Miruna Elena Rosu

The European Public Health Microbiology Training Programme (EUPHEM), Cohort 2023

Istituto Superiore di Sanità (ISS), Italy

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/2022/16 Rev.01 and ECDC/AD/2023/06 govern the European Union (EU)-track and Member State (MS)-track, respectively, of the ECDC Fellowship Programme, field epidemiology path (EPIET) and public health microbiology path (EUPHEM), Cohort 2023.

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

Miruna Elena Rosu is a molecular virologist with a PhD from the Erasmus University Medical Center (Erasmus MC), the Netherlands. Her doctoral research focused on the antigenic evolution of influenza virus and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) glycoproteins and implications for the design of effective vaccines. Miruna’s work involved biosafety level-3+ (BSL-3+) laboratory experimentation, pre-clinical vaccine testing, phylogenetics and antigenic cartography. Prior to her PhD, Miruna completed an MSc degree in Infection and Immunity at the Erasmus MC and a BSc degree in Genetics at the Autonomous University of Barcelona (UAB), Spain. She also conducted laboratory research in the Netherlands and Germany on the molecular determinants of airborne transmission and genome packaging of influenza virus. Driven by a strong interest in global health and international development, Miruna joined the EUPHEM fellowship in 2023 to build competencies in programme implementation and outbreak preparedness and response.

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). Manual for the ECDC Fellowship Programme EPIET and EUPHEM paths. Stockholm: ECDC; 2025. Available at: https://www.ecdc.europa.eu/en/publications-data/ecdc-fellowship-programme-manual

Stockholm, 2025

1. Epidemiological investigations

1.1. Outbreak investigations

1.1.1. Outbreak of Salmonella *Strathcona in Italy, 2023*

Supervisors: Claudia Lucarelli, Laura Villa, Irene Artuso (ISS, Rome, Italy)

Category: Food- and waterborne diseases

Aim: To assess the extent of the multi-country outbreak of *Salmonella* Strathcona (*S.* Strathcona) in Italy, identify the source of infection, characterise the pathogen, and inform mitigation measures.

Methods: A probable outbreak case was defined as any person with a laboratory-confirmed diagnosis of *S.* Strathcona in 2023. Demographic and microbiological data were obtained from the Enter-Net Italia surveillance network. When available, food consumption data were extracted from local health unit questionnaires. Whole genome sequencing (WGS) was performed on a subset of isolates at regional or national reference laboratories. Genomes were assembled and analysed following guidelines by the European Food Safety Authority (EFSA) using core genome multilocus sequence typing (cgMLST), and a minimum spanning tree was generated to assess isolate relatedness.

Results: In total, 62 cases of *S.* Strathcona were reported in 2023 across 12 of Italy's 21 regions, affecting individuals aged 8 months to 88 years (mean age: 33), with 61% females. Isolates were obtained from faeces (78%), blood (13%), urine (4%), and other sources (4%). Travel history showed 91% of cases were infected in Italy. Among those with food consumption data from questionnaires, 55% consumed raw vegetables (31.8% tomatoes), and 45% each reported eating eggs or meat. The cgMLST analysis of 38 isolates revealed high clonality and two clusters: one including a German reference isolate and eight Italian cases; another including 15 human cases and one environmental isolate from Bolzano, Italy.

Public health implications/Conclusions: Heterogeneity in the structure of the questionnaires hindered epidemiological analysis. Genomic data indicated a clonal spread, but no single food item was clearly identified as the source.

Role: Miruna was a co-investigator along with the EPIET fellow, Chiara Sacco (Cohort 2022). She monitored cases reported to the surveillance system, compiled a line list and shared it with European partners, performed data cleaning in R, assisted bioinformaticians with genomic analyses and interpretation of results, attended meetings for the multi-country outbreak investigation, and drafted and edited the national outbreak investigation report.

1.1.2. Autochthonous dengue outbreak in Marche Region, Central Italy, August to October 2024

Supervisor: Giulietta Venturi (ISS, Rome, Italy)

Category: Vector-borne diseases

Aim: To investigate and control an outbreak of autochthonous dengue virus serotype 2 (DENV-2) in Fano, a city in the Marche Region, Central Italy, between August and October 2024.

Methods: The investigation followed Italy's national arbovirus surveillance plan. Probable and confirmed cases were identified using clinical and laboratory criteria. Epidemiological data were collected through case interviews. Entomological surveillance involved mosquito trapping and reverse transcription polymerase chain reaction (RT-PCR) testing. WGS was performed on DENV-positive mosquito pools and human samples. Reproduction numbers and reporting delays were estimated to assess transmission dynamics.

Results: By 28 October 2024, 199 cases (138 confirmed, 61 probable) were identified. All cases were locally acquired, with symptom onset ranging from mid-August to late October. The outbreak peaked in mid-September and was geographically centred in a residential area with vector-friendly environments. The estimated basic reproduction number was 2.66, with a peak net reproduction number of 3.53. WGS of DENV-2 from five human and one mosquito sample revealed high similarity, identifying genotype II, lineage F1, related to strains from the United States and Bangladesh. Three mosquito pools tested positive. Public health measures included vector control, enhanced surveillance, blood and organ donation screening, and risk communication to the community.

Public health implications/Conclusions: This large-scale autochthonous dengue outbreak in Central Italy highlights the increasing risk of vector-borne diseases in temperate regions. Delays in diagnoses were linked to low awareness and the absence of travel history. The findings support the need to include dengue among expected seasonal diseases in Europe and emphasise the importance of preparedness, timely case recognition, and integrated vector management.

Role: Miruna served as co-investigator and contributed to the laboratory confirmation of human dengue cases by performing RNA extractions and real-time RT-PCR testing. She read and reviewed the manuscript detailing the findings as part of the Marche dengue outbreak group (see section 7.1, paper 1).

1.2. Surveillance

1.2.1. National surveillance system for invasive Haemophilus influenzae *disease in Italy: evaluation of simplicity, representativeness, data completeness and timeliness, 2016–2023*

Supervisors: Chiara Sacco, Maria Giufrè, Patrizio Pezzotti (ISS, Rome, Italy)

Type of project: Vaccine-preventable diseases

Aim: To evaluate the Italian National Surveillance System (NSS) for vaccine-preventable Invasive Bacterial Infections (MaBI); in particular, invasive *Haemophilus influenzae* disease (HID) for the attributes of simplicity, representativeness, timeliness and data completeness from 2016 to 2023.

Methods: Simplicity was evaluated by examining the structure of the NSS, its reporting procedures, and data sources. Representativeness was assessed by comparing the age, sex, and regional distribution of cases reported to the NSS with those recorded in hospital discharge registries. Data completeness was measured as the proportion of missing values across key variables, reported at both regional and national levels. Timeliness was defined as the interval between symptom onset and initial notification at the national level, and was also assessed both nationally and by region.

Results: Between 2016 and 2023, 1 286 cases of HID were reported. The NSS reporting structure was well-defined, but multiple stakeholders and regional reporting variations complicated notification and data integration, impacting efficiency. Comparison with hospital discharge records revealed similar age-specific trends, but discrepancies in sex-specific and regional distribution, with three regions reporting no cases to the NSS. Data completeness ranged from 25% to 100% by variable and 71% to 94% by region, showing overall stability or improvement over time. Reporting timeliness improved nationally, with a median of seven days (interquartile range (IQR): 3–20), although regional variation remained substantial (median: 3–27 days).

Public health implications/Conclusions: Regional disparities in efficiency, data completeness, and timeliness suggest the need for follow-up sessions to address reporting deficiencies and enhance data quality.

Role: Miruna conceptualised the study (together with Chiara Sacco and Patrizio Pezzotti), designed the methodology, performed data analysis, data interpretation and visualisation, drafted an abstract for the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2024 and presented a poster as well. She also drafted and submitted a manuscript to a peer-reviewed journal (see section 7.1, paper 2).

2. Applied public health microbiology and laboratory investigations

2.1. Optimisation of amplicon-based whole genome sequencing of dengue virus serotype 1 (DENV-1) using Ion Torrent technology

Supervisors: Valentina Libri, Giulia Marsili (ISS, Rome, Italy)

Aim: To develop a methodological protocol for WGS of dengue virus (DENV) serotype 1 using targeted amplification sequencing with the Ion Torrent technology, with the ultimate goal of supporting the development of molecular tools for genomic surveillance and outbreak investigations of DENV in Italy.

Methods: A previously published amplicon-based WGS protocol for DENV-1 was optimised using three isolates from human and mosquito sources. Three RT-PCR kits were evaluated: SuperScript IV with SuperFi II (two-step), SuperScript III One-Step, and SuperScript IV One-Step with SuperFi. Optimisation involved testing annealing temperatures of 55 °C and 60 °C, and sequencing at depths of 100 000 and one million reads to improve sensitivity and genome coverage. Libraries were prepared using the Ion Plus Fragment Library Kit and sequenced on the Ion GeneStudio S5 Prime System. Bioinformatics analysis used fastp, Bowtie2, SAMtools, BCFtools, and IGV for quality control, mapping, variant calling, and consensus generation.

Results: Among the tested protocols, the SuperScript IV One-Step RT-PCR System with Platinum SuperFi DNA Polymerase resulted in the most efficient amplification of DENV-1 genomes. Optimisation of annealing temperatures showed that 55 °C consistently produced stronger amplicon signals and higher genome coverage across three DENV-1 isolates. At 100 000 reads, genome coverage at 55 °C ranged from 94–98% (1× threshold) and 54–81% (50×), outperforming results at 60 °C. Increasing sequencing depth to one million reads further improved coverage to 98–100% (1×) and 82–92% (50×). Uneven coverage patterns suggested preferential amplification due to variable primer efficiency.

Public health implications/Conclusions: This study optimised a cost-effective amplicon-based WGS protocol for DENV-1, improving amplification consistency and genome coverage using specific enzymes and conditions. While biases and coverage gaps remain, new degenerate primers show promise. Further validation and expansion to other serotypes are needed.

Role: For the validation of the amplicon-based WGS laboratory methodology, Miruna analysed primer binding efficiency and dimer formation in silico, performed RNA extractions, quantitative real-time reverse transcription PCR (qRT-PCRs), RT-PCRs, and gel electrophoresis. She also developed an automated pipeline to analysis Ion Torrent reads, drafted a standard operating procedure (SOP) for the laboratory, and a report detailing experimental outcomes, conclusions and suggestions for subsequent steps moving forward.

2.2. Extensively drug-resistant (XDR) **Salmonella** *Typhi in Italy, 2019–2023*

Supervisors: Irene Artuso, Claudia Lucarelli (ISS, Rome, Italy)

Aim: To conduct a retrospective genomic analysis of all *Salmonella* Typhi (*S.* Typhi) isolates that exhibit an extensively drug resistance (XDR) phenotype, with resistance to first-line antibiotics (ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole), fluoroquinolones, and third-generation cephalosporins, from the Enter-Net Italia collection (2019–2023).

Methods: XDR *S.* Typhi isolates were identified by the European Committee on Antimicrobial Susceptibility Testing (EUCAST)-standardised susceptibility testing. Genomic DNA was sequenced using the Illumina platform. Oxford Nanopore sequencing was carried out only for selected isolates. Reads were processed following EFSA guidelines for quality control, assembly, and annotation. Resistance genes and plasmid replicons were identified via the ResFinder, AMRFinderPlus, and PlasmidFinder databases. Insertion sequences were confirmed with the ISfinder database. Comparative genomics analysis with genomes from the National Center for Biotechnology Information (NCBI) database, were conducted using the pyGenomeViz package.

Results: In total, 217 *S.* Typhi cases were reported in Italy from 2019 to 2023. Among 118 tested isolates, eight (6.7%) were classified as XDR, and six of these were successfully sequenced. All isolates harboured a core of resistance genes consisting of *sul2*, *aph(3")-Ib*, *aph(6)-Ib*, $bla_{\text{TEM-1}}$, *qnrS* and $bla_{\text{CTX-M-15}}$ located either chromosomally, on IncY plasmids, or both. One isolate carried three $bla_{\text{CTX-M-15}}$ copies, two on the chromosome and one in an IncY plasmid, located downstream of IS*Ecp1* elements, forming a mobilisation unit in itself, or as part of larger structures containing elements like IS*26* and *Tn2*, and other resistance genes. Comparative genomics revealed similar resistance gene arrangements in *S.* Typhi, *Escherichia coli* (*E. coli*), and *Klebsiella pneumoniae* (*K. pneumoniae*), suggesting a shared mobile genetic context.

Public health implications/Conclusions: The detection of XDR *S.* Typhi strains in Italy with $bla_{\text{CTX-M-15}}$ copies integrated into both chromosomes and plasmids, indicates stable inheritance and potential for horizontal gene transfer. These findings highlight the risk of importation and spread of high-risk clones. While no local outbreaks were detected between 2019 and 2023, the emergence of XDR *S.* Typhi poses a serious threat to public health due to limited treatment options. Ongoing genomic surveillance is essential for early containment and antimicrobial stewardship.

Role: Miruna developed and wrote the study protocol. She conducted laboratory work (DNA extraction for WGS and antimicrobial susceptibility testing) and data analysis (surveillance data and WGS analysis). She also drafted a manuscript (see section 7.1, paper 3).

2.3. Addressing unique information needs for diverse population groups: insights from the helpline for HIV and other STIs in Italy, 2022–2024

Supervisors: Anna Colucci and Pietro Gallo (ISS, Rome, Italy)

Aim: To characterise users of the helpline for HIV and other sexually transmitted diseases (STIs) in Italy between 2022 and 2024, identifying specific information needs for each user group and informing the design of targeted educational campaigns.

Methods: Helpline users were grouped into: people who use drugs (PWUD), people living with HIV (PLHIV), men who have sex with men (MSM), heterosexual individuals, and those with no identified risk factors. We examined call frequency, number of questions per call, and the type of information requested categorised as related to: pathogens, psychosocial aspects, disinformation, testing, transmission, prevention, symptoms, treatment and research. Descriptive statistics were used to calculate percentages and identify patterns across user groups.

Results: From 2022 to 2024, the helpline received 24 984 calls and addressed 88 652 questions. Most questions were related to transmission (37%), testing (30%) and psychosocial aspects (13%). Users without identified risk factors had the highest call frequency – 25% calling more than five times. MSM and heterosexual users asked the most questions per call, with nearly 50% of them asking more than four questions. Distinct patterns of information needs emerged: PWUD primarily asked about transmission (38%), testing (19%), and disinformation (17%); MSM and heterosexual users focused on transmission (over 40%), testing (over 30%), and psychosocial aspects (over 10%); while PLHIV were most concerned with psychosocial aspects (44%) and topics related to research and treatment (24%).

Public health implications/Conclusions: The study supports national HIV and STI prevention efforts by identifying distinct information needs across population groups. Questions about transmission and testing were recurrent, underscoring the importance of broadly addressing these topics in educational campaigns. Interventions should also target the general population to reduce stigma toward PLHIV, while concurrently providing this group with up-to-date information on treatment options.

Role: Miruna conceptualised the study, designed the methodology, performed data cleaning, transformation, analysis and visualisation in R Studio, and drafted recommendations. She also drafted an abstract for ESCAIDE 2025, and a final report (see section 7.1, paper 4).

3. Biorisk management

3.1. Previous high-containment laboratory experience at European research institutes, 2015–2023

Before starting the EUPHEM fellowship, Miruna accumulated over eight years of BSL-2 laboratory experience working primarily at the Erasmus MC in the Netherlands, the Barcelona Biomedical Research Park (PRBB) in Spain, and the University of Freiburg in Germany. Her work involved virus culture, molecular assays, serology, microscopy and related virological techniques. During her PhD at the Erasmus MC, she gained extensive experience in BSL-3 and BSL-3+ laboratories over a period of three years. In these high-containment settings, Miruna conducted multiple procedures, including virus culture and virus titrations under stringent biosafety conditions. In the BSL-3+ laboratory, she was involved in vaccination-challenge experiments as part of pre-clinical influenza vaccine testing. These experiments required adherence to enhanced biosafety protocols, handling of infectious virus, and managing live-animal models under containment. This combination of BSL-2, BSL-3, and BSL-3+ laboratory experience provided Miruna with a solid foundation in high-containment virology and biosafety practices.

3.2. BSL-3 laboratory introduction for virus cultivation during the DENV-2 outbreak in Fano, Italy, 2024

Supervisor: Giulietta Venturi (ISS, Rome, Italy)

During the 2024 dengue virus serotype 2 (DENV-2) outbreak in Fano, Italy, selected clinical samples were used for virus isolation and culture in a BSL-3 laboratory. Over a two-week period, Miruna was introduced to high-containment procedures specific to arboviruses, including handling infectious material in the biosafety cabinet, viral culture techniques, decontamination protocols, and standard logging practices to ensure biosafety and traceability. This hands-on involvement provided targeted training in DENV culture within a BSL-3 setting.

Role: Miruna participated in hands-on training in a BSL-3 laboratory and assisted in culturing DENV-2 clinical isolates, contributing to the expansion of the isolate collection maintained by the Arbovirus Research Group at the ISS.

3.3. Preparation of **Mycobacterium tuberculosis** cultures for external quality assessment (EQA) of antibiotic susceptibility testing in a BSL-3 laboratory in Rome, Italy, 2025

Supervisors: Angelo Iacobino and Federico Giannoni (ISS, Rome, Italy)

Eight *Mycobacterium tuberculosis* (*M. tuberculosis*) strains, each mono-resistant to either bedaquiline, pretomanid, linezolid, or moxifloxacin, were prepared in a BSL-3 laboratory at the ISS for use in an external quality assessment (EQA) for drug susceptibility testing in peripheral diagnostic laboratories in Italy. Strains were first cultured in Becton Dickinson MGIT tubes and incubated in the automated BACTEC MGIT 960 system. Time to positivity ranged between seven and 21 days, depending on the growth rate of individual isolates. Once sufficient growth was confirmed, each isolate was aseptically sub-cultured onto Becton Dickinson Lowenstein-Jensen slants and incubated at 37 °C for an additional 3–4 weeks to ensure adequate biomass for shipment. Procedures were conducted under high-containment conditions using appropriate personal protective equipment and biosafety protocols. Prepared cultures were packaged in 95 kPa-compliant biohazard bags with absorbent material and shipped in UN3373-classified Bioshipper containers via PHSE for transport logistics, following International Air Transport Association (IATA) and United Nations (UN) regulations for Risk Group 3 pathogens. This activity provided Miruna hands-on experience with slow-growing high-risk pathogens under BSL-3 conditions and contributed to national laboratory quality assurance efforts.

Role: Under the supervision of Angelo Iacobino, Miruna performed *M. tuberculosis* culturing procedures under BSL-3 conditions in the flow cabinet and prepared samples for shipment.

4. Quality management

4.1. Revision of quality management practices of the European Union Reference Laboratory for Parasites (EURLP), Rome, Italy, 2024

Supervisor: Alessia Possenti (ISS, Rome, Italy)

Aim: To describe the quality management practices conducted at the European Union Reference Laboratory for Parasites (EURLP) at the National Public Health Institute in Italy (ISS).

Methods: As part of the 'Biorisk and Quality Management' module, a meeting was organised with the quality manager (supervisor) to discuss the management plan of the EURLP, including procedures for internal and external audits, corrective actions, external validation of laboratory practices, assurance of laboratory test validity, and SOPs.

Results: The Unit of Food-borne and Neglected Parasites has served as the EURLP since its designation by the European Commission in July 2006. It has been accredited under ISO/IEC 17025:2017 (international standard for testing and calibration laboratories) as a competent testing laboratory since 2006, and as a Proficiency Testing Provider (PTP) according to ISO/IEC 17043:2010 since 2013, with accreditation granted by ACCREDIA, Italy's national accreditation body. The EURLP primarily supports EU Member States' National Reference Laboratories (NRLs) in the field of food-borne parasitic diseases – particularly *Trichinella*, *Echinococcus*, and *Anisakis* – by developing, validating, and accrediting analytical methods and organising proficiency testing schemes. The EURLP also organises yearly internal audits for different laboratory procedures or methods. SOPs are periodically revised and are publicly available on the ISS website.

Public health implications/Conclusions: The EURLP plays a critical role in safeguarding public health by enhancing diagnostic capabilities for food-borne parasites across EU Member States. Through method validation, training, and proficiency testing, it ensures harmonised, high-quality surveillance and response, reducing the risk of parasitic infections linked to food consumption.

Role: Miruna reviewed the quality management plan in collaboration with the quality manager (supervisor), and drafted a final report as part of the 'Biorisk and Quality Management' module.

4.2. External quality assessment for antibiotic susceptibility testing of **Salmonella,** *Rome, Italy, 2024*

Supervisors: Claudia Lucarelli, Slawomir Owczarek (ISS, Rome, Italy)

Aim: To evaluate the performance of diagnostic methods used for antibiotic susceptibility testing (AST) of *Salmonella* at the Enter-Net Italia laboratory, within the framework of the EQA for EU/EEA public health laboratories organised by the National Institute for Public Health and the Environment (RIVM) on behalf of ECDC.

Methods: Eight EQA *Salmonella* isolates were tested for antibiotic susceptibility using disk diffusion (DD) for multiple antibiotics, following EUCAST guidelines. Bacterial suspensions were prepared from overnight cultures and spread on Mueller–Hinton (MH) agar before applying antibiotic disks. Inhibition zones were measured and interpreted the next day. Extended-spectrum β-lactamase (ESBL) production was assessed via synergy testing. Colistin susceptibility was evaluated using the ComASP Colistin MIC assay. Control strains included *E. coli* ATCC 25922 and *E. coli* NCTC 13486 for colistin testing. AST results were shared with the RIVM via an online system.

Results: EQA strains were correctly identified as susceptible to colistin by MIC. In most cases, the measured inhibition zones were within +/- 3 mm from the inhibition zones established by the provider. Overall, inhibition zones for pefloxacin exhibited the highest number of deviations and have been noted for future improvements. All ESBL-producing strains were correctly identified.

Public health implications/Conclusions: The antibiotic susceptibility of EQA isolates to most antibiotics was correctly identified. Observed deviations to pefloxacin may be attributed to factors such as an insufficient thickness of the MH agar layer in the plates or a higher-than-recommended inoculum density. Corrective actions should include recalibration of the nephelometer, limiting the storage time of open antibiotic disk cartridges in the fridge, and potential purchase of ready to use MH agar plates.

Role: Miruna assisted with bacteria cultures, DD assays and colistin MIC assays in the laboratory. She read out and interpreted the results, as well as drafted a report.

4.3. Setting up of an external quality assessment for antibiotic susceptibility testing of **Mycobacterium tuberculosis** *in Italy, 2025*

Supervisor: Federico Giannoni (ISS, Rome, Italy)

Aim: Following the 2021 World Health Organization (WHO) redefinition of drug-resistant *Mycobacterium tuberculosis*, the WHO TB Supranational Reference Laboratory aims to evaluate the ability of regional and peripheral laboratories to implement the updated testing guidelines, including susceptibility testing for bedaquiline, pretomanid, linezolid, and the fluoroquinolone, moxifloxacin.

Methods: A questionnaire was distributed to regional and peripheral laboratories in Italy to assess current testing practices and gauge interest in participating in the EQA. Eight *Mycobacterium tuberculosis* strains with defined resistance profiles were cultured on Löwenstein–Jensen medium and shipped to 14 laboratories in compliance with the UN Model Regulations and IATA Dangerous Goods Regulations for Risk Group 3 pathogens as 'Biological Substance, Category B'. Strains were sealed in primary containers, placed in 95 kPa-compliant biohazard bags with absorbent material, and enclosed in rigid, certified Bioshipper containers. Shipments reached designated laboratories within 24 hours.

Results: The EQA results were not available prior to the end of the EUPHEM fellowship.

Public health implications/Conclusions: Implementing an EQA for drug susceptibility testing of *Mycobacterium tuberculosis* ensures that regional and peripheral laboratories maintain high diagnostic standards in line with WHO guidelines. Reliable detection of resistance to key drugs – bedaquiline, pretomanid, linezolid, and moxifloxacin – is critical for guiding effective treatment regimens and curbing the spread of multidrug-resistant TB. Strengthening laboratory capacity through EQA enhances diagnostic accuracy, supports early intervention, and contributes to global TB control efforts by ensuring harmonised testing across laboratories involved in surveillance, diagnosis, and patient care.

Role: Miruna assisted with culturing the eight EQA *Mycobacterium tuberculosis* strains in a BSL-3 laboratory, coordinated their subsequent shipment to regional laboratories, and drafted a final report.

5. Public health microbiology management

Management and communication during international missions, 2024–2025

During the WHO Global Outbreak Alert and Response Network (WHO GOARN) mission to Myanmar/Burma, Miruna served as an epidemiologist supporting the acute watery diarrhoea (AWD)/Cholera response. She coordinated closely with the WHO country office Myanmar, the United Nations Children's Fund water, sanitation and hygiene (UNICEF WASH) team, and the WHO Geographic Information System (GIS) Centre for Health, managing time zone differences and high-pressure conditions with shifting priorities. She navigated intercultural communication, overlapping responsibilities, and tight timelines in a fast-paced emergency setting. As an epidemiologist for the European SARI Vaccine Effectiveness Network (Euro-SAVE) mission, she collaborated with WHO Regional Office for Europe (WHO/Europe) consultants and the Epiconcept technical team to support data analysis and dashboard development, maintaining clear, remote communication to meet deliverables effectively across organisational and technical partners.

Facilitation of intra-action review for the AWD/Cholera crisis response in Myanmar/Burma, 2024

During the intra-action review for the cholera outbreak response in Myanmar/Burma, Miruna co-facilitated the Surveillance Pillar discussions, including aspects of laboratory surveillance. Over the two-day event, she guided conversations among stakeholders from UN agencies, non-governmental organisations (NGOs) and other partners to identify key achievements, challenges, enablers and conducted root-cause analyses for each issue raised. She ensured inclusive participation and coordinated closely with the co-facilitator and note-taker to synthesise discussion outputs into actionable points. Together, they compiled a final report that was submitted to the WHO headquarters (HQ) for publication and dissemination to all participants.

Interview for news section of the National Public Health Institute Ricardo Jorge in Lisbon, Portugal, 2024

As part of the EUPHEM fellowship, Miruna participated in an interview for the news section of Portugal's National Public Health Institute (INSA) following the Project Review Module held in Lisbon. She reflected on the module's impact, highlighting improved data analysis, public health recommendation development, and oral presentation skills. Her comments, featured alongside those of fellow participants, emphasising the value of peer exchange and commending INSA's organisation and infrastructure. The interview served to communicate the fellowship experience to a wider public health audience.

Organisation of 2nd Fellowship Career Series, online, 2024

Miruna organised the 2nd edition of the Fellowship Career Series of Cohort 2023, focusing on public health microbiology. The hybrid event was for an hour and featured three experienced speakers who shared their professional trajectories and valuable lessons with fellows from Cohorts 2022 and 2023. Together with the fellows, Ebba Rosendal and Kaisa Jaakkola, Miruna managed communication with the speakers, coordinated logistics and facilitated discussions during the event.

Management and communication during outbreak investigations and projects, 2023–2025

Between 2023 and 2025, Miruna supported communication and coordination across multiple outbreak investigations and projects. She, together with the Italian outbreak investigation team, represented Italy in international meetings during a multi-country *Salmonella* Strathcona outbreak investigation and contributed to the national response to a local DENV-2 outbreak. At the ISS, she managed several concurrent projects under the guidance of multiple supervisors, requiring effective planning, prioritisation, and reporting. These activities involved strong communication, cross-cultural collaboration, and organisational skills within national and international public health settings.

6. Teaching and pedagogy

Introduction to R for epidemiologists training for WHO Myanmar, 2025

Miruna delivered an intensive four-day remote training, totalling 20 hours, to the WHO Myanmar Epidemiology Team on 'Introduction to R for Public Health Epidemiology'. She developed all training materials – from theoretical lectures to live coding demonstrations and practical exercises with included code solutions using datasets sourced from *The Epidemiologist R Handbook*. The course covered four core modules: data management, descriptive tables and visualisations, GIS basics, and R Markdown. An electronic evaluation survey was designed and administered to participants to collect feedback.

Lectures on **Mycobacterium tuberculosis** *and non-tuberculous* **mycobacteria**, *Sapienza University, Italy, 2024, 2025*

Miruna delivered two-hour lectures to master's students in Medicine and Surgery at Sapienza University, Rome, in June 2024 and 2025 as part of the 'Seminars in Bacteriology and Surgery'. The learning needs were discussed with the course coordinator, Prof. Guido Antonelli and investigator Dr. Federico Giannoni. Slides were updated to include current information and broaden the focus of the lecture. Interactive polls assessed student knowledge and engagement. Feedback was collected via an electronic questionnaire, and both an evaluation report and reflective note were prepared.

Seminar on seasonal influenza – immune escape and implications for vaccine effectiveness, Sapienza University, Italy, 2025

Following the successful delivery of lectures on *Mycobacterium*, Miruna was invited to deliver a 2.5-hour seminar on seasonal influenza to master's students in Medicine and Surgery at Sapienza University in May 2025. She developed the training materials, delivered the lecture, created an evaluation survey, and wrote a reflective note on the teaching experience. The face-to-face seminar covered epidemiology, antigenic evolution and immune escape, the Global Influenza Surveillance and Response System (GISRS) surveillance network, vaccine strain selection and composition, and innovative next-generation influenza vaccines.

7. Communications related to the EPIET/EUPHEM fellowship

7.1. Manuscripts published in peer-reviewed journals

- Sacco C, Liverani A, Venturi G, Gavaudan S, Riccardo F, Salvoni G et al. Autochthonous dengue outbreak in Marche Region, Central Italy, August to October 2024. Euro Surveill. 2024 Nov;29(47):pii=2400713. Available at: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2024.29.47.2400713

- **Rosu ME**, Sacco C, Giufrè M, Pezzotti P. National surveillance system for invasive *Haemophilus influenzae* disease in Italy: evaluation of simplicity, representativeness, data completeness and timeliness, 2016–2023. [Submitted to *Scientific Reports*; under peer review]

- Artuso I, **Rosu ME**, Dionisi AM, Slawomir O, Villa L, Lucarelli C. Genomic characterisation of extensively drug-resistant (XDR) *Salmonella* Typhi in Italy, 2019–2023. [Manuscript in preparation]
- **Rosu ME**, Gallo P, Colucci A. Addressing unique information needs for diverse population groups: Insights from the HIV and other STIs Helpline in Italy. [Manuscript in preparation]

7.2. Conference presentations

- **Rosu ME**, Sacco C, Giufrè M, Pezzotti P. National surveillance system for invasive *Haemophilus influenzae* disease in Italy: evaluation of simplicity, representativeness, data completeness and timeliness, 2016–2023 (poster presentation). Presented at: ESCAIDE; 20 November 2024; Stockholm, Sweden

7.3. Other presentations

- **Rosu ME**. Antigenic evolution of influenza viruses and implications for vaccine design. Presented at: Monthly Infectious Diseases Seminar Series at Istituto Superiore di Sanità; 16 January 2024; Rome, Italy

- **Rosu ME**. *Mycobacterium tuberculosis* and non-tuberculous mycobacteria, Sapienza University; 24 May 2024 and 21 March 2025; Rome, Italy

- **Rosu ME**. Seasonal influenza – Immune escape and implications for vaccine effectiveness, Sapienza University; 27 May 2024; Rome, Italy

- **Rosu ME**. EUPHEM fellows and why they are an asset for MSF. Presented at: EPIET/EUPHEM-MSF kick-of meeting; 16 August 2024; Remote

- **Rosu ME**. Multifactor Priority Index for AWD/Cholera in Myanmar/Burma. Presented at: Inter-cluster Coordination Meeting Myanmar; 17 January 2025; Remote

- **Rosu ME**. Remote GOARN deployment in Myanmar. Presented at: EPIET/EUPHEM International Assignments: Experiences from the Field. 31 March 2025; Remote

8. EPIET/EUPHEM modules attended

- Introductory Course, 25 September−13 October 2023, Spetses, Greece
- Study Protocol and Scientific Writing, 26−27 October and 7−8 November 2023, virtual
- European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2023, 22–24 November 2023, Barcelona, Spain
- Multivariable Analysis, 19−23 February 2024, Berlin, Germany
- Vaccinology, 4−8 March 2024, virtual
- Writing Abstracts for Scientific Conferences, 14 March or 20 March 2024, virtual
- Rapid Assessment and Survey Methods, 15−19 April 2024, Dublin, Ireland
- Public Health Microbiology II – Biorisk and Quality Management, 21−23 May 2024, virtual
- Public Health Microbiology III – Whole Genome Sequencing & Bioinformatics, 3−7 June 2024, Vienna, Austria
- Project Review Module, 26−30 August 2024, Lisbon, Portugal
- European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2024, 20−22 November 2024, Stockholm, Sweden
- Social Behavioural Sciences, 24−28 March, Virtual
- Project Review Module, 25−29 August 2025, Lisbon, Portugal
- Public Health Leadership, 1−3 September 2025, Lisbon, Portugal

9. Other training

- EUPHEM Career Series, 2023–2025
- Introduction to R for Applied Epidemiology, 19−22 September 2023, AppliedEpi, Virtual
- Certified online course: Safety prevention protection - General training, Sector: ATECO 207:M72, Risk type: B, organised by the Directorate General of the Istituto Superiore di Sanità, 15−17 November 2023
- Public Health Preparedness for Mass Gatherings Events, WHO, 15 April 2024, Virtual
- BSAFE, WHO, 3 September 2024
- Prevention of sexual exploitation and abuse (PSEA), 10 September 2024, WHO, Virtual
- United to Respect: Preventing sexual harassment and other prohibited conduct, 11 September 2024, WHO, Virtual
- WHO's New Policy and Strategy on Preventing and Addressing Sexual Misconduct, 11 September 2024, Health Emergencies Programme, WHO, Virtual
- WHO Ethics Empowerment, 11 September 2024, WHO, Virtual
- Management and Facilitation of an After-Action Review (AAR), 1 December 2024, WHO, Virtual
- PETAL seminar – Health Economics, 16 October 2024
- AURORAE Webinar – Introduction to INSaFLU -TELEVIR, 29 October 2024
- Leadership and Management in Global Health, 17 October 2024, 16 January 2025, 17 April 2025, TEPHINET, Virtual
- AURORAE Webinar – Integrated respiratory virus surveillance, 4 March 2025
- WHO Emergency Medical Teams Initiative Monthly Call, 29 April 2025, WHO, Virtual
- WHO Webinar − GOARN’s National Outbreak Response Handbook, 4 June 2025, WHO, Virtual

10. International assignments

WHO GOARN Mission: remote deployment to support the AWD/Cholera protracted emergency G3 in Myanmar/Burma, November 2024–February 2025

Miruna was deployed for a 12-week part-time assignment to support the epidemiology team of the WHO Country Office in Myanmar with the AWD/Cholera response. Her contribution included routine epidemiological analysis, review of internal situational reports, development of the AWD/Cholera priority index by township to enhance preventive measures and surveillance, and development of heat-maps to identify high-risk locations for AWD/Cholera outbreaks based on current cases and other variables such as displacement, WASH, food insecurity, conflict, surveillance availability, vaccination campaigns, health care availability, etc. Additional activities included moderation of the surveillance and lab-based surveillance focus group discussions during the intra-action review of the AWD/Cholera response, technical contribution at the inter-cluster coordination meetings, delivery of R training, and collaboration with GIS team at the WHO HQ and UNICEF Myanmar. Miruna wrote a letter from the field that was published on the GOARN website.

WHO/Europe assignment: remote deployment to develop an interactive R ShinyApp tool to evaluate Euro-SAVE surveillance data, May–July 2025

Miruna was deployed for a 12-week part-time assignment to support WHO/Europe and Epiconcept with the evaluation of the Euro-SAVE surveillance system, which monitors vaccine effectiveness against respiratory viruses in eastern Europe. She provided technical advice on data analysis and contributed to the development of an interactive dashboard using R Shiny to visualise surveillance data. Miruna also presented the new dashboard to stakeholders within Euro-SAVE and WHO/Europe.

11. Other activities

Miruna drafted a testimonial for the WHO GOARN website, describing her experience during a remote deployment to support the WHO Country Office Myanmar in the response to the AWD/Cholera emergency. Available at: https://goarn.who.int/latestnews/stories/miruna-elena-rosu

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