

Summary of work activities

Diana Sofia Pereira Espadinha de Oliveira Costa

The ECDC Fellowship Programme

Public Health Microbiology path (EUPHEM), 2020 cohort

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 in acknowledged training sites across European Union (EU) and European Economic Area (EEA) Member States.

According to Articles 5 and 9 of ECDC's founding regulation (EC No 851/2004) 'the Centre shall, encourage cooperation between expert and reference laboratories, foster the development of sufficient capacity within the community for the diagnosis, detection, identification and characterisation of infectious agents which may threaten public health' and 'as appropriate, support and coordinate training programmes in order to assist Member States and the Commission to have sufficient numbers of trained specialists, in particular in epidemiological surveillance and field investigations, and to have a capability to define health measures to control disease outbreaks'.

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.

This report summarises the work activities undertaken by Diana Sofia Pereira Espadinha de Oliveira Costa, cohort 2020 of the European Public Health Microbiology Training Programme (EUPHEM) at the Public Health Laboratory Health Service Executive Dublin (PHL-HSE), Ireland.

Pre-fellowship short biography

Diana Espadinha de Oliveira Costa holds a BSc in Molecular Biology and Genetics from the University of Lisbon Faculty of Sciences and an MSc in Medical Microbiology and a PhD in Molecular Biology from the NOVA University of Lisbon. She dedicated nine years to academic research, focusing on the molecular epidemiology and virulence of healthcare- and community-associated methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. Having always been interested in the link between microbiology and human health and feeling a strong call for public service, Diana applied to the EUPHEM programme shortly after finishing her PhD, in order to obtain experience and training in different areas of the public health sector, improve her microbiological and epidemiological analytical skills, and broaden her knowledge in different categories of infectious diseases affecting human health.

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Methods

This report accompanies a portfolio that demonstrates the competencies acquired during the EUPHEM fellowship by working on various projects, activities and theoretical training modules.

Projects included epidemiological investigations (outbreaks and surveillance); applied public health research; applied public health microbiology and laboratory investigation; biorisk management; quality management; teaching and public health microbiology management; summarising and communicating scientific evidence and activities with a specific microbiological focus.

The outcomes include publications, presentations, posters, reports and teaching materials prepared by the fellow. The portfolio presents a summary of all work activities conducted by the fellow, unless prohibited due to confidentiality regulations.

Results

The objectives of these core competency domains were achieved partly through project or activity work and partly through participation in the training modules. Results are presented in accordance with the EUPHEM core competencies, as set out in the ECDC Fellowship Manual ¹.

1. Epidemiological investigations

1.1 Outbreak investigations

1.1a Management of Legionella pneumophila contamination in the water distribution system at a long-term health care residential facility

Supervisor: Eleanor McNamara

On 13 May 2021, the regional Public Health Laboratory (PHL) in Dublin alerted the local Community Healthcare Organisation (CHO) about an unexpected rise in the levels of *Legionella pneumophila* (serogroup 2-14) in the main water distribution system at three long-term healthcare residential units, part of a larger long-term care facility campus with a total of nine residential units. At the time of the incident, 139 beds were occupied on the campus: 123 beds by older people in need of long-term residential care, 10 were addiction service beds for people undergoing treatment for addiction to drugs; and six beds were occupied by younger people with disabilities, distributed across eight physically discreet units. With regard to gender distribution: 53 residents were male and 86 were female. Combined mean age, excluding addiction beds, was 81.4 years. The water for all residential units came from a treated public water supply and was stored in tanks, before being fed through the main water tower distribution system. Although no cases of human infection were registered at the time of the alert, the Infection Prevention and Control (IPC) and Water Safety Committees at the facilities were convened and after an initial risk assessment, regular meetings were held with a multidisciplinary team to define and implement a plan of action for preventive and mitigation measures.

Initial samples from the three residential units were taken on 7 May 2021, as part of the annual *Legionella* preventative & maintenance scheme. This was planned to take place in the first quarter of 2021, but had to be postponed due to COVID-19 restrictions. All water samples demonstrated levels of *Legionella* species growth (>100 cfu/L) that were non-compliant with the national regulations for *Legionella* management in healthcare settings. The PHL provided continuous updates on the laboratory testing results for the samples collected during the sampling rounds (a total of 62 water samples were collected and processed). The most affected units were in an older area of the campus. The IPC & Water Safety Committees promptly implemented remedial and preventive action to protect the health of residents and healthcare workers at the long-term care facility which reduced and eliminated *Legionella* water contamination. Alert surveillance was also initiated for clinical symptoms of Legionnaires' disease among residents and healthcare workers.

Long-term priority actions listed in a previous *Legionella* risk assessment, compiled out by an external environmental services (EES) company on 26 April 2021, were revised by the above-mentioned committees and recommendations were provided to the Health Estates Management (HEM) and the Regional Health Authority at a water safety meeting held in August 2021, for a future quality-managed water system to mitigate *Legionella* contamination.

Role: Diana participated in all phases of the investigation: attending the meetings, following up on the laboratory results and making site visits to overview implementation of corrective measures. She also collated data and wrote the outbreak report in collaboration with the IPC specialist nurse practitioner.

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

1.1b Gastroenteric illness outbreak at a crèche in south-east Ireland

Supervisor: Eleanor McNamara

In October 2021, the Public Health Laboratory (PHL) of the Health Service Executive (HSE) Dublin was involved in the investigation of a suspected verotoxigenic *Escherichia coli* outbreak at a crèche in south-east Ireland. A total of 95 staff and children attended the crèche, and 28 (10 staff and 18 children) became symptomatic. Dates of symptom onset ranged from 22 October 2021 to 22 November 2022. Symptoms reported included vomiting, diarrhoea and abdominal pain. Stools were received, processed and analysed by the VTEC National Reference Laboratory. One VTEC VT2 culture-positive case and one VT2 PCR positive/culture-negative case were detected. Both children had been in the same room at the crèche. No other VTEC positive cases were detected. All other symptomatic cases were interviewed and no common exposure, other than crèche attendance, was found. The index case had a private well but the water was deemed safe after laboratory investigation. Further testing for other enteropathogens was not possible as the alert arrived late at PHL and no suitable samples were collected.

Role: Diana participated in the outbreak control team meetings and followed-up on the laboratory results.

1.1c The first description of a nosocomial breakthrough outbreak caused by SARS-CoV-2 Eta (B.1.525) variant, Germany, June 2021

Supervisor: Silvia Herrera-Leon

On 14 June 2021, two fully immunised patients on Ward A of a general hospital in Germany tested positive for SARS-CoV-2. The hospital reported a high (>90%) COVID-19 immunisation coverage among healthcare workers and all patients had been tested by PCR for SARS-CoV-2 infection on admission and on Day five. The hospital infection control team investigated the outbreak, gathering epidemiological, contact tracing and microbiological data to identify transmission routes and prevent further infections. On 25 June 2021, it was confirmed that the outbreak involved the SARS-CoV-2 Eta (B.1.525) variant, and the Nordrhein-Westfalen State Centre for Health was notified. Overall, the outbreak involved 14 cases: seven patients, six healthcare workers, and one visitor to a patient case. Patients had no contact with one another and were all accommodated in ensuite single rooms on three wards (A, B and C) on different floors. All six healthcare workers worked in indirect patient care all over the hospital. Vaccinated cases were older than non-vaccinated (median 69.5 versus 27 years) and more likely to be asymptomatic (60% versus 33%). No healthcare workers working in direct patient care tested positive for SARS-CoV-2. The attack rate (AR) for non-medical healthcare workers was 2.6% among 38 healthcare workers employed in patient transport, and 4.5% among 22 healthcare workers in the bed preparation unit. The infection rate was 17.2% for 29 patients at risk on Ward A. Whole-genome sequencing data was available for five cases, all placed together in the SARS-CoV2 Eta variant clade (B.1.525) with a maximum difference of 0-2 nucleotides, indicating that the cases probably all belonged to the same outbreak cluster. The infection was probably introduced by an asymptomatic or minimally symptomatic healthcare worker, followed by healthcare worker-to-healthcare worker transmission and healthcare worker-to-patient transmission.

Role: This outbreak investigation was led by the EPIET fellow Katja Siling. Diana was responsible for the phylogenomic analysis of the Eta-variant SARS-CoV-2 strains and helped to write and review the manuscript.

1.1d COVID-19 outbreak in an acute healthcare facility

Supervisor: Eleanor McNamara

In February 2020, Diana was asked to provide assistance for a COVID-19 outbreak investigation taking place at an acute hospital in Dublin. The outbreak was ongoing, involving both patients and healthcare workers and had already affected nine wards of the hospital.

Role: Diana's role, in collaboration with a medical doctor, was to formulate a case definition, set up a line listing, collect and collate data from the hospital's IT system, build an Excel database and provide the first descriptive data for the outbreak. Diana also prepared a presentation with the tables and graphs obtained from the data analysis. The data analysis provided useful information for the outbreak investigation team on the rates and trends of SARS-CoV-2 infection among patients and healthcare workers at the hospital. It also facilitated nearly 'real-time' monitoring of the outbreak as it evolved on the wards.

1.1e COVID-19 outbreak in a long-term healthcare residential unit

Supervisor: Eleanor McNamara

Diana participated in the outbreak team's weekly/bi-weekly meetings concerning a COVID-19 outbreak at a long-term healthcare residential unit between October 2020 and December 2021. These meetings provided exposure to the reality of outbreak management and the various aspects involved: line listing, contact tracing, screening, infection and prevention control measures and communication with the various relevant stakeholders.

Role: Diana was responsible for collating the data and generating an epidemiological curve that could show the evolution of the COVID-19 outbreak among the patients and healthcare workers of the long-term care residential unit.

Training modules related to the assignment/projects

EPIET/EUPHEM Introductory Course – this module provided the fellow with an introduction to public health microbiology and field epidemiology. It also provided an overview of basic concepts of logistical and analytical approach to outbreak investigations, including the ten steps of an outbreak investigation (establish the existence of an outbreak, confirm the diagnosis, case definition, case-finding and data collection/collation, describe findings and formulate hypothesis, analytical studies, draw conclusions, conduct additional investigations, communication of findings, and implement control and prevention measures).

Outbreak Investigation Module – the fellow was taught how to perform analytical epidemiological studies within outbreak investigations using various software packages (MS Excel, EpiData, R and STATA). Practical training was also given on when and how to perform analytical studies for an outbreak investigation, including descriptive, cohort and case-control studies.

Management, Leadership and Communication in Public Health module - the fellow learned about various aspects and styles of management, leadership, and communication within a public health context, also including outbreak investigations.

Educational outcome

Participation in the different outbreak investigations allowed the fellow to apply concepts of microbiology and epidemiology in outbreak situations; develop competencies in the 10 stages of an outbreak investigation; actively participate and engage with a multidisciplinary outbreak team, manage datasets, perform laboratory typing methods, and write reports.

1.2 Surveillance

1.2a Evaluation and comparison of WGS-based analytical methods for molecular surveillance of *Mycobacterium tuberculosis* at a National Reference Laboratory

Supervisors: Margaret Fitzgibbon and Tom Rogers

Whole genome sequencing-based methodologies have become extremely relevant in the context of molecular surveillance of human pathogens and have been increasingly introduced into public health laboratory services. With this study, we aimed to evaluate core-genome Multi-Locus Sequence Typing (cgMLST) and whole genome single-nucleotide polymorphism (wgSNP) analysis in terms of ease-of-use of the respective WGS platforms, and concordance of clustering and discriminatory power with Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat (MIRU-VNTR) typing. A total of 188 Irish *M. tuberculosis* clinical isolates collected from 1999 to 2019, and previously grouped in 13 cluster by MIRU-VNTR and sequenced by Illumina's MiniSeq platform, were analysed using both cgMLST and wgSNP analysis. cgMLST was performed using Ridom SeqSphere+ (v.7): 2 891 core-genome genes scheme with a threshold of ≤ 12 alleles to identify clusters. SNP analysis was performed using the MTBseq pipeline, with clusters defined with a maximum distance threshold of five SNPs. From the original 13 MIRU-VNTR clusters, cgMLST and SNP analysis produced a smaller number of clusters (10 and 15) and a greater number of 'sporadic' strains (26 and 51), with three MIRU-VNTR clusters completely disassembled. SNP analysis showed the highest discriminatory power ($p < 0.001$) of the three methods, and concordance of results was 88% with MIRU-VNTR and 76% with cgMLST and wgSNP-analysis, respectively. Overall, cgMLST and SNP-analysis are both suitable for inference of genetic relatedness. However, due to the simplicity of use and streamlined analysis provided by the SeqSphere+ platform, cgMLST is recommended as the first-line approach for molecular typing of *Mycobacterium tuberculosis* isolates in the context of routine surveillance work. wgSNP-analysis can be reserved for a more in-depth analysis of new isolates showing a distance of ≤ 12 alleles to isolates from already existing cgMLST clusters.

Role: Diana performed the WGS analysis using both SeqSphere+ software and the MTBseq pipeline; evaluated and compared the two analytical strategies by carrying out statistical analysis and a qualitative assessment; wrote a final internal report and submitted a manuscript to a peer-reviewed journal.

1.2b Transmitted drug-resistance in HIV-1 in Ireland, 2019

Supervisors: Cillian de Gascun (NVRL), Kate O' Donnell (HPSC)

With increasing global access to antiretroviral therapy (ART), HIV drug resistance (HIVDR) in patients with no prior exposure to ART has become a reality, posing a threat to the long-term goal of controlling the HIV pandemic. In Ireland, transmitted HIV drug resistance (TDR) in ART-naïve individuals has been monitored since 2017, in an effort to inform public health action and treatment guidelines. This study aimed to assess the prevalence of HIV TDR for the year of 2019. Epidemiological data from the national Computerised Infectious Diseases Reporting system (CIDR) were linked to genotypic antiretroviral testing (GART) data from the National Virus Reference Laboratory (NVRL), for individuals diagnosed with HIV-1 in Ireland during 2019. Susceptibility to any of the 24 available antiretroviral drugs in the four main drug classes was tested: nucleoside-analogue reverse transcriptase inhibitors (NRTIs), non-nucleoside analogue reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs),

and integrase strand transfer inhibitors (INSTIs). TDR was categorised on the basis of WHO's 2009 List of Mutations For Surveillance of Transmitted Drug Resistant HIV Strains and the 2019 INSTI list, with 95% confidence intervals calculated using the Wilson score interval for binomial proportions. Those under the age of 18 years were excluded from this analysis. Of 529 HIV-1 diagnoses in 2019, 161 were identified as ART-naïve and underwent GART: the prevalence of TDR to any drug class was found to be 8.6% (95% CI 5.2-14), a non-significant decrease compared with 2018 (10.8%, 95% CI 7.2-16). TDR prevalence was 6.2% for NNRTI, 1.9% for PI, and 1.2% for NRTI. In total, 13 cases showed ≥ 1 mutation conferring resistance to a single drug class. Combined resistance to NRTIs, NNRTIs and INSTIs was detected in one person (0.6%), for the first time since TDR surveillance commenced in Ireland in 2017. Continued TDR surveillance is essential to monitor trends and guide early public health action and national/regional ART guidelines.

Role: Diana was responsible for the management and merger of the epidemiological and GART data, data validation, descriptive analysis, and write-up of the national surveillance report published online. The work was submitted and presented as a poster at the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) in 2022.

Training modules related to the assignment/projects

EPIET/EUPHEM Introductory course – the fellow was taught various aspects of surveillance, including the different types of surveillance; the basic functions and elements required for the establishment of a surveillance system; simple statistics to analyse epidemiological data; how to interpret surveillance data and how to communicate public health information to various stakeholders.

Rapid Assessment and Survey Methods module – this module covered aspects related to surveillance such as rapid risk assessment and surveillance planning in resource-poor or crisis situations, geographical information systems and sampling.

Educational outcome

The fellow conducted data analysis of surveillance data at national level; interpreted current trends in the surveillance data and developed corresponding recommendations; participated in regular feedback on surveillance data to stakeholders and wrote scientific reports using the analysed data.

2. Applied public health research

2.1 Investigation of the genomic and epidemiological factors associated with Haemolytic Uraemic Syndrome among confirmed cases of Verotoxigenic Escherichia coli infection in Ireland, 2017-2020

Supervisors: Anne Carroll, Eleanor McNamara, and Robert Cunney

This was a joint project conducted by both Diana and the EPIET fellow Melissa Brady at the Health Protection Surveillance Centre in Dublin. The overall aim was to investigate the genomic and epidemiological factors associated with the development of the Haemolytic Uremic Syndrome (HUS) among confirmed VTEC cases in Ireland, in order to improve early diagnosis, surveillance and public health management of VTEC-associated HUS.

A total of 108 cases (HUS VTEC) and 416 unmatched controls (non-HUS VTEC) were selected for a case-control study among VTEC notifications in Ireland between the period of 2017–2020. A gene presence/absence pan genome-wide association study (GWAS) was conducted to identify novel genes associated with HUS. Routinely collected epidemiological data on VTEC epidemiological data notifications were combined with GWAS results and logistic regression was used to estimate adjusted odds ratios (aOR).

GWAS analysis indicated a positive association between HUS and the presence of 26 genes ($OR > 1$; Benjamini-Hochberg and pairwise comparison p -values < 0.05) involved in processes including toxin production, sugar metabolism, stress response and phage life cycle. Seven genes had unknown functions.

In multivariable analyses, younger patients (0–9 years) had a three-fold likelihood of contracting HUS compared to patients aged 10 years and older (aOR=3.3; 95% CI=1.7-6.4). The odds of contracting HUS were higher among patients with *vtx2a* alone (aOR=154.3; 95% CI=27.1-1567.3), both *vtx1a/* and *vtx2a* (aOR=36.7; 95% CI=7.3-358.4) or both *vtx1a/* and *vtx2c* (aOR=31.3; 95% CI=2.9-447.4), when compared to patients with *vtx1a* alone. The odds were higher among patients with both *ygM/* and *mokC* homologs (aOR=5.4; 95% CI=1.8-18.6) compared to those with neither, and among patients with both *pfkA/* and *fiE* genes (aOR=1.8; 95% CI=1.0-3.3) compared to those with neither.

The findings reaffirm known risk factors, including young age and presence of specific *vtx* genes/gene combinations, and additionally suggest that *ygM/* *mokC* homologs and *pfkA/* *fiE* genes are associated with developing HUS. Further investigation could improve early diagnosis, surveillance and public health management of VTEC infection.

Role: Diana was involved in all stages of the study: conceptualisation, literature review, development of research question, study design, development of the study protocol, dataset management and validation, genomic DNA library preparation, whole-genome sequencing and genomic analysis, including the GWAS. Together with the EPIET fellow, Diana wrote and submitted an abstract to ESCAIDE 2022 and prepared a manuscript for publication.

Training modules related to the assignment/projects

EPIET/EUPHEM Introductory Course - introduction to statistical tools, public health microbiology, whole genome sequencing, phylogeny, sensitivity and specificity, and a brief introduction to operational research.

Multivariable Analysis Module – provided a comprehensive understanding of the principles of statistical analysis and how to build an optimal model using linear, logistic, Poisson and Cox regression in R/STATA.

Management, Leadership and Communication in Public Health Module - provided training in communication, and time and person management, all necessary aspects of project management.

Educational outcome

The fellow enhanced her knowledge in the areas of public health microbiology, bacteriology and bioinformatic analysis of WGS data together with epidemiological data. She also planned, designed and developed a study protocol, and applied ethical principles regarding data security and protection.

3. Applied public health microbiology and laboratory investigations

3.1 Validation of Tecan Fluent for increased capacity of COVID-19 diagnostics

Supervisors: Anne Carroll and Eleanor McNamara

During the COVID-19 pandemic, in-house RT-qPCR testing methods targeting the viral RNA in nasopharyngeal swabs became the gold-standard for diagnosing suspected cases of COVID-19. Laboratory testing can be streamlined using robotic molecular platforms for the initial step of high-throughput batch processing of clinical specimens - i.e using robotic platforms such as MagNa Pure 96, Life River and TECAN Fluent® for viral RNA extraction. This project aimed to implement and validate TECAN's Fluent® Automation Workstation into routine COVID-19 laboratory testing at the PHL to complement the existing automated extraction platforms.

Viral RNA from 145 nasopharyngeal swabs was newly extracted by Fluent Workstation. All samples had been previously extracted using the reference method/platform MagNa Pure 96 (non-contemporaneous testing). RNA samples were amplified by RT-qPCR and new Ct values were registered. RNA extraction efficiency was compared for Fluent and MagNa Pure 96 by assessing the congruence of RT-qPCR Ct results obtained for the two platforms, since RNA extraction efficiency directly affects the amount of viral RNA obtained per sample and, consequently, the Ct value obtained by the RT-qPCR. Fluent showed 92% sensitivity, 96% specificity, 99% PPV, 74% NPV and 93% accuracy when compared to the already established reference extraction method, MagNA Pure 96. Even though the specificity was quite satisfactory, the sensitivity and accuracy were lower - deviating by more than 5% compared to MGP. These values were too low to be able to validate the use of Fluent at the PHL. It is important to note that 6% of the samples (n=9/145) reported as 'SARS-CoV-2 detected' by MagNa Pure would have been reported as 'SARS-CoV-2 not detected' by Fluent. However, external factors not related to the Fluent workstation may have caused the discrepancy between the two platforms (e.g. time elapsed between the collection of the swabs and their processing by Fluent). Our results suggested that there is a need for immediate processing of swabs to achieve optimal RNA extraction and accurate Ct values, as time can lead to an increase in the Ct values obtained, probably due to viral degradation. In light of the results obtained, it was decided that further testing was warranted, with a new batch of samples processed in a contemporaneous manner by the two platforms, before the Fluent® Automation Workstation could be validated and implemented as an additional platform for viral RNA extraction at the PHL.

Role: Diana was responsible for the whole of the study design process, laboratory testing, data analysis and elaboration of the final report. She was also responsible for writing SOPs, carrying out risk assessments and providing training to PHL staff.

3.2 Fungicidal activity of Voriconazole in *Candida* species

Supervisor: Johannes Wagener

Candida species are natural inhabitants of the human body, being common colonisers of the skin, gastrointestinal and genital tracts. However, they are also important opportunistic pathogens, of particular relevance for more vulnerable groups such as the elderly and hospitalised, or immunosuppressed patients. It is important to note that invasive candidiasis is one of the most common fungal infections globally and, according to ECDC's last point prevalence survey for 2016–2017, it is one of the 10 leading causes of healthcare-associated infections. Furthermore, an increase in resistance to antifungal drugs among *Candida* species has been observed, which has serious implications for the clinical management of invasive fungal infections.

Azole antifungals have been widely used in clinical practice and are generally classified as a fungistatic drug against *Candida* species. However, previous studies have demonstrated that voriconazole can have a partial fungicidal effect against certain isolates/*Candida* species. This has raised the question as to whether voriconazole fungistatic activity against *Candida* applies to all species or if it is strain dependent.

In this study, the fungicidal activity of voriconazole was investigated using microscopy imaging for five of the *Candida* species most frequently recovered from clinical infections: *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida krusei*. Fungicidal activity was assessed by calculating the proportion of dead cells in comparison to the total number of cells at two incubation time points. The results obtained confirmed that voriconazole has both a fungistatic and fungicidal effect on *Candida* cells, at varying degrees and depending on the species and strains tested. In general, a longer incubation time increased the fungicidal effect of voriconazole. These preliminary results showed the need for a better understanding of the mechanism involved in resistance to azoles in *Candida* species, in the hope that the fungicidal effect of azoles can be used in new therapy regimens, possibly in combination with other drugs.

Role: Diana was responsible for the study design, experimental laboratory work, microscopy and data analysis, and wrote a report to serve as basis for future publication of results.

Training modules related to the assignment/projects

EPIET/EUPHEM Introductory Course – provided an introduction to statistical tools, the concepts of sensitivity, specificity, positive and negative predicted values of a diagnostic test.

Biorisk and Quality Management Module – the module provided fellows with important concepts about laboratory quality management, which were applied throughout the laboratory investigations.

Educational outcome

The fellow applied concepts of virology, molecular biology, mycology and antifungal resistance to public health; acquired experience in project management, development of laboratory methods to improve diagnostic procedures and surveillance, understanding of the use and limitations of diagnostic methods and their interpretation.

4. Biorisk management

4.1 Validation of Tecan Fluent for increased capacity of COVID-19 diagnostics

As part of the project for the validation of Fluent® Automation Workstation, Diana carried out risk assessments on the handling of COVID-19 swabs transported in a new lysis buffer, and equipment and chemical reagents used for nucleic acid extraction of the SARS-CoV-2 virus. To perform these tasks, the fellow had to familiarise herself with the existing PHL safety manual on the handling of chemical reagents and waste disposal, and the guidelines on how to conduct a risk assessment using a risk assessment matrix.

4.2 Observational training of mycobacterial sample processing at the BSL3 facility in St. James's Hospital

In March 2021, as an introduction to the project on the evaluation of WGS analytical methods for the molecular surveillance of *Mycobacterium tuberculosis* isolates, Diana had the chance to spend one morning at the national reference BSL3 laboratory of an acute hospital in Dublin. This experience offered the fellow the chance to observe and get acquainted with the procedures used in the reception, processing and analysis of mycobacterial samples at the National Reference Laboratory (NRL). These included an overview of the services provided by the laboratory, a short explanation of the structure and organisation of the BSL3 laboratory, the standard safety measures to be observed in a BSL3 facility, and how to work in the safety cabinet with different types of samples collected from patients (sputum, pleural fluid, biopsies, etc.). There was also a brief explanation of the different methods used in the laboratory to diagnose tuberculosis: mycobacterial culturing media, GeneXpert for rapid identification of *M. tuberculosis* complex in the sample and detection of rifampicin resistance; and a short practical session on smear microscopy and interpretation of results.

4.3 Audit of BSL3 facility at PHL HSE, Dublin

Diana carried out an internal audit for the Biosafety Level 3 (BSL3) laboratory at the PHL. This involved reviewing the laboratory safety manual, quality system documentation relating to the BSL3 laboratory and the BSL3 Code of Practice, as well as interviewing the Chief Medical Scientist. The audit focused on biosafety, process management, quality control indicators and documentation. The audit yielded a general indicator compliance percentage of 98%. The overall quality of operational and facility management was high and no major deviations affecting biosafety were identified at the facility.

Training modules related to the assignment/projects

Biorisk and Quality Management Module – In this module, the fellow received training on biorisk assessment and mitigation measures; biorisk and quality management in biomedical laboratories in accordance with ISO15189, and working in a high-risk containment laboratory.

Educational outcome

The fellow developed her understanding of biosafety regulations, appropriate measures for the safe transport of pathogenic samples and hazardous substances. In addition, she obtained experience in applying the concepts of biorisk assessment and mitigation measure to produce risk assessments and audit a BSL3 facility.

5. Quality management

5.1. Update and elaboration of SOPs on SARS-CoV-2 swabs RNA extraction by Fluent® Automation Workstation, new pre-lysed swabs

As part of the validation project for the Fluent® Automation Workstation, the fellow wrote standard operating procedures for the handling of COVID-19 samples received in swabs containing a new lysis buffer, the nucleic acid extraction kit and the Fluent® Automation Workstation equipment.

5.2. Revision and update of chapter in PHL's Safety Manual

Diana reviewed, updated, and wrote a new section in the chemical waste management chapter of the PHL's safety manual.

5.3. Observing the accreditation process for ISO standards ISO17025:2017 and ISO15189 at the Public Health Laboratory

In March 2021, Diana attended the two-day virtual visit of the Irish National Accreditation Board (INAB) to the PHL as an observer. In preparation for the meeting, Diana attended PHL's accreditation meetings and reviewed the ISO standards that were to be assessed: ISO17025:2017 and ISO15189. Diana attended the introductory meeting where the schedule for visit was set out, observed discussion on quality system documentation (e.g. personnel training files, user manual updates, service provider personnel training documentation and review of previous non-conformities). Diana also observed the review of the following: test interpretation; technical validation and result reporting including use of LIMS; checking of equipment service and calibration records; assessment of preventive action (e.g. temperature monitoring of fridges/freezers).

5.4. Development of an SOP for sample reception at PHL HSE during a cyber-attack on Health Service Executive's informatics system, May 2021

Diana was responsible for creating a flowchart to serve as guidance for sample reception and processing at PHL during an IT outage and was involved in the writing of an SOP for a paper-based logging of samples.

5.5. Internal vertical audit of water samples processing during IT outage resulting from a cyber-attack on Health Service Executive's informatics system, May 2021

Diana carried out an internal vertical audit of water sample processing to assure that the timeliness and quality of the service provided by the laboratory was not affected by the IT issues resulting from a cyber-attack on the HSE informatics system. A checklist was used to assess the workflow and detect any constraints.

Training modules related to the assignment/projects

Biorisk and Quality Management Module – the fellows were introduced to quality management practice in biomedical and public health laboratories in accordance with the ISO 15189 norm, and procedures for laboratory accreditation and certification. A tool to assess process management, quality control and documentation was made available to fellows and there was a simulation exercise for a BSL3 audit.

Educational outcome

The fellow became familiar with accreditation procedures and quality control measures in the laboratory and carried out internal audits at PHL.

6. Teaching and pedagogy

6.1. Training on setting up TECAN's Fluent Workstation for RNA extraction of COVID-19 nasopharyngeal swabs

Diana organised a morning training session for two staff members at the PHL. The training consisted of a brief introduction to the equipment and controlling software, and a demonstration of how to set up the equipment, perform the daily maintenance steps and run the implemented extraction protocol with a small set of samples. Diana then wrote some reflections on the teaching experience, detailing what went well and what needed to be improved.

6.2. Facilitation of a case study during Phylogeny Inject days of the Introductory Course for Cohort 2021

Diana participated in the preparatory session and the facilitation of a case study on multidrug resistance for *Salmonella Kentucky*. The fellow facilitated this case study alongside a EUPHEM alumni and co-supervisor, Anne Carrol, for a group of seven fellows. Throughout the activity, the conversation was very dynamic and productive. Diana explained several terms and concepts relating to whole genome sequencing, antimicrobial resistance and virulence, and the tools available for genomic analysis. All learning objectives were achieved in the allotted time. The feedback from the fellows on the facilitation was very positive.

6.3. Journal Club at St. James's Hospital in Dublin

Early in the fellowship, Diana was enrolled onto the Journal Club schedule of St. James's Hospital and had the opportunity to present the results of two of her projects to a multidisciplinary audience consisting of medical doctors, nurses, medical scientists, and researchers.

Training modules related to the assignment/projects

EPIET/EUPHEM Introductory Course – this module familiarised the fellows with the core concepts of teaching and public health communication. Strategies for effective teaching and evaluation were used in the teaching assignments.

Management, Leadership and Communication in Public Health Module – this module complemented the EPIET/EUPHEM introductory course and further developed the fellows' communication skills in the public health context. Project management and feedback tips from the module were also used during the teaching assignments.

Project Review module – This module gave the opportunity to the fellows to give constructive feedback, to practise for oral presentations, to learn how to keep an audience engaged during a presentation, and to present results clearly and effectively.

Educational outcome:

The fellow identified learning objectives, planned and organised training sessions, taught laboratory methods and concepts of microbiology and genomics and prepared presentations on public health topics for a multidisciplinary team.

7. Public health microbiology management

7.1 Application to CIDR peer review group for use of epidemiological data in a project

Together with EPIET fellow Melissa Brady, Diana prepared a successful 12-page application for review by the Computerised Infectious Disease Reporting (CIDR) peer review group, to seek approval for the use of epidemiological data linked to confirmed VTEC cases included in the study population of the project 'Investigation of the genomic and epidemiological factors associated with HUS among confirmed cases of VTEC infection in Ireland, 2017-2020'.

7.2 GDPR and health research regulation courses

During the course of the fellowship, Diana attended two workshops on general data protection and health research regulations, delivered by Trinity College, Dublin and the Health Service Executive Ireland via their e-learning platform HSeLanD. Through these courses the fellow learned about the legal landscape for data protection and health research in Ireland; key definitions and principles of GDPR and health research regulations; obligations and explicit consent under the health research regulations and the role of the data protection officer. She also participated in case studies on GDPR roles (controller/processor).

7.3 Public Health Microbiology Management as part of EUPHEM projects

Public health microbiology management was an essential component in all projects and activities carried out during the fellowship. This was undertaken in the form of project and activities management, including laboratory management and time management; collaboration with external parties; respect for cultural differences in multicultural environments and work in multidisciplinary teams.

Training modules related to the assignment/projects

Management, Leadership and Communication in Public Health Module – This module provided an overview of the different management styles, team roles and team evolution, delegation of tasks, conflict management, effective communication, how to deliver structured feedback, communicating with higher authorities, and work under pressure in a complex situation (simulation exercise).

Educational outcome

The fellow gained experience working in multidisciplinary public health teams, both at the training site and with external collaborators; considering ethical implications for data collection and use and developing her competencies and skills in planning, organising and time-management by carrying out several projects during the course of the fellowship.

8. Communication

8.1. Publications related to the EUPHEM fellowship

1. Espadinha D, Roycroft E, Flanagan P, Mok S, McNamara E, Fitzgibbon M, et al. 2021. Evaluation of WGS-based methods for molecular surveillance of *Mycobacterium tuberculosis* complex isolates in Ireland, 1999-2019 [submitted].
2. Siling K, Freudenau I, Espadinha D, Jensen W, Greeve J, Diermann R, et al. 2021. The first description of a nosocomial breakthrough outbreak caused by SARS-CoV-2 Eta (B.1.525) variant, Germany, June 2021 [submitted].
3. Espadinha D, Brady M. et al. Investigation of the genomic and epidemiological factors associated with haemolytic uraemic syndrome among confirmed cases of verotoxigenic *E. coli* infection in Ireland, 2017-2020. (in preparation).

8.2. Reports

1. Validation of TECAN's Fluent for increased capacity of COVID-19 diagnostics at PHL [internal report].
2. National Report of HIV-1 Transmitted Drug Resistance in Ireland, 2019 [published: <https://www.hpsc.ie/a-z/hivandaids/hivdrugresistance/>].
3. Executive summary - COVID-19 outbreak investigation in a hospital in Dublin.
4. Executive summary - COVID-19 outbreak investigation in a long-term healthcare residential unit in Dublin.
5. Evaluation and comparison of WGS-based methods for molecular surveillance of *Mycobacterium tuberculosis* complex isolates at a National Reference Laboratory [internal report].
6. Report on *Legionella* contamination of water distribution system in long term healthcare facilities [internal report].
7. Fungicidal effect of voriconazole antifungals in *Candida* spp. [internal report].

8.3. Conference presentations

1. Espadinha D, Roycroft E, Flanagan P, Mok S, McNamara E, Fitzgibbon M, et al. 2021. Evaluation and Comparison of WGS-based methods for molecular surveillance of *Mycobacterium tuberculosis* complex isolates in Ireland, 1999-2019. Abstract 209 in Surveillance: Implementation and Evaluation. In ESCAIDE 2021, online. November 16-19, 2021. Oral presentation.
2. Espadinha D, O' Donnell K, Brady M, Neary M, Moran J, Dean J, et al. 2022. HIV transmitted drug resistance in Ireland, 2019. PS001 - HIV/AIDS (inc. diagnostics & epidemiology, anti-retroviral drugs, vaccines, treatment & susceptibility/resistance). In ECCMID 2022, Lisbon, Portugal. April 23-26, 2022. Poster presentation.

8.4. Other presentations

1. Evaluation and comparison of WGS-based methods for molecular surveillance of *Mycobacterium tuberculosis* complex isolates in Ireland, 1999-2019 – Presentation at St. James' Hospital Journal Club.
2. Evaluation and comparison of WGS-based methods for molecular surveillance of *Mycobacterium tuberculosis* complex isolates in Ireland, 1999-2019 – Presentation to Senior Staff at PHL HSE.
3. Evaluation and comparison of WGS-based methods for molecular surveillance of *Mycobacterium tuberculosis* complex isolates in Ireland, 1999-2019 – Presentation during Project Review Module 2021.
4. HIV transmitted drug resistance in Ireland, 2019 – Presentation at St. James' Hospital Journal Club.
5. Investigation of the genomic and epidemiological factors associated with haemolytic uraemic syndrome among confirmed cases of verotoxigenic *E. coli* infection in Ireland, 2017-2020 – Presentation during Project Review Module 2022.

9. Other activities

9.1. Manuscript reviewing

Diana reviewed a manuscript for PLOS One on *Staphylococcus epidermidis*.

9.2 EUPHEM representative for Cohort 2020

In the beginning of the fellowship, Diana was elected as one of the EUPHEM representatives. In this role, Diana liaised with fellows, the fellowship office and coordination to ensure an open communication channel between all the parties, which was particularly relevant during the first year and half of the programme due to the restrictions caused by the COVID-19 pandemic. The fellow also participated in numerous meetings with fellow representatives from cohorts 2019 to 2021, the training site forums and an ECDC working group. She also performed many other tasks (organisation of meetings, taking of minutes, satisfaction surveys, social activities, etc.) in order to contribute to the continuous improvement of the EPIET & EUPHEM fellowship.

9.3 Member of Curricular Revision Working Group of EPIET/EUPHEM training programme

Diana volunteered to join the Curricular Revision Working Group whose primary objective was to decide on the composition of a review panel, and to approve a standard operational procedure on how curricular revision of the fellowship should progress. This involved attending meetings, giving feedback and reviewing documentation.

10. EPIET/EUPHEM modules attended

1. Introductory Course part 1, (28/09/2020 to 16/10/2020), virtual
2. Introductory Course part 2 - Operational Research Inject Days, (9-10/11/2020), virtual
3. Outbreak Investigation, (07-11/12/2020), virtual
4. Multivariable analysis, (15-19/02/2021), virtual
5. Multivariable analysis – Cox Regression Inject Day, (18/03/2021), virtual
6. Introductory Course Part 3, (26/04/2021 to 07/05/2021), virtual
7. Rapid Assessment and Survey Methods, (5-16/05/2021), virtual
8. Project Review 2021, (23-27/08/2021), virtual
9. Biorisk and Quality Management, (17-18/01/2022), virtual
10. Vaccinology, (14-18/02/2022), virtual
11. Time Series Analysis, (4-8/04/2022), ISS, Rome, Italy
12. Management, Leadership and Communication in Public Health, (13-17/06/2022), ECDC, Stockholm, Sweden
13. Project Review 2022, (29/08/2022 to 02/09/2022), CIDNUR, Lisbon, Portugal

10. Other training

14. European Society of Clinical Virology conference on COVID-19, 23-26 September 2020 (online).
15. Introduction to the COVID-19 national surveillance system, 23 September 2020, PHL HSE, Dublin
16. Short tutorial on COVID-19 samples testing at PHL (Nasopharyngeal swab processing, DNA extraction and RT-qPCR), 24 September 2020, PHL HSE
17. Host site laboratory training on COVID-19 samples testing: sample handling, RNA extraction using River Life method, RT-qPCR setup and validation/authorisation of results.
18. GDPR & health research regulations workshop, 24 November 2020 (1 hour, online).
19. ESCAIDE, 24-27 November 2020 (online).
20. '20 years of Campylobacter disease surveillance and research – PubMLST impact' online forum, 29 March 2021.
21. 'Rapid Risk Assessment (RRA)' e-Learning course on EVA
22. Hands-on training on WGS (DNA extraction, library preparation, sequencing, importation of sequences into BioNumerics and bioinformatic analysis), February–December 2021.
23. Observational training on *Legionella* sample processing at PHL, 26 May 2021.
24. Observational training on water sample processing at PHL, 1-4 June 2021.
25. Observational training on quality control setup at PHL, 10 June 2021.
26. Observational training on food sample processing at PHL, 22-25 June 2021.
27. Observational training on VTEC sample processing in BSL3 at PHL, 28-30 June 2021.
28. Online talks on genomic population structures of microbial pathogens, 28 September 2021.

29. PAHO – Webinar on genomic surveillance of COVID-19, 26 October 2021.
30. WHO Public Health Laboratory Knowledge Sharing Webinars – Laboratory Management Series, 27 October 2021.
31. ESCAIDE, 16-19 November 2021.
32. HIV drug resistance prevention and response in light of the WHO HIV Drug Resistance Report 2021, 24 November 2021.
33. Focus on Infection - webinar, 6 December 2021.
34. Consultation on global genomic surveillance strategy for pathogens with pandemic and epidemic potential, 8 December 2021.
35. GDPR course by HSE land, 17 December 2021 (online).
36. Manual Handling and People Handling e-learning Theory Module through the Health Service Executive Ireland's e-learning platform HSeLand, 5 September 2022 (online).
37. Think Tank series by ECDC (online).

Discussion

Coordinator's conclusions

One of the main goals of the EUPHEM programme is to expose fellows to diverse and multidisciplinary public health experiences and activities, enabling them to work across different disciplines. This report summarises all activities and projects conducted by Diana Sofia Pereira Espadinha de Oliveira Costa during her two-year EUPHEM fellowship (cohort 2020) as an EU-track fellow at the Public Health Laboratory of the Health Service Executive in Dublin (PHL-HSE), Ireland.

During the two years of the fellowship, Diana has shown a high level of competency and this portfolio is a reflection of her dedication and hard work. The laboratory and epidemiologically based projects covered a diverse range of disease programmes and a wide range of pathogens (bacteria, viruses and fungi) which is an achievement that should be acknowledged against the background of the COVID-19 pandemic. Her projects involved multidisciplinary tasks and teamwork, showing her ability to work within a varied and extensive environment. Diana also showed clear management and leadership skills during her role as cohort representative. All her activities were in line with the 'learning by doing' principles of the EUPHEM programme and fulfilled the core competency domains described for professionals in their mid-career and above. The activities were complimented by training modules, providing theoretical and practical knowledge and skills. During the two year period, she not only achieved her primary objectives, but also contributed to the COVID-19 pandemic response in Ireland in a number of different areas. In addition to the COVID-19 response, she played an important role in the investigation of national outbreaks and contributed to increasing laboratory capacity by, for example, comparing different WGS schemes for the molecular surveillance of *Mycobacterium tuberculosis*. Diana also enhanced her research knowledge and analytical skills by participating in the study of the genomic and epidemiological factors associated with Haemolytic Uraemic Syndrome caused by verotoxigenic *Escherichia coli*. Finally, I would like to comment on Diana's personal attitude toward the fellowship. She was an independent and hard worker, always eager to learn and apply her new competences in her projects.

The EUPHEM Coordinator Team concludes that the fellow has succeeded in performing all her tasks to a very high standard and with a professional attitude. We wish the fellow every success in her future career and congratulate the training site for the successful training of the fellow.

Supervisor's conclusions

Diana Sofia Pereira Espadinha de Oliveira Costa from Portugal commenced her two-year EUPHEM Fellowship in the middle of a severe national lockdown due to the COVID 19 pandemic in Ireland. However, despite these difficulties, she completed a unique fellowship that encompassed all the challenges and learning opportunities associated with a global pandemic in public health microbiology (PHM). The scope of her fellowship at the Public Health Laboratory (PHL), HSE, Dublin was very broad in that she worked along with a consortium of National Reference Laboratories and the Health Protection Surveillance Centre. Her resilience, fortitude and ability to work independently enabled her to quickly become immersed in a COVID diagnostics project, addressing project aims and objectives resulting in the validation of a new piece of equipment and a standard operating procedure, to enhance large-volume DNA extraction for COVID PCR diagnostics. This increased the capacity for COVID diagnostics at PHL Dublin. Added to these attributes, the fellow demonstrated excellent communication skills, participated in multi-disciplinary *Legionella* outbreak teams, carried out trouble shooting on projects, wrote clear reports, gave presentations at international meetings and showed her ability to learn quickly in the 'field'. Her project supervisors complimented her on these attributes and her developing skills. Meanwhile, she successfully gained public health microbiology competencies by completing a variety of projects, including a review of HIV antiviral resistance in Ireland, a comparison of a variety of genomic typing methods for MTB, and an investigation into fungicidal azole activity in invasive *Candida* infections. Her confidence and professional knowledge in coordinating public health microbiology and field epidemiology in collaboration with her EPIET colleagues has grown steadily throughout the fellowship, and was specifically demonstrated in a joint EPIET/EUPHEM Verotoxin *E. coli* project. She is now a competent public health microbiology specialist, who can contribute to this speciality at both national and European level. We are confident that Diana will have a fulfilling career in public health microbiology and are pleased that we could facilitate her specialist development during such a unique fellowship period.

Personal conclusions of fellow

I am extremely grateful to have been given the opportunity to join the EUPHEM programme. Despite all the restraints imposed by the COVID-19 pandemic, these were two fantastic years filled with opportunities to receive training and develop my skills and competencies in a broad range of disciplines and categories of infectious diseases within the scope of public health. The 'learning-by-doing' approach is undoubtedly one of the strengths of this programme, combined with the opportunity to connect both at a personal and professional level with other public health professionals across Europe and worldwide, thereby strengthening the public health network.

Acknowledgements of fellow

I would like to thank my main supervisor Eleanor McNamara, and my co-supervisors Anne Carroll (C2013) and Robert Cunney for their excellent supervision, support, and mentoring throughout my fellowship, for which I am very grateful. Thank you to all my colleagues at PHL who welcomed me so warmly and were a pleasure to work with over the past two years.

I would like to thank all my project supervisors, for giving me their time and the opportunity to deepen my knowledge and work on such a diverse range of topics, in particular Tom Rogers, Margaret Fitzgibbon, Cillian de Gascun, Kate O'Donnell, and Johannes Wagener. Thanks to all other collaborators at the Irish Mycobacteria Reference Laboratory, St James' Hospital Clinical Microbiology Department, the National Virus Reference Laboratory, the Health Protection Surveillance Centre, and Trinity's Translational Medicine Institute.

Many thanks to my EUPHEM scientific co-ordinator, Silvia Herrera Leon, for her guidance and encouragement; to the fellowship faculty office and coordination, especially Adam Roth and Aftab Jasir, for all their hard work and for continuously trying to improve our cohort experience during the difficult pandemic period.

A very special thank you goes to my C2020 fellows Melissa Brady and Katja Siling for being amazing collaborators, for their support and friendship; and to Charlotte Salgaard Nielsen (C2021) for her companionship and making life easier in Dublin. Finally, I feel very privileged to be part of cohort 2020, who are such an amazing bunch of people with whom I have shared this journey. Whether at a distance or face-to-face, their friendship and support has been invaluable, and I hope our paths will continue to cross within the world of public health and beyond.