of the assignment for the Biorisk and Quality Management module completion, was to assess the biosafety risk associated with working with a fictional pathogen in the laboratory using the Biosecurity Risk Assessment Methodology (BioRAM) tool. The exercise consisted of using the information about the pathogen, the type of work, and the laboratory environment from the provided scenario to discuss and fill the different sections of the BioRAM module, and to come up with an assessment of the risk for both the individual performing the in vitro work and the community at large (including also animals). The module included a set of questions designed to provide a quantitative estimate of the likelihood of transmission and the severity of infection. The fellow was an active member of the group which included other fellows of the same cohort. At the end of the exercise, which was completed during an online meeting, the group submitted the results of the risk assessment.

### 3.2 Working in an arthropod containment level 3 (ACL3) facility fellow previous experience

Prior to starting the Fellowship, the fellow got extensive experience in working with vector-borne biosafety level 3 viral and parasitic pathogens during his postdoc at the Liverpool School of Tropical Medicine in the UK. The fellow performed mosquito infections with arboviruses (Dengue, Zika) in the arthropod containment level 3 (ACL3) facility after completing full training and authorisation by the facility biosafety officers. He also got experience in developing protocols and performing risk assessments for the establishment of mosquito infections with viruses and malaria parasites. He also familiarised with the shipping requirements, use of personal protective equipment, and decontamination procedures for biosafety level 3 vector-borne pathogens.

### **Educational outcome**

The fellow familiarised himself with the concepts of likelihood and consequences of exposure to a pathogen in a laboratory setting and he learnt how to use information to perform a biosafety risk assessment, not only limited to the level of the operator but also considering the risks for the human and animal community outside the laboratory environment. He also learnt how to discuss these topics in a group and to mediate between different views and perceptions of risk.

## **4. Quality management**

### **4.1 Poliovirus proficiency test external quality assessment (EQA) simulation**

Supervisor: Paula Palminha

The Global Polio Laboratory Network (GPLN) supports the global polio eradication activities through laboratory detection of polioviruses. All network laboratories undergo annual accreditation by WHO via proficiency tests (External Quality Assessment, EQA). The fellow was guided through a simulation of the poliovirus intratypic differentiation (ITD) proficiency test at the INSA National Reference Laboratory for Vaccine Preventable Diseases, which is a member of WHO European Regional Polio Laboratory Network. Ten unknown RNA samples were tested with two real time PCR kits provided by the WHO Collaborating Centre for Enteroviruses and Polioviruses at the CDC for differentiating Non-Polio Enterovirus (NPEV) or Poliovirus (PV), and within these whether they are Wild Type (WT), Sabin-Like (SL) or Sabin Like Discordant virus (VDPV virus). Samples were first screened with the real time RT-PCR ITD 5.2 kit targeting all enteroviruses (including non-polio viruses) (PanEV), all polioviruses (PanPV), Sabin polioviruses (S1-3), wild poliovirus type 1 (WPV1), wild poliovirus type 3 (WPV3), and poliovirus type 2 (PV2). Results were interpreted with the decision algorithm, and depending on the result samples were further screened with the VDPV 5.2 kit. Results were entered into the EQA worksheet and interpreted using the decision algorithm as described above. Compared to the original EQA results, some discordances were found possibly due to degradation of RNA samples.

Role: The fellow prepared and ran the real time PCR reactions, and interpreted the results using the decision algorithm.

### **4.2 Audits at the National Reference Laboratories for Gastrointestinal and Respiratory Infections**

The National Institute of Health serves as a state and national reference laboratory for infectious diseases. As such, the diagnostic and analytical work conducted in the Department of Infectious Diseases (DDI) must adhere to international quality standards. The department is accredited as a medical laboratory according to the standard EN ISO 15189:2014 (1). Audits and accreditation are provided by the Portuguese Accreditation Institute (Instituto Português de Acreditação, IPAC), a signatory of the European Accreditation Multilateral Agreement (EA MLA) and the International Laboratory Accreditation Cooperation Mutual Recognition Arrangement (ILAC MRA). Laboratories of the DDI are subjected to regular audits. The fellow observed two external audits taking place at the National Reference Laboratory for Gastrointestinal Infections – *Salmonella* (URGI) and the National Reference Laboratory for Respiratory Infections – *Legionella* and *Neisseria meningitidis* (URIR) on the 12 and 13 of October 2021, respectively. The audit included questions and checks on both management and technical activities performed in the laboratories. The purpose of the audit is to ensure that all the procedures, from specimen arrival in the laboratory up to the communication of the results to who requested them, are included in a quality management system and are adhered to. Various aspects were subject to examination, including quality control in both the pre-analytical and post-analytical phase, equipment checks, storage of reagents, record keeping and documentation (in both paper and electronic form), data analysis, and communication of results. Both laboratories completed the audit successfully.

### **4.3 Biosafety management module homework: Audit of the National Reference Laboratory for Legionella**

The aim of this exercise, part of the assignment for the Biorisk and Quality Management module completion, was to simulate the audit of a laboratory of choice at the training site using a laboratory quality evaluation spreadsheet tool. The fellow chose to audit the National Reference Laboratory for Legionella. The laboratory receives specimens for *Legionella* testing, and provides advice and guidelines for specimens' collection (which happens outside the lab). The audit form questions were related to every aspect of quality management of the laboratory and its diagnostic activities, and were divided between process management and quality control and documentation. The audit results showed that the laboratory followed all the procedures for process management, quality control, and the associated documentation was thorough.

### **Educational outcome**

The fellow learnt how audits and EQAs are performed, and he also appreciated their role and importance in a clinical and public health microbiology laboratory. He also learnt about the role of the Global Polio Laboratory Network and the importance of having good laboratory tests and practices in place, particularly to detect the resurgence of pathogens nearing elimination or eradication.

## 5. Public health microbiology management

### ***5.1 Molecular surveillance of Chlamydia trachomatis, Neisseria gonorrhoeae, and other sexually transmitted infections in a Lisbon community centre for men who have sex with men, and impact of the COVID-19 pandemic study protocol (for ethical approval)***

The aim of the project was to describe the burden of sexually transmitted infections (STIs) in men who have sex with men (MSM) presenting for testing or healthcare at a Lisbon community-based sexual health clinic (GAT-CheckpointLX). In order to proceed for data collation and analysis, it was important to protect personal identifiable information and at the same time gather sensitive information such as the HIV status. Hence it was necessary to obtain ethical clearance from INSA to use the data from the National Reference Laboratory for Sexually Transmitted Infections, and to ensure their safe sharing and collating with the study collaborators at the clinic and the EPIUnit at the Institute of Public Health, University of Porto. The fellow, with the help of all the collaborators, wrote the study protocol and gathered all the documentation needed for the submission to the ethics committee on the 4 March 2022. Clarifications were requested from the committee in April, and updated documentation with the modifications required were re-submitted at the end of the month. Further clarifications were requested in May 2022. The requests were answered in an updated submission at the end of June, and final approval was officially obtained on the 12 July 2022.

### ***5.2 Participation to the RONDA meetings***

Once a week, an online RONDA (Reunião sobre Observações, Notícias, Dados e Alertas) meeting is organised by the Support Unit of National Health Authority and the Emergency Management in Public Health based at the Centre for Public Health Emergencies (CESP) of the Portuguese Directorate General of Health (Direção-Geral da Saúde, DGS). Participants include several of the DGS and focal points from the Regional Health Administrations, the Autonomous Region of the Azores and of Madeira, and INSA. The objective of these meetings is to provide information on events with a potential impact on public health at both national and international level. RONDA meetings provide important and regular updates on the epidemiological situation regarding infectious threats in Portugal and in relation to the rest of Europe and the world. Between October 2022 and March 2023 the fellow assisted to a series of RONDA meetings to learn more about them and their importance in public health and infectious threats management at the national level.

### ***5.3 Shigellosis enhanced surveillance questionnaire for Portugal***

Following the analysis of the data on the circulation of multidrug resistant (MDR) *Shigella* in Portugal, it became apparent that the surveillance of shigellosis could be improved. In particular, since the whole-genome sequencing of *Shigella* MDR isolates has become routine at INSA, it would be important to collect more detailed exposure information from the cases in order to complement the genomic evidence and better understand the contribution of person-to-person, travel-related, and food and waterborne transmission to the epidemiology of the disease in Portugal. For this reason, the fellow prepared an enhanced surveillance questionnaire for future use by INSA. The questionnaire (translated in Portuguese for immediate use) included a series of questions on symptoms, antibiotic treatment, occupation, and travel abroad or within the country, water and food consumption, and person-to-person contacts (including those of sexual nature) in the week preceding the symptoms onset.

### ***Educational outcome***

The fellow gained knowledge on the issue related to personal identifiable information linked to laboratory diagnostic results, how to consider and acknowledge ethical issues in the use of sensitive information in research protocols, how to write a research protocol for ethical approval, and how to interact with an ethical committee to address concerns. The fellow also learnt more about how public health meetings are done, and their utility in keeping focal points and public health managers updated with ongoing and emerging threats, and he understood better how emerging epidemiological information is collected and shared at the national level and between the different regions for preparedness and response. Overall, across the different projects the fellow learnt how to interact with different public health workers and specialists, including those on the frontline of infection management and control, and how to manage and summarise information from different sources always taking into account the different perspectives and with an applied public health angle.

## 6. Teaching and pedagogy

### 6.1 Tuberculosis case study for the outbreak investigation course (for resident physicians in public health)

The aim of the course was to give medical residents in public health the basics of field epidemiology, and to train them in conducting and interpreting outbreak investigations. The training was held face to face, at the National Institute of Health Doutor Ricardo Jorge over two days (14-15 July 2022). The fellow took part in the more practical session on the second day, by preparing and facilitating a case study on a tuberculosis outbreak involving the use of whole-genome sequencing. The fellow adapted one of the Introductory Course case studies for this occasion. The participants were able to interact, ask questions and work in parallel with the fellow to understand the use and interpretation of molecular typing and whole genome sequencing evidence during an outbreak investigation. The participants also provided feedback by filling a short online questionnaire. The comments were very positive, and the two aspects which were particularly appreciated included the practical component and use of what was taught, as well as the high levels of interaction between people during the sessions. The level of knowledge and teaching abilities of the facilitators were also positively noted, and nearly all participants found the course useful for their work and career.

### 6.2 Xenomonitoring and xenosurveillance webinar (for EAN members)

The aim of the webinar was to give EAN members the basics of using mosquitoes to detect vector-borne (xenomonitoring) and non-vector-borne (xenosurveillance) pathogens, focussing specifically on threats in Europe. The one-hour webinar was promoted within the EAN network and by the fellow himself, and it was held online on the 26 October 2022. The fellow organised the presentation with a first part covering the aims, methods, and data interpretation of xenomonitoring/xenosurveillance studies, followed by examples from the published literature on West Nile Virus and filariasis to better clarify the use of these approaches in public health. At the end of the webinar, the participants were able to ask questions and they provided feedback through an online form prepared and shared by the fellow. Overall, the comments were very positive and most participants rated the overall webinar as excellent noting the clarity and enthusiasm of the speaker. More than half of the participants said that they were considering using xenomonitoring and/or xenosurveillance in the future. The recording of the webinar was uploaded on the EAN website.

### 6.3 Molecular diagnostic tools for endemic and imported (vector-borne) blood and tissue parasitic diseases online lecture

The aim of the lecture was to give public health microbiologists and other professionals an updated overview on the molecular diagnostic (and typing) tools used for imported and endemic blood and tissue vector-borne parasites of medical interest for Europe and Portugal. The one-hour lecture was held online on the 16 June 2023 using the Microsoft Teams online platform, after being promoted by INSA and the fellow himself. The fellow organised the presentation with a first part introducing the basic epidemiological and diagnostic features of the parasitic diseases, followed by a second part on the advantages and formats of molecular tools (including whole-genome sequencing and metagenomics) for the clinical management, surveillance, and outbreak investigations of such pathogens. Examples from the published literature were included. At the end the participants were able to ask questions and they provided feedback through an online form prepared and shared by the fellow. The majority of attendees worked in a public health institute, followed by those working in academia and in the private sector. The main reason to attend the lecture was a personal and professional interest (people working in the same area) in the topic, and to keep updated with the latest tools in the field. Overall, the comments were very positive and most participants rated the overall lecture very good to excellent, and thought the learning objectives were met. The lecture aspects that were most appreciated included the topic itself (particularly the presentation of metagenomics and the latest advancements), the clarity, and also the communication style of the speaker.

#### *Educational outcome*

The fellow learnt how to facilitate a case study, putting in use what he learnt during the various fellowship modules, and how to appropriately plan and adapt the content and the style of delivery according to different training needs, purposes, and format (online versus face-to-face lectures). He also learnt how to adapt and deliver the topic and the public health message in the clearest way to people from diverse backgrounds. Using the feedback received after the teaching activities, the fellow will pay particular attention to include more practical examples and ensuring there is time to engage more his audience using online interactive tools.



## 7. Communication

### 7.1 Publications related to the EUPHEM fellowship

#### 7.1.1 Manuscripts published in peer-reviewed journals

1. **Minetti C\***, Manageiro V\*, Ourique M\*, Lucas M, Vicente A, Roque C, Coelho A, Oleastro M, de Sousa R, Rodrigues N. Epidemiological and genetic characterization of Norovirus outbreaks occurred in LVT region, Portugal 2017-2022. (\*co-first authorship) (manuscript in preparation)
2. Perez Duque M, Ribeiro S, Martins JV, Casaca P, Leite PP, Tavares M, Mansinho K, Duque LM, Fernandes C, Cordeiro R, Borrego MJ, Pelerito A, de Carvalho IL, Nuncio S, Manageiro V, **Minetti C**, Machado J, Haussig JM, Croci R, Spiteri G, Casal AS, Mendes D, Souto T, Pocinho S, Fernandes T, Firme A, Vasconcelos P, Freitas G. Ongoing monkeypox virus outbreak, Portugal, 29 April to 23 May 2022. *Euro Surveill.* 2022 Jun;27(22). <https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2022.27.22.2200424> (published)
3. Sá R, Roque J, Marques Mendes P, Gonçalves I, Sousa J, Matos C, Júnior A, Coelho A, Belo Correia C, Manageiro V, **Minetti C**, de Sousa R, Horta Correia F, and Outbreak Investigation Team. Prevention, Protocols, and Lab Capacity: Lessons from a Norovirus Outbreak in the Algarve. *Arch Virol.* 2023 Nov 28;168(12):299. <https://link.springer.com/article/10.1007/s00705-023-05926-z> (published)
4. **Minetti C**, Rocha M, Miguel Duque L, Meireles P, Correia C, Cordeiro D, João I, Manita C, Soeiro S, Almeida Santos J, Matos R, Almeida C, Cortes Martins H, Vinagre E, Lopo S, Borrego MJ. Orogenital and anal infection by *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and other sexually transmitted infections in MSM in Lisbon. (Submitted to International Journal of STD and AIDS. Submission ID: IJSA-23-255)
5. **Minetti C**, Barton R, Farley C, Spiller OB, Rodrigues R, Gonçalves P. Antimicrobial susceptibility testing reveals reduced susceptibility to azithromycin and other antibiotics in *Legionella pneumophila* serogroup 1 isolates from Portugal. (Submitted to European Journal of Clinical Microbiology & Infectious Diseases. Submission ID: EJCM-D-23-01095). (Also available as preprint in medRxiv: <https://doi.org/10.1101/2023.09.25.23296014>)
6. Jacqueline C\*, **Minetti C\***, Silveira L, Pista Â, Herrera-Leon S. Circulation of fluoroquinolone-resistant strains of *Shigella sonnei* and *Shigella flexneri* in the Iberian Peninsula from 2015 to 2022: a retrospective study. (\*co-first authorship) (Submitted to Eurosurveillance. Submission ID: EMID:e9382425e8d2970d)

#### 7.1.2 Other reports

1. Epidemiological investigation of a Norovirus GII.4 Sydney [P16] variant outbreak in a long-term care facility, 2022
2. Molecular surveillance of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and other sexually transmitted infections in a Lisbon community centre for men who have sex with men, and impact of the COVID-19 pandemic
3. Antibiotic susceptibility profile of *Legionella pneumophila* isolates from laboratory surveillance and outbreak investigations in Portugal, 2006-2022
4. A retrospective study of the antimicrobial susceptibility profile and genomic epidemiology of *Shigella sonnei* and *S. flexneri* strains in Portugal from 2015 to 2022

### 7.2 Conference presentations

- ECCMID 2023, 15-18/04/2023, Copenhagen, Denmark. **C. Minetti**, L. Silveira, A. Pista. Antibiotic resistance profile of multidrug-resistant *Shigella sonnei* and *Shigella flexneri* in Portugal, 2015-2022. ePoster Flash presentation (Accepted and presented).
- ESCAIDE 2022. **C. Minetti**, M. Rocha, L. M. Duque, C. Correia, I. João, D. Cordeiro, S. Almeida, M. J. Borrego. Prevalence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and other bacterial sexually transmitted infections in men who have sex with men referring to a Lisbon community-based sexual health centre, 2019-2021. (Submitted)
- ESCAIDE 2022. M. Ourique, V. Manageiro, **C. Minetti**, A. Vicente, C. Roque, A. Coelho, M. Oleastro, N. Rodrigues, R. de Sousa. Epidemiological investigation of a Norovirus GII.4 Sydney variant outbreak in a long-term care facility in Portugal, 2022. (Submitted)
- XLI Reunión Anual de la Sociedad Española de Epidemiología (SEE) y XVIII Congresso da Associação Portuguesa de Epidemiologia (APE) 2023. M. Ourique, V. Manageiro, **C. Minetti**, A. Vicente, C. Roque, A. Coelho, M. Oleastro, N. Rodrigues, R. de Sousa. Epidemiological investigation of a Norovirus GII.4 Sydney [P16] variant outbreak in a long-term care facility in Portugal, 2022. (Accepted for oral communication)
- 7<sup>th</sup> ESCMID Study Group for Legionella Infections (ESGLI) meeting 2023. C. Minetti, R. Barton, C. Farley, O.B. Spiller, R. Rodrigues, P. Gonçalves. Antimicrobial susceptibility testing reveals reduced susceptibility to azithromycin and other antibiotics in *Legionella pneumophila* serogroup 1 isolates from Portugal. (Accepted and presented as a poster by P. Gonçalves)

## 8. EPIET/EUPHEM modules attended

1. Introductory Course part 1, 20/09/2021 to 08/10/2021, virtual
2. Introductory Course Inject Day – Operational Research, 27-28/10/2021, virtual
3. Introductory Course Inject Day – Data Management, 10-11/11/2021, virtual
4. Outbreak Investigation, 06–10/12/2021, virtual
5. Biorisk and Quality Management, 17–18/01/2022, virtual
6. Multivariable Analysis, 14–18/03/2022 (Inject Day 30/03/2022), virtual
7. Mid-term Project Review 2022, 20-22/04/2022, Spetses, Greece
8. Introductory Course part 2, 25-29/04/2022, Spetses, Greece
9. Rapid Assessment and Survey Methods, 06-10/06/2022, Solna, Sweden
10. Project Review Module, 29/08/2022 to 02/09/2022, Lisbon, Portugal
11. Time Series Analysis, 07-11/11/2022, Bilthoven, Netherlands
12. Qualitative Research – Optional Inject Days, 31/01/2023 and 03/02/2023, virtual
13. Vaccinology, 13-17/02/2023, virtual
14. Management, Leadership and Communication in Public Health, 08–12/05/2023, ECDC, Stockholm, Sweden
15. Project Review Module, 28/08/2023 to 01/09/2023, Lisbon, Portugal

## 9. Other training

1. WHO Introduction to International Health Regulations (IHR) e-learning course, 17/09/2021, online
2. ESCAIDE 2021, 16-19/11/21, online
3. WHO Public Health Preparedness for Mass Gathering Events e-learning course, 26/05/2022, online
4. UNDSS BSAFE e-learning course, 26/05/2022, online
5. ECDC Epidemic Intelligence e-learning course, 27/05/2022, online
6. Technical University of Denmark (DTU)/Coursera Whole genome sequencing of bacterial genomes - tools and applications e-learning course, 01-03/08/2022, online
7. EAN Mini-Module on Media and Infodemic Management, 21-22/1/2022, Stockholm, Sweden
8. ESCAIDE 2022, 23-25/11/22, Stockholm, Sweden
9. ECDC Assessing and grading evidence in public health (PRECEPT) e-learning course, 04.04.2023, online

## 10. Missions

Not applicable

## 11. Other activities

1. ASM satellite workshop 2022 - Diagnostics workshop: mobile detection platforms for One Health diagnostics applications, 14/04/2022, online
2. Developing teaching material and facilitating case studies in the EAN Mini-Module on molecular epidemiology (20-21/11/2023)

## Acknowledgements

I would first like to thank my supervisor, Rita de Sousa, and both my frontline coordinators, Silvia Herrera Leon and Jennifer Bender, for their invaluable support, feedback and good advice during the entire fellowship. I also want to mention all the ECDC lecturers, facilitators, and the whole administrative team for all the training and support they provided.

A big thank goes to all the people of the National Reference Laboratories at INSA that contributed with their data and helped me in the projects and activities with their expertise. I would like to mention in particular Ricardo Mexia and Maria João Gargate for involving me in teaching and training activities. Also I would like to thank the people from the Public Health Unit Moinhos for their availability in involving me in the field component of the outbreak investigation, and the people from the GAT-CheckpointLX clinic for their help with the projects.

Last but not least, I would like to thank my fellowship cohort for the support and unforgettable experiences during the training modules. A particular thank goes to Vera Manageiro, Christina Merakou, Eftychia Kotronia, Lida Politi, Gordan Sarajlic, Camille Jacqueline, Ioanna Spiliopoulou, and Alvaro Roy Cordeiro for the wonderful time we spent together and a friendship that will surely continue after the fellowship. You all made these two years very special.