EPIET & EUPHEM

João Pedro do Couto Pires

The European Public Health Microbiology Training Programme (EUPHEM), Cohort 2022
Norwegian Institute of Public Health, Norway

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. The Administrative Decisions ECDC/AD/2023/23 and ECDC/AD/2023/06 govern the EU-track and MS-track, respectively, of the ECDC Fellowship Programme, field epidemiology path (EPIET) and public health microbiology path (EUPHEM).

Both curriculum paths provide training and practical experience using the 'learning by doing' approach at acknowledged training sites across the European Union/European Economic Area (EU/EEA). This final report describes the experiences and competencies the fellow 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

João Pedro do Couto Pires has a Master's degree in Pharmaceutical Sciences from the Faculty of Pharmacy of the University of Porto (Porto, Portugal, 2006–2012). In the course of the degree, he joined the Microbiology Department to work in projects related to bacteriology and antimicrobial resistance (AMR). He obtained a PhD in Molecular Biology and Biochemistry from the University of Bern (Bern, Switzerland, 2014–2017) for which he studied the epidemiology of extended-spectrum cephalosporin-resistant Enterobacterales in healthy humans, their pets, and on returning travellers from high AMR-prevalence areas. During his postdoctoral work at the ETH Zürich (Zürich, Switzerland, 2018–2022), he shifted from wet-lab to data-driven projects. There, he worked on several projects on the use of public data on antimicrobial susceptibility testing to infer AMR trends in food-producing animals in low-and-middle income countries, as well as using genomes from public repositories to infer resistance trends based on the presence of antimicrobial resistance genes.

Results

The objectives of the 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/EUPHEM core competencies, as set out in the ECDC Fellowship Manual[1].

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[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

Stockholm, 2024

1. Epidemiological investigations

1.1. Outbreak investigations

Outbreak of Mycobacterium bovis*, in Rogaland, Norway, 2022–2023*

Supervisors: Umaer Naseer, Karine Nordstrand

Category: Emerging and re-emerging diseases

Aim: To investigate the factors associated with probable transmission of bovine tuberculosis from an infected cow to slaughterhouse workers.

Methods: Slaughterhouse workers were tested for interferon gamma release assay (IGRA) to identify exposure to *M. tuberculosis* complex (MTC) and a retrospective cohort study was conducted. Questionnaires were used to identify possible risk factors associated with IGRA positivity, including demography, previous exposure to MTC, activity within the slaughterhouse, workstation, and probability of exposure to aerosols.

Results: Four out of 18 individuals were IGRA positive (22.2%). No demographic factors or previous exposure was associated with IGRA positivity. Individuals working in stations associated with aerosol generation (p = 0.023) or directly manipulating airway organs (p = 0.006) were associated with IGRA positivity.

Public health implications: i) Norwegian slaughterhouses need to ensure that they have updated and well documented infection-prevention practices; ii) personal protective equipment (PPE) needs to be readily available and slaughterhouse workers need to be trained to use it correctly; iii) PPE should be used when bovine tuberculosis is suspected, irrespective of the low incidence in the country.

Role: The fellow was a co-investigator on the outbreak investigation team and led the analytical epidemiology. The fellow was responsible for performing data extraction, cleaning, and analysis. Furthermore, the fellow also updated the analysis with new information, presented the results to other stakeholders including the Veterinary Institute and Food Safety Authority, and prepared data for submission to conferences and a Norwegian outbreak report.

1.2. Surveillance

Evaluation of the wastewater surveillance for SARS-CoV-2 in Norway, June 2022–March 2023

Supervisor: Ettore Amato

Aim: To evaluate a pilot national wastewater surveillance (WWS) system for monitoring SARS-CoV-2 and its variants in Norway, operated between June 2022 and March 2023.

Methods: The evaluation adapted guidelines from ECDC and CDC for surveillance system assessment to fit WWS., including: i) detailed description of the WWS system; ii) identification of users and stakeholders; and iii) performance analysis of the following attributes: sensitivity, specificity, timeliness, usefulness, representativeness, simplicity, flexibility, stability, and communication. Cross-correlation analysis was conducted to assess the system's ability to provide early warning signals for new infection waves.

Results: The pilot WWS system used existing wastewater surveillance infrastructure from major Norwegian municipalities and was effective in tracking infection trends. It was found to be sensitive, timely, useful, representative, simple, flexible, acceptable, and stable. The system also showed potential for early detection of changes in variant distribution over time. Nonetheless, the following challenges were identified: i) specificity of the system can be affected by temporary viral RNA fluctuations in wastewater; ii) representativeness when downscaling the surveillance; and iii) flexibility and acceptability when upscaling the system due to limited resources and/or capacity.

Public health implications: Evaluation showed that WWS met most surveillance objectives, effectively monitored population-level infections, especially when individual testing was low, and provided early warning signals 1–2 weeks in advance. Recommendations include standardising and validating trend assessment methods for novel variants; evaluating the system for new variants over a longer period; identifying trend fluctuation causes; and conducting population prevalence studies to improve data interpretation.

Role: The fellow coordinated and performed data analysis and visualisation, drafted part of the manuscript related to variant detection, and revised the manuscript [1].

Mapping of the Group A Streptococcus *(GAS) laboratory data in the Norwegian Surveillance System for Communicable Diseases Laboratory Database (MSIS-Lab), Norway, January 2023–March 2024*

Supervisors: Pascale Renée Cyr, Nina Therese Aasand

Aim: To map the data on laboratory testing reported to the Norwegian Surveillance System for Communicable Diseases (MSIS) Laboratory Database (MSIS-Lab) for Group A *Streptococcus* (GAS) and to automatically identify GAS to be notified to MSIS to enable real-time surveillance of this pathogen and identify potential missing or unreported cases.

Methods: The *tidyverse* R packages were used to perform data cleaning, standardisation, and algorithm development. Cases of invasive GAS (iGAS) were identified based on Norwegian notification criteria: 'Systemic disease with laboratory detection of *S. pyogenes* from normally sterile sample material by isolation, nucleic acid or antigen testing'. Information on iGAS identified through MSIS-Lab was quality assured against those in MSIS.

Results: Between January 2023 and March 2024, a total of 25,509 tests associated with *S. pyogenes* were conducted. 2997 (11.7%) tests were associated with the 695 cases of iGAS reported to MSIS. Based on the current algorithm, all but 40 cases of iGAS (5.8%) were identified in MSIS-Lab. Unidentified cases were associated with biological samples not typically categorised as sterile based on laboratory data (e.g. wound secretions). Nine cases (1.3%) did not have the same testing material in both MSIS and MSIS-Lab. 20 additional cases of iGAS cases identified via MSIS-Lab alone, where iGAS was identified in blood (n =11), synovial fluid (n = 9), and one lymph node biopsy.

Public health implications: Implemented systematic identification of cases of iGAS through laboratory databases and identification of unreported cases of iGAS. Provided information to laboratories to include information on sample sterility to improve iGAS identification in MSIS-Lab in the future.

Role: The fellow created code that performs data cleaning and standardisation, as well as created an algorithm to identify invasive GAS from laboratory information. The fellow also generated a report with the main findings of iGAS in the MSIS Laboratory Database.

Surveillance of cases of mpox in Norway, 2023

Supervisor: Tone Brunn

Aim: To describe the epidemiological situation of cases of mpox in Norway, 2023.

Methods: Cases of mpox were retrieved from the Norwegian Surveillance System for Communicable Diseases (MSIS) and descriptive epidemiology was performed.

Results: In 2023, 10 cases of mpox were reported to MSIS. The 10 cases reported were all among males aged 23–62 years. Most cases were reported as sexually transmitted, with 60% occurring among men who have sex with men (MSM). All cases were residents of Oslo, 40% were infected in Norway, 40% in various other countries, and the remaining 20% unknown.

Public health implications: Continuous monitoring of the national and international situation, promoting health education in high-risk groups, and increasing the awareness of healthcare professionals about high-risk groups.

Role: The fellow performed data analysis and data visualisation for mpox data and drafted the text in Norwegian for the mpox section in the yearly report 'Blod- og seksuelt overførbare infeksjoner i Norge. Årsrapport 2023' (see, 'Other reports').

2. Applied public health microbiology research and laboratory investigations

Molecular epidemiology of Yersinia enterocolitica *infections in Norway, 2018–2021*

Supervisors: Umaer Naseer, Lin Catherine T Brandal

Aim: To develop a core-genome multilocus sequence typing (cgMLST) scheme for *Yersinia enterocolitica* and to assess its usefulness in outbreak investigations and surveillance.

Methods: Ridom SeqSphere+ was used to develop the cgMLST scheme. A good quality finished reference genome was used to identify all genes from *Y. enterocolitica* and 15 seed genomes were used to refine the gene targets. The scheme's performance was evaluated on 920 *Yersinia* spp. genomes (561 Norwegian *Y. enterocolitica*, 327 available in public databases, and 32 non-*Y. enterocolitica*). The performance was compared to available schemes for *Yersinia* spp. Known outbreaks were used to establish the single-linkage cluster threshold that groups all outbreak isolates into individual groups.

Results: The final cgMLST scheme included 2,582 genes (59.9% of the reference genome). Among the evaluation genome set, a median of 97.9% (interquartile range 97.6–98.8%) targets were found. The developed scheme performed better than schemes developed for *Yersinia* spp. based on the distributions of the gene targets found (adjusted p value < 0.001). Within the outbreak evaluation genome set, the single-linkage cluster threshold at 4 allelic differences grouped all isolates into one outbreak cluster within each outbreak.

Public health implications: Implementing a stable cgMLST scheme for *Yersinia enterocolitica* in Norway and elsewhere, inclusion of the cgMLST in SeqSphere, and improving surveillance and comparison between diverse geo-temporal scales.

Role: The fellow was responsible for the study design, and also wrote the protocol, developed the cgMLST and performed data analysis, wrote and revised the manuscript, and submitted the manuscript to a peer-reviewed journal [2].

Epidemiological pattern of antibiotic resistance in Shigella *spp. isolated between 2018–2022, Norway*

Supervisors: Umaer Naseer, Lin Catherine T Brandal

Aim: To describe the antimicrobial resistance (AMR) determinants in non-travel-associated *Shigella* spp. strains in Norway between 2018 and 2022.

Methods: Antibiotic susceptibility testing results for *Shigella* spp. isolates were collected from the National Reference Laboratory (NRL) for a panel of antibiotics. Multidrug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antibiotic classes. Whole genome sequencing (WGS) was performed at the NRL using Illumina technology, and antibiotic resistance genes (ARGs) were identified using AMRFinderPlus within SeqSphere. Descriptive statistics characterised genotypic information.

Results: Out of 377 sequenced isolates, *S. sonnei* (56.8%) and *S. flexneri* (34.5%) were predominant. Resistance was most common against quinolones (38.5%) and extended-spectrum cephalosporins (21.5%). A total of 92.3% of isolates were MDR. Travel-associated cases had more ARGs than non-travel-associated cases: median 3 (IQR 2–4) versus 2 (IQR 1–2) for *S. sonnei* and 3.5 (IQR 2–4) versus 2 (IQR 2–3) for *S. flexneri* (Wilcox-test p<0.001). Frequent resistance determinants included *gyrA* mutations (34.4% in *S. sonnei*; 24.6% in *S. flexneri*), $bla_{\text{CTX-M-15}}$ (27.4% in *S. sonnei*; 17.7% in *S. flexneri*), and *qnrS1* (16.6% in *S. sonnei*; 40.6% in *S. flexneri*). AMR in non-travel-associated cases rose from 9.1% in 2021 to 64.3% in 2022, especially associated with *gyrA* mutations.

Public health implications: Increased awareness of public health professionals on the potential of importation of antimicrobial-resistance from abroad. Increased awareness of potential horizontal gene transfer from travel-related *Shigella* spp. into non-travel related *Shigella* spp.

Role: The fellow managed and supervised the lead investigator, provided input data analysis, partially analysed data, and contributed to abstracts and the national report (NORM and NORM-VET: Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway).

Implementation of whole genome sequencing-based typing for Corynebacterium diphtheriae *in Norway*

Supervisors: Tone Kristin Bjordal Johansen, Kristian Alfsnes

Aim: To implement a core-genome multilocus sequencing typing (cgMLST) scheme and genome-based toxin prediction at the NRL to support *Corynebacterium diphtheriae* diagnostics and outbreak investigations.

Methods: 56 Norwegian *C. diphtheriae* isolates (20 toxin- and 36 non-toxin producers) collected between 2010 and 2023 were sequenced and typed with the two available cgMLST schemes – SeqSphere and Pasteur. Performance was assessed based on the percentage of detected genes. 107 publicly available genomes from the 2022 European multi-country outbreak of *C. diphtheriae* were used to establish a single-linkage cluster (SLC) outbreak threshold for both schemes. 125 additional genomes were included in the analysis to assess the stability of this threshold. In silico detection of the toxin was performed within SeqSphere and mapped on a core-genome phylogenetic tree.

Results: The Pasteur scheme performed better (median 98.9% genes, IQR [98.7–99.3%]) than SeqSphere (median 98%, IQR [97.7–98.3%], Wilcox test p<0.001). The outbreak threshold was defined for both Pasteur and SeqSphere schemes at SLC at 5 allelic differences. Bioinformatic toxin detection matched 100% with NRL diagnostics. However, the phylogenetic tree showed clustering of toxin and non-toxin-producing isolates, hindering toxin production prediction based on the isolates' position within the phylogeny.

Public health implications: Implementation of the Pasteur scheme at the Norwegian Institute of Public Health using SLC at 5 allelic differences for outbreak investigation and maintain current toxin diagnostics (ELEK testing and *tox* gene PCR) at the National Reference Laboratory.

Role: The fellow designed the study, performed data analysis, provided guidance for the validation report, wrote the assessment privacy, and an abstract for the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE).

3. Biorisk management

Biosafety level-3 Laboratory Training, Norwegian Institute of Public Health, Norway, 2023

Supervisors: Tone Kristin Bjordal Johansen, Margrete Solheim

Working in a high containment level 3 laboratory – BSL-3 - in Norway requires training and induction. The Norwegian Institute of Public Health provides both theoretical and practical training. The theoretical aspects cover biosafety and biosecurity, laboratory safety levels, microbial risk group classification, risk assessments, handling dangerous goods, and how to proceed in case of emergency or spillovers. Furthermore, the training talks about the microorganism part of the Norwegian preparedness programme as they can be used in bioterrorism, such as – *Bacillus anthracis*, *Burkholderia mallei* and *B. pseudomallei*, *Coxiella burnetiid*, *Brucella* spp., *Francisella tularensis*, *Yersinia pestis*, Middle East Respiratory Syndrome, Crimean-Congo haemorrhagic fever, Lassa fever, Marburg virus, and Ebola virus. The practical component is two half-days' visit to the BSL-3 laboratory, where the fellow had the opportunity to perform donning and doffing personal protective equipment for BSL-3, use different safety cabinets, inactivating pathogenic samples, RNA extraction, quantitative PCR, and use the commercial solution FilmArray® BioThreat Panel.

Biosafety management module homework: risk assessment using the BioRAM tool, Online, 2023

Supervisor: Aftab Jasir

Aim: To assess biosafety and biosecurity risks associated with culturing Multi-Drug-Resistant Titan Blue (MDR-TB) in a laboratory.

Methods: The BioRAM Lite tool was used to conduct this assessment. The tool assesses biosecurity and biosafety through a systematic scoring process that considers factors such as factors of the biological agent (e.g. modes of transmission and infectious dose), laboratory practices, and mitigation strategies.

Results: The assessment indicated that this fictional pathogen had a moderate risk for inhalation and ingestion (2.71 and 1.89, respectively) to the individual. For the community, the risk of inhalation was moderate, but a low risk of ingestion was identified. Finally, a low risk of inhalation and ingestion was identified for animals.

Public health implications: This simulation exercise was important to understand how different aspects affect the risk of diverse transmission modes to the individual, community, and animals.

Role: The fellow discussed and filled the BioRAM tool as part of the group work.

Infodemiology of Vibrio *and* Shewanella *infections in Norway, 2018–2023*

Supervisor: Ettore Amato

Aim: To assess the communication of risk of developing *Vibrio vulnificus* and *Shewanella* spp. performed by the media in Norway (2018-–2023)

Methods: Cases of *Vibrio* and *Shewanella* infections potentially causing necrotising fasciitis (also known as flesh-eating disease) available in the Norwegian Surveillance System for Communicable Diseases (MSIS) were mapped against the number of Norwegian media reports on 'flesh-eating bacteria' ('kjøttetende bakterier'), '*Vibrio*' or '*Shewanella*' infections, and Google Trends on the same topic. To allow comparison with Google Trends, the number of cases and media reports were converted into relative proportions, where 100% corresponds to the month with the highest number of cases and media reports, respectively.

Results: In the exceptionally warm summer of 2018, cases of *Vibrio* and *Shewanella* spp. doubled compared to the average from 2019–2023. Media reports declined from 2018–2022 but peaked in June–July 2023. No correlation was observed between the MSIS cases and the number of media reports over the summer months. Higher relative proportion of media reports were identified in 2019 (relative proportion 45% vs 37.3%) and 2023 (relative proportion 100% vs 37.3%). Increased media attention in 2019 was likely associated with outbreak in the previous year, whereas in 2023 it was likely due to media's concern of an exceptionally warm summer.

Public health implications: Revising communication strategies and recommending targeted public health risk communication at the municipal/local level to raise awareness among at-risk groups such as individuals with a weakened immune system or elderly individuals with existing open wounds before or during swimming-related activities.

Role: The fellow performed data analysis, generated figures, and drafted parts of the manuscript.

4. Quality management

Early detection of SARS-CoV-2 variants through environmental wastewater surveillance: a systematic review and meta-analysis, 2021–2022

Supervisors: Jose Antonio Baz Lomba, Ettore Amato

Aim: To perform a systematic review of Environmental Wastewater Surveillance (ES) as an Early Warning System (EWS) for SARS-CoV-2 that focus on: i) timeliness, sensitivity, and specificity of ES; ii) the ability to detect the early introduction of new variants into wastewater; iii) the public health impact and control measures associated with ES at both national and international levels.

Methods: A total of 1,588 publications published between June 2021 and July 2022 were screened. Following an initial screening process, 331 articles were selected for full-text review. Of these, 151 publications met the inclusion criteria for assessing the effectiveness of Environmental Surveillance (ES) as an Early Warning System (EWS) for SARS-CoV-2 and the early detection of its variants. Additionally, 30 relevant publications from the grey literature were included in the analysis.

Results: ES was mostly performed in high income countries [44% (n = 67) in North America, and 33% (n = 50) in Europe]. Sensitivity of SARS-CoV-2 detection varied between 44% to 99%, and specificity between 86% and 99%. ES had a 1–2 weeks lead time in identifying new SARS-CoV-2 infections for most publications. This was also similar for EWS for the detection of variants of concern (VOC) (median lead time was 11.2 days). The studies also displayed a wide range of methods associated with RNA extraction, next-generation sequencing (NGS) indicating that a standardisation of these methods will be fundamental for inter-country comparisons.

Public health implications: ES could function as an EWS for SARS-CoV-2 infection waves and new VOCs, especially in areas with no registered cases or limited clinical capacity. Recommendations for ES include harmonisation of data collection, and harmonisation of methods used for VOC identification and quantification to ensure comparability between geographical regions. Furthermore, information on sample collection, RNA extraction, library preparation, NGS and pipelines were crucial to interpret and contextualise information of the project, 'Evaluation of the Wastewater Surveillance for SARS-CoV-2 in Norway, June 2022–March 2023'.

Role: The fellow extracted and standardised data on VOC, performed descriptive data analysis on VOC surveillance, produced figures for manuscript, drafted the VOC section of manuscript, and revised the manuscript [3].

Audit of the Unit 5 at the National Reference Laboratory, Norwegian Institute of Public Health, Norway, 2023

Supervisor: Ragnhild Bardal Roness

Aim: To audit the Unit 5 laboratory of the National Reference Laboratory which is responsible for the diagnostics of *Corynebacterium* spp. and *Streptococcus pneumoniae.*

Methods: A standardised form was used to assess different quality aspects regarding the process management and quality control, as well as the documentation of the Unit 5 lab. This includes among others: accommodation and environmental conditions, pre-analytical process and specimen management, analytical process, technical records, equipment logbooks, and biosafety documentation.

Results: The Unit 5 lab showed a high level of quality (general indicator 90%) in both process management and quality control (90%) and documentation (90%). Notably, some of the questions within the pre-analytical process and specimen management did not apply and this led to an artificial reduction of the quality score. However, no documentation on stock tracking was implemented for the Unit 5 lab. This is important considering that some of the consumables for some of the tests have a short shelf life and thus could impact timely laboratory testing in the future.

Public health implications: Improving stock documentations to avoid future delays in laboratory testing.

Role: The fellow audited the Unit 5 lab and wrote the audit report in collaboration with his co-EUPHEM fellow, Arne Michael Taxt.

5. Public health microbiology management

Epidemic Intelligence Rotation, Norwegian Institute of Public Health, Norway, 2023–2024

The fellow was a duty officer on the Epidemic Intelligence team at the NIPH. The objectives of this team are to: i) monitor national and international health events; ii) rapidly disseminate information of public health concern; iii) immediately inform the responsible officers when assistance requests reach the institute. Each week, a duty officer for epidemic intelligence is responsible for monitoring national and international health alerts via several platforms and is responsible for assessing and redirecting information, summarising event information, and preparing weekly reports to be disseminated internally and to the Ministry of Health. This activity highlighted the significant information flow at the NIPH and how it reveals areas requiring additional resources and training.

Improvement of Public Health Microbiology in the ECDC Fellowship Curriculum, 2022–2024

Over the course of João's fellowship, the curricular revisions for the ECDC fellowship were underway. During this period, the fellow actively participated in discussions on how to address the training needs of EUPHEM and EPIET fellows in public health microbiology. For EUPHEMs, João provided input into the concept note and final content of the module, 'Public Health Microbiology III - WGS & Bioinformatics' in addition to other training resources for the module. For EPIETs, the fellow actively participated in discussing the content and organising the module, 'Public Health Microbiology I - Basic phylogeny and AMR', particularly the antimicrobial resistance content which the fellowship did not have previously.

EUPHEM Cohort-Representative, 2022–2024

João was one of the representatives from the EUPHEM Cohort 2022. In collaboration with other cohort representatives, he gathered and summarised the views and issues of the fellows, supported routine fellowship activities, and acted as a liaison between fellows and the fellowship faculty office and head of the fellowship programme to improve it. The fellow partook in multiple meetings including the Active Supervisors and Training Site Forum (TSF), National Focal Points for Training and TSF, and the EPIET Alumni Network. João's majorly contributed to the improvement of the Public Health Microbiology training available for fellows, and provided necessary information to trigger salary revisions for EU-track fellows.

Organisation of the EPIET Alumni Network (EAN) Molecular Epidemiology Mini-Module, Public Health Agency of Catalonia, Barcelona, Spain, 2023

To mark the 15th anniversary of EUPHEM, the EPIET Alumni Network (EAN) organised the yearly mini-module on Molecular Epidemiology and Antimicrobial Resistance (AMR). The course was aimed at individuals with limited microbiology experience who wanted to improve their knowledge on molecular typing and whole genome sequencing. It included current fellows, but also EAN members with diverse levels of work experience. João organised the mini-module, identified training needs, developed the course curriculum, disseminated and facilitated the course.

Organisation of Training of Trainers in Applied Molecular Epidemiology of Infectious Diseases, Noguchi Memorial Institute for Medical Research, Accra, Ghana, 2023–2024

Through the project, 'Building Stronger Public Health Institutions and Systems', the fellow participated in the organisation of the five-day course, 'Train of the Trainers in Applied Molecular Epidemiology of Infectious Diseases' which was held in Accra, Ghana. This course was aimed at different public health professionals who will be responsible to teach molecular epidemiology to students at the University of Accra and Ghana Field Epidemiology and Laboratory Training. As part of the organisation team, João contributed to the management of the curriculum, and discussion of the course curriculum and timeline. Furthermore, João organised the literature and further training materials to be delivered to the trainers.

Project Management

Alongside the EUPHEM site supervisor, João had a leading role in supervising the EPIET fellow, Melanie Stecher on the project, 'Epidemiological pattern of antibiotic resistances in *Shigella* spp. isolated between 2012–2022 in Norway'. During this period, João provided the scientific support to guide Melanie through microbiology, antimicrobial resistance, genotypic data, and phylogenetics. Furthermore, João also provided R code support throughout the project. This project led to an oral communication at the Congress of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID Global 2024), contribution to a national report, and a manuscript is under preparation.

6. Teaching and pedagogy

Training of Trainers in Applied Molecular Epidemiology of Infectious Diseases, Noguchi Memorial Institute for Medical Research, Accra, Ghana, 2024

João led the development of two technical modules – 'Introduction to Molecular Epidemiology' and 'Interpretation of Genotypic Data' – which covered topics on sampling to sequencing, principles of molecular typing (fingerprint and sequenced-based methods, whole genome sequencing-based), comparison methods associated with different typing methods, and phylogenetics. The fellow was also a facilitator of the two case studies developed to put in practice the knowledge gained from the course: 'Whole Genome Sequencing in a Food-and-Waterborne Outbreak' and 'Whole Genome Sequencing in a Respiratory Disease Outbreak'. The course was a success, with an overall satisfaction of 9/10.

EPIET Alumni Network (EAN) Molecular Epidemiology Mini-Module, Public Health Agency of Catalonia, Barcelona, Spain, 2023

The fellow participated in the organisation of the module and developed the content of AMR in collaboration with Carmen Espinosa Gongora. The fellow developed a lecture on AMR covering the interpretation of antimicrobial susceptibility testing, understanding which isolates have developed resistance mechanisms, identifying which resistance mechanisms are important for public health, and understanding the importance of horizontal gene transfer. The AMR section of the mini-module was evaluated a 5/5.

Public Health Microbiology I (PHM I) ECDC Fellowship Module (Online), 2024

Given the success of the EAN mini-module and the lack of this content in the fellowship, the module's content was adapted and incorporated into the fellowship module, 'Public Health Microbiology I - Basic phylogeny and AMR' aimed at EPIET fellows. For that, the fellow lectured an adapted version of the AMR lecture fitted to the fellowship's needs. Additionally, the fellow also provided feedback on the AMR case study developed by Jennifer Bender. Finally, João facilitated the case studies, '*Salmonella* Adjame – Is it an outbreak?' and 'Hospital outbreak with $bla_{\text{NDM-1}}$ in Germany'. Despite the complexity of some of the contents, the fellows had positive feedback and evaluated that the content of PHM I would be useful in their careers.

Communication Workshop with Ghana Field Epidemiology and Laboratory Training Program, Oslo, Norway, 2022

During the four-week exchange visit of the Ghana Field Epidemiology and Laboratory Training Programme (GFELTP) at NIPH, João conducted a two-hour workshop addressing common presentation pitfalls such as text-heavy slides and lack of structure, as requested by the visiting fellows. Following this, João also conducted individual sessions to aid the fellows' preparation for their final presentations scheduled at the end of their stay. These efforts significantly improved the fellows' communication skills.

R Code Support

Over the course of the fellowship, João provided R support functioning as an ad-hoc facilitator during the Outbreak Investigation Module, Multivariable Analysis, Time Series Analysis, and Public Health Microbiology III (herein also BASH). Additionally, João participated in the R code revision of the case study, 'Outbreak of gastroenteritis after a high school dinner in Copenhagen, Denmark, November 2006'. Finally, the fellow also provided guidance on R practices and analysis for some fellows outside of the modules and is acknowledged in the manuscript under preparation, 'Diverging trends in new HIV diagnoses among four subpopulations in France from 2010 to 2022 and the impact of pre-exposure prophylaxis at population level' led by David Kelly (EPIET 2022 Marseille, France).

7. Communications related to the EPIET/EUPHEM fellowship

7.1. Manuscripts published in peer-reviewed journals

1. Amato E, Hyllestad S, Heradstveit P, Langlete P, Moen LV, Rohringer A, **Pires J**, Baz Lomba JA, Bragstad K, Feruglio SL, Aavitsland P, Madslien EH, Evaluation of the pilot wastewater surveillance for SARS-CoV-2 in Norway, June 2022 – March 2023. 2023;23(1714). BMC Public Health. Available at https://doi.org/10.1186/s12889-023-16627-2

2. **Pires J**, Brandal LT, Naseer U. Development and Implementation of a Core Genome Multilocus Sequence Typing scheme for *Yersinia enterocolitica*: A Tool for Surveillance and Outbreak Detection. Journal of Clinical Microbiology. 2024. Available at: https://doi.org/10.1128/jcm.00040-24

3. Baz Lomba JA, **Pires J**, Myrmel M, Arnø JK, Madslien EH, Langlete P, Amato E; Hyllestad S. Effectiveness of environmental surveillance of SARS-CoV-2 as an early-warning system: Update of a systematic review during the second year of the pandemic. 2024;22(1):197-234. Journal of Water & Health. Available at: https://doi.org/10.2166/wh.2023.279

4. Amato E, **Pires J**, Naseer U, Hyllestad S. Infodemiology of *Vibrio* and *Shewanella* infections in Norway, 2018-2023. [Submitted]

5. **Pires J**, Roness RB, Solheim M, Bjørnstad ML, Mengshoel AT, Alfsnes K, Johansen TB. Implementation of whole genome sequencing-based typing for *Corynebacterium diphtheriae* in Norway. [Under preparation]

6. **Pires J**, Roness RB, Solheim M, Bjørnstad ML, Mengshoel AT, Alfsnes K, Johansen TB. Increase in Non-*Corynebacterium diphtheriae* in Norway. [Under Preparation]

7. Stecher M, **Pires J**, Brandal LT, Naseer U. Increasing number of resistance strains to quinolones among non-travel-associated *Shigella* spp. in Norway between 2018 and 2022. [Under Preparation]

7.2. Other reports

1. Brunn T, Caugant, Kløvstad H, Labberton AS, Nilsen Ø, Olsen AO, **Pires J**, Salamanca BV, Whittaker RN. Blod- og seksuelt overførbare infeksjoner i Norge. Årsrapport 2023. Oslo: Norwegian Institute of Public Health; 2014. Available at: https://www.fhi.no/publ/2024/blod--og-seksuelt-overforbare-infeksjoner-i-norge.-arsrapport-2023
2. NORM og NORM-VET: Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway 2023. Increasing number of resistance strains to quinolones among non-travel-associated *Shigella* spp. in Norway between 2018 and 2023. page 109–111. [Publication pending].

7.3. Conference presentations

Pires J, Brandal LT, Naseer. Development and Validation of a cgMLST scheme for *Yersinia enterocolitica*: A Tool for Outbreak Management and Surveillance (Poster). Presented at: European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), 22–24 November 2023; Barcelona, Spain

Stecher M, **Pires J**, Brandal LT, Naseer U. Increase of multidrug resistance in *Shigella* spp. among travel-acquired cases in Norway, 2012-2021 (Poster). Presented at: European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), 22–24 November 2023; Barcelona, Spain

Pires J, Brandal LT, Naseer. Development and Validation of a cgMLST scheme for *Yersinia enterocolitica*: A Tool for Outbreak Management and Surveillance (ePoster Flash Session). Presented at: ESCMID Global 2024 – Congress of the European Society of Clinical Microbiology and Infectious Diseases, 27–30 April 2024; Barcelona, Spain

Stecher M, **Pires J**, Brandal LT, Naseer U. Increasing number of resistance strains to quinolones among non-travel-associated *Shigella* spp. in Norway between 2018 and 2022 (Oral Presentation). Presented at: ESCMID Global 2024 – Congress of the European Society of Clinical Microbiology and Infectious Diseases, 27–30 April 2024; Barcelona, Spain

Pires J, Roness RB, Solheim M, Bjørnstad ML, Mengshoel AT, Alfsnes K, Johansen TB. Implementation of whole genome sequencing-based typing for *Corynebacterium diphtheriae* in Norway (Oral Presentation). Presented at: European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), 20–24 November 2024; Stockholm, Sweden

7.4. Other presentations

Pires J. Surveilance and outbreak detection of *Corynebacterium diphtheriae* in Norway using cgMLST. Presented at: Departmental Seminar. 5th September 2024. Oslo, Norway

Pires J. Whole genome sequencing based typing of *Corynebacterium diphtheriae* in Norway. Presented at: Interpartmental Seminar. 20 June 2024. Oslo, Norway

Pires J. Whole genome sequencing based typing of *Corynebacterium diphtheriae* in Norway. Presented at: Bacteriology Seminar. 22 March 2024. Oslo, Norway

Pires J. Mapping of the Group A *Streptococcus* (GAS) laboratory data in the of the Norwegian Surveillance System for Communicable Diseases Laboratory Database (MSIS). Presented at: Nordic Mini-Module; 29 February 2024. Helsinki, Finland

Pires J and Nordstrand K**.** Outbreak of *Mycobacterium bovis* among beef cattle in Vestland («Utbrudd av *Mycobacterium bovis* hos storfe på Vestlandet»). Presented at: Interdepartmental Seminar; 4 May 2023, Oslo, Norway

Pires J. Molecular Epidemiology of *Yersinia enterocolitica* infections in Norway 2018–2021: Factors associated with Seasonality and Source. Presented at: Nordic Mini-Module; 14 March 2023, Copenhagen, Denmark

Pires J. Antimicrobial resistance in *Escherichia coli* from humans and pigs recovered at small and medium farms in Thailand. Presented at: Gene Technology and Bioinformatics Seminar; 19 January 2023, Oslo, Norway

Pires J. Molecular Epidemiology of *Yersinia enterocolitica* infections in Norway 2018–2021. Presented at: Project Review Seminars; 1 November 2022, Oslo, Norway

8. EPIET/EUPHEM modules attended

- Introductory Course, 26 September–14 October 2022, Spetses, Greece
- Outbreak Investigation, 5–9 December 2022, Berlin, Germany
- European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2022, 23–25 November 2022, Stockholm, Sweden
- Biorisk and Quality Management, 16–17 March 2023, virtual
- Multivariable Analysis, 22–26 May 2023, Frankfurt, Germany
- Rapid Assessment and Survey Methods, 19–23 June 2023, Stockholm, Sweden
- Project Review Module 2023, 28 August–1 September 2023, Lisbon, Portugal
- European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2023, 22–24 November 2023, Barcelona, Spain
- Time Series Analysis, 11–15 December 2023, Rome, Italy
- Vaccinology, 4–8 March 2024, virtual
- Writing Abstracts for Scientific Conferences, 20 March 2024, virtual
- ESCMID Global 2024 – Congress of the European Society of Clinical Microbiology and Infectious Diseases , 27–30 April 2024, Barcelona, Spain
- Public health microbiology III – Whole Genome Sequencing & Bioinformatics, 3–7 June 2024, Vienna, Austria
- Management, Leadership and Communication in Public Health, 24–28 June 2024, Stockholm, Sweden
- Project Review Module 2024, 26–30 August 2024, Lisbon, Portugal
- European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2024, 20–22 November 2024, Stockholm, Sweden

9. Other training

- BSAFE, United Nations Department of Safety and Security, Online
- B1 and B2 Norwegian Course, September 2023 – December 2023, Oslo, Norway

10. International assignments

One-week Training of Trainers in Applied Molecular Epidemiology of Infectious Diseases, Noguchi Memorial Institute for Medical Research, Accra, Ghana, 18–25 February 2024. The deployment was to support the 'Building Stronger Public Health Institutions and Systems' project which is coordinated by the Department for Global Health, Norwegian Institute of Public Health

11. Other activities

Analysis of the non-travel related cases infected with Carbapenemase-producing organisms in Norway (2012–2021)

The fellow contributed with his previous experience in antimicrobial resistance and in particular β-lactamase molecular epidemiology expertise on devising a methodological plan to compare clonal transmission of carbapenemase-producing versus plasmid-mediated transmission among Enterobacterales in Norway. Furthermore, the fellow participated in several meetings with Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-res).

ECDC Fellowship Information Outreach

João participated in the ECDC Fellowship information outreach at the ECDC stand during : ESCMID Global 2024 – Congress of the European Society of Clinical Microbiology and Infectious Diseases, 27–30 April 2024, Barcelona, Spain.

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