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The European Public Health Microbiology Training Programme (EUPHEM), Cohort 2022
Agentur für Gesundheit und Ernährungssicherheit, Austria

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

Maria João Cardoso has a bachelor's degree in Nutritional Sciences and a master's degree in Food Service Management with a focus on food safety, both from the University of Porto. She also has a second master's in Applied Microbiology from the Catholic University of Portugal, where she studied *Campylobacter* spp. cross-contamination events in household settings as part of a large European project aimed at reducing the health burden of food-borne illnesses.

Prior to starting the EUPHEM fellowship, Maria João gained experience in food microbiology research across academic, public health, and private sectors. She served as a visiting research fellow at the European Union Reference Laboratory for *Escherichia coli*, including *Shiga* toxin-producing *E. coli* (EURL-VTEC, Italy), and worked at Colab4Food as a researcher. Additionally, she has experience in hospital environment research from an international stint in Spain. Her strong interest in infectious disease epidemiology and genomics, particularly within the One-Health framework, drove her to pursue this fellowship.

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 Manual¹.

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. Epidemiological investigations

1.1. Outbreak investigations

Measles outbreaks in Austria, 2023

Supervisors: Andreas Reich (AGES), Lukas Weseslindtner (Reference laboratory for measles, mumps, rubella and hepatitis viruses, Centre for Virology, Medical University of Vienna)

Category: Vaccine-preventable diseases

Aim: In 2023, 186 laboratory-confirmed cases of measles were notified in Austria, mainly in Graz and Vienna. The aims of this project were to identify transmission chains through epidemiological investigation and genotyping, combine genotyping and epidemiological data to show the absence of endemic transmission and pinpoint the benefit of comprehensive genotyping for epidemiological outbreak investigation.

Methods: Cases were reported via the epidemiological reporting system (EMS). At the National Reference Laboratory, cases of measles were confirmed by PCR and genotyped by Sanger sequencing of the nucleoprotein 450-nucleotide region. Short-read whole genome sequencing was performed when higher resolution was needed. Identified genotypes were linked with results from epidemiological investigations obtained by local public health authorities and data extracted from EMS.

Results: Eleven different genotypes were identified, which supported the epidemiological distinction of at least 14 different outbreak events. The most common genotype, D8-5963, was imported into Austria at least three times in 2023, as index cases had travel history to endemic countries during exposure period. Epidemiological and genotyping data have demonstrated that endemic measles transmission has been absent in Austria for at least 12 months, thereby maintaining the elimination status first achieved in 2018.

Public health implications: The integration of genotyping and epidemiological investigation synergistically improved the identification and/or confirmation of reimportations and transmission chains in multiple locations, especially when epidemiological links were not obvious or epidemiological data were unavailable/inaccessible. Thus, genotyping at high frequency is particularly beneficial in refining epidemiological investigations for countries at elimination phase.

Role: Maria João was an observer in this outbreak investigation and managed inter-institutional communications. She also contributed to aggregate datasets from diverse sources for further analysis. She authored and presented a scientific poster at an international conference (Conference presentation i).

Salmonella Enteritidis ST11 international outbreak related to chicken meat consumption

Supervisor: Sabine Maritschnik (AGES)

Category: Food- and waterborne diseases

Aim: Following a Danish EpiPulse event in July 2023, the National Reference Centre for Salmonellae (NRZS-AGES) identified two microbiological clusters among cases of *S. Enteritidis* using core genome Multilocus Sequence Typing (cgMLST). The aim of this investigation was to describe the outbreaks and identify its vehicle(s).

Methods: Cases were defined as a person with a clinical sample positive for *S. Enteritidis* Sequence Type (ST) 11, belonging to Complex Types (CT) 2114 or 13755, with up to seven allelic differences, between 1 January 2023 and 1 October 2023. A descriptive study was conducted including case interviews, assessments of food frequency and purchasing behaviour among 16 cases. Food traceback investigations were performed.

Results: Between March and July 2023, 7/9 federal states in Austria reported six and 11 cases of *S. Enteritidis* ST11 belonging to Outbreak-1 (CT2114) and Outbreak-2 (CT13755). Median age was 17 years (range: 1–75), 14 were male. 16/17 cases reported chicken consumption prior to illness. One fatal case had eaten chicken kebab. Sampling from that restaurant identified both outbreak strains in one chicken kebab skewer and cleaning cloth. Another six food investigations identified both outbreak strains in kebab restaurants across Austria. Food traceback investigations showed a high level of widespread contamination responsible for 257 cases from 13 EU Member States, and the United Kingdom (UK). The complex supply chain identified through international efforts showed Polish food business operators as the common source of this contaminated meat served in Austria and Denmark.

Public health implications: Effective international and cross-sector collaboration was essential in identifying a common source for these outbreaks. This investigation emphasises the importance of collaborative efforts among all stakeholders to mitigate *Salmonella* and protect public health through the enhancement of control measures on hygiene and biosafety from farm-to-fork.

Role: Maria João was part of the outbreak investigation team as a co-investigator, she co-authored the reports for the Ministry of Health, participated in the revision of the Rapid Outbreak Assessment report generated by EFSA (other reports I) and authored a manuscript submitted at a peer-reviewed journal with colleagues from another public health institute (Manuscript 1).

Salmonella *Enteritidis* ST11 travel-associated outbreak

Supervisor: Sabine Maritschnik (AGES)

Category: Food- and waterborne diseases

Aim: In October 2023, the NRZS-AGES reported a suspected outbreak of *Salmonella enterica* serovar Enteritidis ST11 CT1915 (2 cases) and CT9611 (13 cases) across federal states with a probable foreign source. An EpiPulse report created by the United Kingdom further confirmed the identification of cases in travellers returning from Türkiye in nine other countries. An investigation was undertaken to provide Turkish authorities with information for action.

Methods: Cases were defined as a person residing in Austria who has an infection with *S. Enteritidis* ST11 CT9611 or CT 1915 as of 1 January 2023, with a maximum of five allelic differences by cgMLST to the reference strains from the United Kingdom and who has travelled to Türkiye as of January 1, 2023. A descriptive study was conducted including case interviews and assessments of food frequency.

Results: In Austria, 15 cases with a positive sample for the outbreak strains were interviewed about their recent travels and food consumption during the same period. Median age for all cases was 7 years (range: 1–65), 11 were male. Travel to Türkiye was reported by 13/15 cases, and those who hadn't, or who didn't stay in an all-inclusive resort, were excluded. Among the 12 cases who stayed in an all-inclusive resort, common food exposures included chicken meat, eggs, and ice cream. Data was shared with the Food-and Waterborne Diseases team at ECDC coordinating this investigation.

Public health implications: This project underscores the value of international collaboration through platforms like EpiPulse. Aligning outbreak investigation methods for pooling data across countries allowed for more effective assistance in providing data to Turkish authorities and drawing conclusions that would be unattainable from the limited case numbers of individual countries.

Role: Maria João was part of the outbreak investigation team as a co-investigator. She co-authored the reports for the Ministry of Health and established communication with the Food-and Waterborne Diseases team coordinating this investigation at ECDC.

1.2. Surveillance

Surveillance of invasive mosquitoes in Austria in 2023

Supervisor: Karin Bakran-Lebl (AGES)

Aim: To monitor and detect alien mosquitoes in Austria, such as the Asian tiger mosquito, to assess the entomological risk of mosquito-borne disease transmission.

Methods: Surveillance activities were conducted from April to October 2023 using BG-Sentinel traps and ovitraps to capture adult mosquitoes and mosquito eggs, respectively. Traps were strategically placed in urban, suburban, and transport hub locations. Additionally, one BG-Sentinel trap was set up in late April at AGES' premises for a week, as part of the West Nile Virus monitoring programme targeting *Culex* mosquitoes. Weekly, ovitrap samples were collected, counted, and analysed. Mosquito eggs were identified both morphologically and genetically. Additionally, the effectiveness of *Bacillus thuringiensis israelensis* (Bti) in controlling mosquito populations was evaluated after its application at specific sites in Graz.

Results: A total of 323 traps were set across 57 sites, yielding 6,520 samples, with *Aedes* eggs being detected in 1,986 samples (30.5%). The study identified 105,456 eggs of *Aedes albopictus*, *Aedes japonicus*, *Aedes koreicus*, and *Aedes geniculatus*. Significant numbers of *Ae. albopictus* were found in Vienna and Graz. The effectiveness of Bti application varied, showing different results on egg count across the treated traps.

Public health implications: This project enables the early detection of new alien mosquito populations and monitors the development of existing ones, allowing for the timely adoption of countermeasures. The established presence of *Aedes albopictus* in specific areas poses a significant public health risk due to its role as a vector for diseases. The detection of mosquito eggs at transportation hubs underscores the important role played by these sites as well as the need for its vigilant monitoring. Further research is needed to assess the effectiveness of Bti under local environmental conditions.

Role: Maria João contributed to invasive mosquito surveillance, assisting in trap setup, sample collection, egg counting, and data analysis of Bti application. She gained valuable insights into entomological research methodologies, enhancing their understanding of vector surveillance and the importance of thorough monitoring.

Increase of Group A Streptococcus infections in Austria in 2023 – a retrospective genome-based characterisation of invasive and non-invasive Streptococcus pyogenes isolates

Supervisors: Markus Hell (MEDILAB), Adriana Cabal (AGES)

Aim: To provide an in-depth understanding of the circulating strains of Group A *Streptococcus* (GAS) in Austria for the 2022/23 season, as well as gain new insights not only into the epidemiology of invasive GAS infections (iGAS), but also the pathogenesis and evolution of the organism to support real time decision-making, and inform more effective future interventions.

Methods: Eight hospitals, clinics, and medical laboratories were invited to participate in this retrospective study by sharing iGAS and non-iGAS isolates and their respective patient data. Isolates were subjected to short-read sequencing. Sequence type (ST) and *emm* type were extracted from each genome, and genetic relatedness was assessed with cgMLST. Further characterisation of cluster 1 was performed by Single Nucleotide Polymorphism (SNP) analysis. A case-control study was performed to determine the association of virulence factors with invasive disease using logistic regression.

Results: Sequencing was successful for 318 isolates out of 336, 72 were classified as iGAS (cases), 243 as non-iGAS (controls) and three remained unclassified. Patients' median age was 14 years (range 0–95), with a median age of 37 years for iGAS cases. Nine patients died. The most prevalent clones were ST28/*emm*1 for iGAS (28%) and ST36/*emm*12 for non-iGAS (17%). Forty-eight clusters were identified, of which the largest comprised 10 iGAS and 25 non-iGAS isolates carrying 16 characteristic SNPs, potentially representing a local sub-lineage (Cluster 1 – ST28/*emm*1). No association between virulence genes and invasiveness was observed in this case-control study.

Public health implications: Continued laboratory-based surveillance such as this study has important public health implications. It allows timely detection of circulating clones with potentially altered transmission or virulence, as shifts in *emm* types can signal changes in the bacterium's behaviour. This study informs infection control policies, enhances antibiotic stewardship by targeting treatments based on genomic profiles, and enables a rapid response to emerging GAS strains or outbreaks.

Role: Maria João developed the study protocol, created the line list template, and assisted in preparing the documents for ethical approval. She conducted the literature review, handled sample reception, and contributed to the wet lab work. Maria João was responsible for data cleaning, management and statistical analysis, drafting a scientific article (Manuscript 2), and preparing and delivering a poster and an oral presentation at two conferences (Conference presentation ii and iii). She also prepared and delivered two presentations on preliminary results to study participants and prepared a report on a group of isolates at the request of one participant.

2. Applied public health microbiology research and laboratory investigations

Multi-country surveillance of paediatric invasive Group A Streptococcus (iGAS) infection in the EU/EEA during the 2022–2023 season

Supervisors: Vivian Leung, Agoritsa Baka, Dorothee Obach and Emma Löf (European Centre for Disease Prevention and Control)

Aim: To assess the availability of iGAS surveillance data across the EU/EEA, describe paediatric iGAS cases that occurred in participating countries during the 2022–2023 season, identify potential factors associated with progression to severe illness and death with the goal of developing targeted prevention messages and potential recommendation for surveillance for future GAS seasons.

Methods: ECDC National Focal Points for Surveillance from all 30 EU/EEA countries were invited to complete a questionnaire on GAS surveillance. Countries with available paediatric iGAS data were also asked to contribute to the study by submitting case report forms through national iGAS contacts. For case-case analysis, 'severe outcome' was defined as a case being admitted to the ICU or dying. The odds of severe outcome were estimated by logistic regression analyses.

Results: Nineteen countries responded to the questionnaire about GAS surveillance. Systematic surveillance for GAS infections has been uncommon among EU/EEA countries, making it challenging to assess the overall level of circulation in Europe. Eight countries provided case data on 1,277 paediatric iGAS infections. Females, children aged 0–2 with iGAS disease, cases infected with an *emm*1 strain (OR 1.73, 95% CI 1.13–2.67) and patients with sepsis (OR 1.73, 95% CI 1.11–2.73) or a lower respiratory diagnosis (OR 4.14, 95% CI 2.70–6.44) had higher odds of severe outcome.

Public health implications: This study highlights the importance of surveillance and international collaboration for iGAS. The EU/EEA would benefit largely from a harmonised case definition for multiple countries, allowing for comparability of case information and data to be pooled, producing more robust results than single-country analyses.

Surveillance of other infections known to be proxies for iGAS, such as viral infections and scarlet fever, can provide valuable insights into the epidemiology of iGAS infections.

Role: Maria João contributed to the development of the study protocol, the surveillance questionnaire and to the preparation of two presentations (at the ECDC Advisory Forum meeting and National Health Protection Service Ireland Conference). The fellow did the literature review and developed the case report form. She was responsible for the data management, cleaning and statistical analysis, drafting the manuscript (Manuscript 3), and preparing and delivering four presentations on preliminary results for participating countries and ECDC Public Health Functions unit.

Genotypic characterisation and susceptibility to antimicrobials in *B. parapertussis* isolated from a protracted course of whooping cough in Austria, 2023

Supervisors: Daniela Schmid (Medical University of Vienna), Adriana Cabal (AGES)

Aim: To characterise a *Bordetella parapertussis* isolated from a case of whooping cough with a protracted clinical course in a four-year-old patient who had received three doses of diphtheria-tetanus-acellular pertussis-hepatitis B-poliomyelitis-*Haemophilus influenzae* type b vaccine.

Methods: Short- and long-read sequencing were performed, as well as antimicrobial susceptibility testing (AST) with minimum inhibitory concentration (MIC) gradient strips for erythromycin, azithromycin, clarithromycin, and trimethoprim-sulfamethoxazole. An ad hoc cgMLST scheme comprising 3,479 core targets was created using the strain NCBI:txid257311 as reference and 35 *B. parapertussis* query genomes from GenBank. Additionally, the genome was analysed for the presence of antimicrobial resistance genes, virulence genes, and mobile genetic elements. Due to the lack of AST breakpoints, susceptibility results were compared with a closely related strain (10 allelic differences) isolated in France (PRJEB29316).

Results: The *B. parapertussis* isolate showed ST19. The cgMLST analysis identified genetic relatedness with two isolates from GenBank (NZ_CP043074.1, PRJEB29316) that differed from the Austrian isolate by 10 and 16 alleles, respectively. The gene encoding pertactin (*prn*) showed a deletion, and the *prn* promoter was absent, rendering it non-functional. The gene encoding for tracheal colonisation factor (*tcfA*) was absent. This case's isolate carried *bla_{BOR-1}*, three efflux pumps, and had 50-, 12-, and 8-fold higher MICs for trimethoprim-sulfamethoxazole, erythromycin, and clarithromycin than the French strain.

Public health implications: This is the first report of a *B. parapertussis* isolate with elevated MICs for trimethoprim-sulfamethoxazole, erythromycin, and clarithromycin, causing a protracted clinical course of whooping cough in Austria. These findings outline the need for the immediate development of international recommendations for AST breakpoints or epidemiological cut-off values (ECOFFs) for *Bordetella* spp.

Role: Maria João performed the isolation of *B. parapertussis* from a nasal swab, species identification by MALDI-TOF MS, antibiotic susceptibility testing by disk diffusion and E-test and DNA extraction. In addition, she assisted in the library preparation for long-read sequencing. She also prepared a report for the clinical staff at the paediatric hospital, drafted and submitted a manuscript to a peer-reviewed journal (Manuscript 4), and designed and delivered a poster communication at a scientific conference (Conference presentation iv).

MiniSeq implementation for rapid outbreak investigation

Supervisor: Adriana Cabal (AGES)

Aim: To implement a sequencing workflow using MiniSeq Rapid Reagent Cartridge, in order to provide a rapid turnaround for urgent clinical cases including those implicated in outbreaks.

Methods: In order to design a sequencing workflow for 1x101bp single-indexed sequencing using the MiniSeq Rapid Reagent Kit and 1/5 volume Nextera XT Library prep, all the steps — library preparation, cluster amplification and sequencing — were adapted to the chosen instrument and kits. The protocol was tested, refined, and developed in consultation with Illumina Inc. Testing of the protocol was conducted with two different species, *Legionella pneumophila* and *Pseudomonas aeruginosa*, and the results were referenced against those obtained from NextSeq 2000.

Results: The protocol and accompanying spreadsheets for the experiments were successfully developed. In the wet laboratory, challenges arose with the instrument and kit. The same genomic results as those generated by the NextSeq 2000 in cgMLST were obtained. However, due to the lower and uneven coverages for all isolates using 1/5 of the volume of the Nextera XT Library prep, it was decided to switch to a different library preparation kit (Illumina DNA Prep) that was also in use for the NextSeq 2000. Ultimately, the implementation of the Rapid Reagent kit was successful with Illumina DNA Prep, and a workflow for the rapid sequencing of clinical samples is now in place.

Public health implications: Molecular epidemiological investigations are vital in establishing and extinguishing source and transmission networks of infectious diseases. Rapid turnaround time will make it possible for the outbreak investigation team to take actions against an ongoing outbreak sooner.

Role: Maria João developed and wrote the protocol. She also established contact with Illumina for consultation.

Study of antimicrobial resistance and epidemiology in *Enterobacter*, *Bacteroides* and *Parabacteroides* species clinical isolates from Austria, 2010–2022

Supervisor: Heidrun Kerschner (Analyse BioLab GmbH)

Aim: To describe the antimicrobial resistance (AMR) genes and the Verona Integron-encoded Metallo- β -lactamase (VIM) subtypes present in the genomes of Enterobacterales with a focus on *Enterobacter cloacae* complex and *Bacteroides* clinical isolates, through assessing genotypic susceptibility to different antimicrobials and compare them with the phenotypic results performed by the diagnostic laboratory for the isolates, when information was available.

Methods: Species identification of all 221 isolates was confirmed using MALDI-TOF MS. Seventy isolates were randomly selected for sequencing. Patient and sample data were collected. In addition, minimum inhibitory concentrations were determined for meropenem, metronidazole, clindamycin, and amoxicillin-clavulanic acid for randomly selected anaerobic bacteria, as well as meropenem and colistin for randomly selected *Enterobacter cloacae* complex isolates.

Results: For patients with available data, the median age was 63 years (range: 6–96; n=58) and 117 were male (n=189). Among the 221 isolates, 163 were identified as belonging to the *Enterobacter cloacae* complex, 51 as *Bacteroides* spp. and 7 as *Parabacteroides* spp. Of the 70 randomly selected isolates, 62 were successfully sequenced, 43 were *Enterobacter* spp. isolates. While all the anaerobes were associated with healthcare-associated infections (HAI), only 27/45 *Enterobacter* isolates were linked to HAI. Among *Enterobacter* isolates, 41 carried VIM-1, one carried VIM-2 and another one carried both. MIC determination among sequenced *Enterobacter* spp. isolates showed no phenotypic resistance to colistin (0/28) and 6/18 to be non-susceptible to meropenem. However, 17/28 *Enterobacter* spp. carried *mcr-1* and 17/18 carried *bla_{VIM-1}*. Among sequenced anaerobes, 5/12 proved to be phenotypically resistant to clindamycin, despite the presence of *erm* genes in 9/12 isolates. For meropenem, 9/13 isolates were phenotypically resistant, and 8/13 genomes had *cfiA* genes present.

Public health implications: This study provides valuable insights into resistance mechanisms and their spread, providing information of utmost importance to understand the epidemic's dynamics in Austria. Understanding the genetic variations and phenotypic expressions of resistance, particularly in VIM-producing pathogens, is crucial for informing targeted surveillance, infection control strategies, and antibiotic stewardship programs.

Role: Maria João was responsible for isolate reception, data management and analysis. She also contributed to the wet laboratory work (culture of shared isolates, species confirmation by MALDI-TOF, DNA extraction and library preparation for one short read sequencing run). Maria João prepared and delivered a presentation at the Project Review Module 2024, in Lisbon.

3. Biorisk management

Biosafety Level-3+ (BSL-3+) experience - basic veterinary virology techniques

Supervisor: Julia Figl (AGES)

Aim: To develop the fellow's experience in working in biosafety level 3 laboratories and in basic virology methods.

Methods: Titrations, virus neutralisation tests (VNT), and virus isolation were performed on pathogens routinely handled at the Institute for Veterinary Medical Examinations: Foot-and-Mouth Disease Virus (FMDV), West Nile Virus (WNV), and Lumpy Skin Disease Virus (LSDV). During the second week, a VNT experiment for WNV was performed as part of a study conducted by the University of Veterinary Medicine of Vienna. This project intends to test a commercial vaccine against WNV.

Results: The fellow observed and learnt the specific requirements for entering and exiting the BSL-3+ containment area. In addition, specific biosafety measures were applied when working in the Viral Zoonoses labs or with FMDV in the serology laboratory. Virus isolation was successfully performed for FMDV, and its concentration was subsequently measured in a titrations experiment. Titrations for stocks of LSDV and two lineages of WNV were successfully performed, identifying concentrations between 2.37×10^3 and 1.00×10^5 TCID₅₀/50 μ L. The results obtained for the VNT for WNV were confidential and for the LSDV stock was 3.16×10^3 TCID₅₀/50 μ L.

Public health implications: Training to work at a BSL-3+ laboratory enhances safety, improves disease surveillance, and strengthens response capabilities to emerging threats. Additionally, understanding basic virology techniques is crucial, as these methodologies underpin virology research and epidemiological surveillance of viral infections. This type of training builds expertise in handling high-risk agents, thereby enhancing the overall effectiveness of public health laboratories.

Role: Maria João was a trainee in the laboratory. She participated hands-on in all the basic virology experiments, performed the VNT for testing the WNV commercial vaccine by herself and wrote the activity report.

Risk assessment as part of the Biorisk and Quality Management module

As part of the Biorisk and Quality Management module, Maria João (along with other EUPHEM fellows) was tasked with conducting a biosafety and biosecurity risk assessment for a laboratory that cultures Multi-Drug-Resistant Titan Blue (MDR-TB) for antibiotic susceptibility testing (scenario) by using the Biosecurity Risk Assessment Methodology

(BioRAM) tool. This group exercise required analysing information from the provided scenario — covering the pathogen, work type, and laboratory environment — to complete the BioRAM tool's sections. The tool featured questions designed to quantitatively estimate the likelihood of transmission and the potential consequences of disease. This assessment aimed to evaluate the risks for both the individual performing the in vitro work and the broader community, including animals.

In the given scenario, the likelihood of direct percutaneous exposure and infection for the individual performing the in vitro work was calculated as 3.85. For the community, the risk was lower, at 2.32 for both humans and animals. The likelihood of direct contact exposure and infection was 1.64 for the worker, while for the community it was 0.46 for humans and 0.55 for animals. The consequence of disease for the operator was rated at 3.69, compared to 3.87 for the community's human risk and 1.87 for animals.

4. Quality management

External Quality Assessment (EQA) to support diagnosis of mpox in Austria, 2024

Supervisor: Christian Borsodi (National Reference Laboratory for Virology, Medical University of Vienna)

Aim: To set up a proficiency test to diagnose mpox for participating accredited laboratories.

Methods: The preparation of this proficiency test involved selecting clinical samples with specific CT values, inactivating the mpox virus for safety, and preparing samples with varying concentrations. HeLa-Ohio (HOE) cells were used for cellular control. The decision was to include two core samples, an educational sample (with challenging viral concentration) and a negative sample. Stability tests were performed mimicking shipment conditions. The samples were then packaged in a triple-layer system, labelled for biohazard safety, and shipped to participants. Detailed protocols and controls were employed to ensure accuracy, consistency, and safe transport of the samples. Results were analysed blindly.

Results: The EQA included two samples with a CT value of 26.7, one with 32.7 and a negative control. Four laboratories participated, correctly identifying all samples. While the small number of participants limited detailed analysis, no false positives were obtained, and all labs were able to identify the challenging samples as positive. The NRLV concluded all participants performed perfectly and the recommended action is to include a more challenging educational sample with a CT value of 36–38 in future EQAs.

Public health implications: An EQA for mpox diagnostics ensures accurate and standardised testing across laboratories, enhancing surveillance, outbreak early detection and control. In addition, it strengthens laboratory capacity, supports effective disease surveillance, fosters global health security, and builds public trust by confirming that health responses are based on reliable data.

Role: Maria João was an observer of all stages. She participated in the shipment of samples and drafted a report summarising all retrieved lab data.

Audit of the Sequencing laboratory as part of the Biorisk and Quality Management module

As part of the Biorisk and Quality Management module, the fellows were asked to simulate the audit of a laboratory of choice at the training site using a laboratory quality evaluation spreadsheet tool. Maria João chose to audit the Sequencing laboratory of the Clinical Molecular Microbiology Department. The laboratory receives bacteria pellets and isolated inactivated viruses for nucleic acid extraction and short-read sequencing. The questions in the audit form covered all aspects of the laboratory's activity and quality management, and were categorised into process management, quality control, and documentation. Few non-conformities or improvement opportunities were identified, demonstrating that the laboratory is working under the quality standards. The overall quality of operational and facility management was high and no major deviations affecting biosafety were identified at the facility.

5. Public health microbiology management

Project management and liaising with partners

Maria João was involved in project management for most of her projects, covering several aspects of public health microbiology management. Her duties included project development, drafting of study protocols, meeting agendas and minutes, working in multidisciplinary teams and communicating findings for stakeholders. During the fellowship, she collaborated with other teams within AGES, external Austrian partners, the Austrian Ministry of Health, national reference laboratories, several public health institutes in the European Union, ECDC and EFSA. In addition, to develop a case report form to apply in multiple countries, Maria João turned to her network to gather information about the most commonly adopted antibiotic therapy for invasive group A Streptococcal disease in the respective country where each colleague was working at the time or prior to starting the fellowship.

6. Teaching and pedagogy

'Microbiological Analysis in the food chain for the Catering and Food Service sector' course

Supervisor: Patricia Antunes (Faculty of Nutrition and Food Sciences, University of Porto)

The fellow was part of the teaching team for the course, developing and revising materials for practical sessions and one theoretical class. She remotely facilitated discussions on seven case studies across three editions of this training and taught one theoretical class on food-borne viruses. The 80-hours course aims at updating the knowledge of professionals in the food service sector and developing competencies in microbiology and food safety, focusing on contamination control, microorganism proliferation in food/water and improving communication with the auditing laboratory.

7. Communications related to the EUPHEM fellowship

7.1. Manuscripts published in peer-reviewed journals

1. Dibba White E*, **Cardoso MJ***, Küffel F, Novacek A, Handra N, Kornschöber C, Milanov M, Mateva G, Ruppitsch W, Ribert Larsen A, Nielsen S, Gymoese P, Kold Munch P, Ethelberg S, Müller L and Maritschnik S. International outbreaks of *Salmonella* Enteritidis related to chicken meat from Poland, 2023. (In preparation)
2. **Cardoso MJ**, Cabal Rosel A, Hyden P, Richter L, Stöger A, Hasenberger P, Stadlbauer S, Kerschner H, Dopita A, Zeitlberger EM, Leitner E, Diab-Elschahawi M, Hobisch-Pirkel M, Govrins M, Indra A, Hell M, Ruppitsch W. Increase of Group A *Streptococcus* infections in Austria in 2023 – a retrospective genome-based characterization of invasive and non-invasive *Streptococcus pyogenes* isolates. (In preparation)
3. **Cardoso MJ**, Obach D, Löf E, Marrone G, Cornelissen L, Charalambous M, Vohnova S, Plainvert C, Tazi A, Georgakopoulou T, Ó Maoldomhnaigh C, Cotter O, McKeown P, de Gier B, Mäkitalo B, Baka A, Leung V, and the iGAS Study Group. Multi-country surveillance of paediatric invasive Group A *Streptococcus* (iGAS) infection in the EU/EEA during the 2022–2023 season. (In preparation)
4. **Cardoso MJ**, Rouhani D, Cabal Rosel A, Daza Prieto B, Hopfgartner M, Stöger A, Hasenberger P, Stadlbauer S, Moesbacher T, Hyden P, Wiedermann U, Schmid D, Ruppitsch W. Complete genome sequence of *Bordetella parapertussis* strain 400431-b, isolated from a protracted course of whooping cough in Austria, 2023. Microbiol Resour Announc. 2024 Jan; 13(1): e00976-23. Available at: <https://doi.org/10.1128%2FMRA.00976-23>
5. Daza-Prieto B, Raicevic N, Cabal A, Hyden P, Mösenbacher T, Ladstätter J, Richter S, Stöger A, **Cardoso MJ**, Chakeri A, Hasenberger P, Stadlbauer S, Mach RL, Martinovic A and Ruppitsch W. *Enterococcus montenegrensis* sp. nov., isolated from artisanal Montenegrin dry sausage. Int J Syst Evol Microbiol. 2024;74(1) Available at: <https://doi.org/10.1099/ijsem.0.006206>

* Co-first authorship

7.2. Other reports

- I. European Centre for Disease Prevention and Control, European Food Safety Authority, 2023. Three clusters of *Salmonella* Enteritidis ST11 infections linked to chicken meat and chicken meat products – 26 October 2023. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/ROA_S-Enteritidis-ST11_chicken-meat_2023_amended.pdf
- II. Weekly outbreak reports to the Ministry of Health on *Salmonella* Enteritidis ST11 international outbreak related to chicken meat consumption: Update 6, 8, 9 and 10 and final report
- III. Weekly outbreak reports to the Ministry of Health on *Salmonella* Enteritidis ST11 travel-associated outbreak: Update 1 and final report
- IV. Report on the genomes of shared Group A *Streptococcus* isolates for one study participant
- V. Report on the genome of one shared *Bordetella parapertussis* isolate for one study participant

7.3. Conference presentations

- (i) **Cardoso MJ**, Borsodi C, Brait V, Springer D, Ruppitsch W, Aberle SW, Reich A, Weseslindtner L. Comprehensive genotyping supports the epidemiological investigation of Austrian measles virus outbreaks in 2023 (Poster presentation). Presented at: ESCMID Global 2024; 27–30 April 2024; Barcelona, Spain.
- (ii) **Cardoso MJ**, Cabal Rosel A, Richter L., Stöger A, Hasenberger P, Stadlbauer S, Kerschner H, Dopita A, Zeitlberger E-M, Leitner-Meyer E, Diab-Elschahawi M, Hobisch-Pirkel M, Govrins M, Indra A, Hell M, Ruppitsch

W. Increase of Group A Streptococcus (GAS) infections in Austria in 2023 a genome based characterization of invasive and non-invasive Streptococcus pyogenes isolates (Moderated poster presentation). Presented at: ESCAIDE 2023; 22–24 November 2023; Barcelona, Spain.

- (iii) **Cardoso MJ**, Cabal Rosel A, Hyden P, Richter L., Stöger A, Hasenberger P, Stadlbauer S, Kerschner H, Dopita A, Zeitlberger E-M, Leitner-Meyer E, Diab-Elschahawi M, Hobisch-Pirkel M, Govrins M, Indra A, Hell M, Ruppitsch W. Increase of Group A Streptococcus infections in Austria in 2023 – a retrospective genome-based characterization of invasive and non-invasive Streptococcus pyogenes isolates (Oral presentation). 38 Jahrestagung der Österreichischen Gesellschaft für Hygiene, Mikrobiologie und Präventivmedizin (OGHMP). 13–16 May 2024; Salzburg, Austria.
- (iv) **Cardoso MJ**, Rouhani D, Cabal Rosel A, Daza Prieto B, Hopfgartner M, Stöger A, Hasenberger P, Stadlbauer S, Simeoni D, Schmid D, Wiedermann U, Ruppitsch W. Genotypic and phenotypic characterization of a *Bordetella parapertussis* isolate from a patient with a protracted course of whooping cough, Austria 2023 (Poster presentation). Presented at: EUPertStrain Congress 2023; 5–6 October 2023; Barcelona, Spain.

7.4. Other presentations

- I. Cardoso MJ. 2022. Kick-off meeting for EUPHEM: presentation to introduce the fellow's background to potential project supervisors from the different groups within AGES and external partners, 9 November 2022, Virtual.
- II. Cardoso MJ. 2023. Surveillance project preliminary results presentation for one study participant, 27 April 2023
- III. Leung V, Cardoso MJ, Obach D, Romani A, Baka A. Invasive Group A Streptococcus (iGAS) study: 1st meeting with prospective participating member states, 25 July 2023, Virtual
- IV. Cardoso MJ. 2023. Surveillance project preliminary results presentation for eight study participants, 7 December 2023
- V. Cardoso MJ, Baka A. Surveillance of iGAS in the EU/EEA: Preliminary findings from the ECDC iGAS Project, ECDC Public Health Functions Unit meeting, 20 March 2024, Virtual
- VI. Cardoso MJ. Surveillance of iGAS in the EU/EEA: Preliminary findings from the ECDC iGAS Project, Participants meeting, 30 May 2024, Virtual
- VII. Leung V, Cardoso MJ. Findings from the ECDC iGAS Project, Potential new participant meeting, 13 December 2023, Virtual
- VIII. Cardoso, MJ. Study of antimicrobial resistance and epidemiology in clinical isolates from Austria, 2010–2022. Presentation at the Project Review Module, session 2; Lisbon, Portugal, 26 August 2024

5. 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
- Qualitative Research – Elective course, 19 and 22 March 2024, virtual
- European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 2024, 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

6. Other training

- Course 'Infectious disease epidemiology' winter semester 2022/23 (Robert Koch Institut), 27 October 2022 to 23 February 2024, Virtual
- Microbial Genomics Workshop HERA 2: Bacterial Typing and Antimicrobial Resistance, 2–3 May 2023, Heraklion, Greece
- Workshop on Plasmids as Vehicles of AMR Spread (International Centre for Theoretical Physics), 18–22 September 2023, Virtual
- GenEpi-BioTrain – Virtual training 1: Whole-genome sequence-based detection of antimicrobial resistance in bacteria, 4 and 8 September 2023, Virtual.
- GenEpi-BioTrain – Virtual training 2: From sequencer to polished reads for bacteria, 12 and 14 September 2023, Virtual.
- GenEpi-BioTrain – Virtual training 3: Introduction to bioinformatic analysis of SARS-CoV-2 amplicon sequencing data, 25 and 26 September 2023, Virtual.
- GenEpi-BioTrain – Virtual training 4: Introduction to Avian influenza NGS data analysis for outbreak investigation from a one health perspective, 20 and 24 November 2023, Virtual.
- GenEpi-BioTrain – Virtual training 5: Bacterial genome assembly and quality control, 4 and 7 December 2023, Virtual.
- GenEpi-BioTrain – Virtual training 7: Phylogenetics and alignments, 19 and 20 March 2024, Virtual.
- GenEpi-BioTrain – Virtual training 8: Waterborne disease (Leptospirosis) and Water Surveillance, 22 and 24 July 2024, Virtual.
- GenEpi-BioTrain – Virtual training 9 and 10: Unix for beginners & Introduction to the Conda ecosystem, 20–22 August 2024, Virtual.

7. International assignments

Risk factors for developing invasive Group A Streptococcus (iGAS) infection in the EU/EEA during the 2022–2023 season, EU Health Task Force (ECDC), 7 July – 22 October 2023, Remote. See: Public Health Microbiology Research project.

8. Other activities

- Annual EU Health Task Force Meeting, 25–26 January 2024, Stockholm, Sweden
- The fellow contributed to the laboratory work carried out for the publication '*Enterococcus montenegrensis* sp. nov., isolated from artisanal Montenegrin dry sausage' through the semi-quantification of enzyme activities, assimilation and acid production using API ZYM, API STREP and API RAPID ID 32 STREP kits following the instructions of the manufacturer (bioMérieux).
- The fellow participated as early-career researcher in co-reviewing an article submitted for publication in Eurosurveillance in 2023.

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