

Summary of work activities

Álvaro Roy Cordero
European Public Health Microbiology Training Programme
(EUPHEM), 2021

Background

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

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

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

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

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

Pre-fellowship short biography

Álvaro Roy Cordero completed his bachelor of veterinary medicine in 2013 at the Complutense University of Madrid, Spain. He holds a Master of Tropical Medicine from the Autonomous University of Madrid. In 2020, he obtained an industrial PhD from the Complutense University of Madrid, in partnership with CZ Vaccines, during which he wrote his dissertation on the evaluation of tuberculosis vaccines and advances in the immunological diagnosis of animal tuberculosis. After his PhD, he worked as a postdoctoral researcher at the Mycobacteria Unit of the VISAVET Health Surveillance Centre in Madrid. He also collaborated with the tuberculosis advisory group of the Spanish Ministry of Agriculture, Fisheries and Food and the European Union Reference Laboratory for Bovine Tuberculosis.

Álvaro has always been interested in infectious diseases, especially zoonoses, public health microbiology and epidemiology. Álvaro joined the EUPHEM fellowship at the Hospices Civils de Lyon (HCL), France, in order to enhance his microbiological and epidemiological analytical skills and broaden his knowledge of human infectious diseases.

Results

The objectives of these core competency domains were achieved partly through project and activity work and partly by participating in the training modules. Results are presented in accordance with the EUPHEM core competencies, as set out in the ECDC Fellowship Manual¹.

1. Epidemiological investigations

1.1. Outbreak investigations

1.1.1. Outbreak of carbapenem-resistant Enterobacteriaceae in two wards of the Hepatogastroenterology Unit in a hospital in Lyon, France, 2022

Supervisor: Cédric Dananché

Category: Healthcare-associated infections and antibiotic resistance

An increase of carbapenem-resistant Enterobacteriaceae (CRE) occurred in two wards of the hepatogastroenterology department of a hospital in Lyon, France, between 1 June and 30 September 2022. Rapid control measures were implemented and an epidemiological investigation was conducted to investigate possible sources of infection. Environmental samples from shower drains and other locations in patients' rooms were collected and characterised by whole genome sequencing (WGS), along with rectal swab samples from patients. Immediately after, patients were transferred to an empty ward and both wards were thoroughly disinfected.

Eleven out of fourteen patients with CRE were detected during regular screenings in rectal swabs (colonisations) and three were detected incidentally in other biological samples such as urine or surgical drains (infections). All environmental samples were positive for CRE and identical plasmids responsible for harbouring resistance genes encoding different types of carbapenemases (VIM, NDM and OXA-48) were found in patients and the shower drains of the rooms they stayed in, but also in shower drains from other rooms. After the affected wards were disinfected, CRE were detected in 14/19 (74%) shower drains of ward A and 1/19 (5%) shower drains of ward B.

Shower drains were identified as reservoirs of CRE, as they harboured CRE with the same resistance genes detected in patients, which were also temporally related. For this reason, transmission via splashing could not be ruled out. Reinforced disinfection procedures reduced the presence of CRE in environmental reservoirs and the number of cases decreased in the following months. However, non-compliance with the disinfection protocol was detected in one of the wards. Future assessments of the protocol's efficacy and the optimal interval between reinforced cleanings will be conducted. We recommended promoting training of health professionals and cleaning staff in hygiene and prevention measures, as well as special disinfection procedures.

Role: Co-investigator. The fellow participated in all phases of the outbreak investigation, including case definition, data collection and the integrated analyses of epidemiological data and laboratory results. He also wrote the outbreak report [6] and participated in the environmental sampling of one of the wards under the supervision of Cédric Dananché (Head of the Infection Control Unit).

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

1.1.2. Patient characteristics and clinical presentations of an outbreak of skin ulcers with unknown origin in children in Muyinga province, Burundi, 2021–2022

Supervisor: Temmy Sunyoto, Julita Gil Cuesta, Ankur Rakesh and LuxOR/MSF group

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

In December 2019, an outbreak of skin ulcers of unknown origin started in the Health District of Giteranyi in the northeast province of Muyinga in Burundi. Médecins Sans Frontiers (MSF) responded by investigating this outbreak in January 2020 in two municipalities, Buthinda and Giteranyi. MSF supported the provision of free and effective wound care and investigated the origin of the outbreak. The objective of this retrospective outbreak investigation was to describe the sociodemographic and clinical characteristics of the patients, their ulcers and the clinical evolution during the outbreak to better identify and control future outbreaks. Sociodemographic and clinical data were collected from consultations with patients that met the case definition and were registered in seven MSF intervention health centres (HCs) in Muyinga province from April 2021 to the end of January 2022.

There were a total of 4 202 patients included in the study, most of whom resided in the Giteranyi municipality (69.2%). The majority of the population were young patients aged 5–15 years (72%), with a median age of 11 years (interquartile range (IQR): 8–14 years). The male-to-female ratio was balanced and most patients were students (61%) and/or working in agriculture or farming (31.8%). The ulcers were mainly located on the lower legs (71%), presented little pain (median: 2/10 on a numeric pain scale), were itchy (100%) and non-infected (87%), and had a median size of 1 cm² (IQR: 1.00–2.25 cm²). The median number of ulcers was 1 (IQR: 1–2 ulcers), dressings needed during appointments was 4 (IQR: 3–7 dressings) and the treatment duration in days was 26 (IQR: 15–42 days), but these numbers varied greatly between HCs ($p < 0.001$). Microbiological investigations were not conclusive and *Streptococcus pyogenes* and *Staphylococcus aureus* were isolated from the small number of collected samples.

The descriptive results of this study may guide future analytical epidemiological studies to respond to the remaining unknowns of this outbreak.

Role: Co-investigator. Álvaro performed all the data analysis from the MSF databases, attended the team meetings and drafted a manuscript for submission to a peer-reviewed journal [3].

Educational outcome

The fellow gained experience in the 10 steps of outbreak investigation, the analysis of integrated microbiological and epidemiological data, and the communication of results to internal and external stakeholders. Álvaro participated in two different kinds of outbreaks: an ongoing outbreak at a hospital and another in the framework of a retrospective and remote international mission. In the first one, the fellow collected and analysed clinical, epidemiological and microbiological data, and participated in the environmental samplings. In the second, the fellow gained experience in the curation and analysis of large databases, and in working with international organisations such as MSF.

1.2. Surveillance

1.2.1. Monitoring epidemiological changes for toxoplasmosis: a six-year longitudinal study in pregnant women in Lyon, France, 2017–2022

Supervisors: Martine Wallon, Laurent Gaucher

France has a congenital toxoplasmosis prevention programme based on identifying women at risk at the beginning of pregnancy and monthly monitoring of their serological status. However, national data on *Toxoplasma* seroprevalence is limited to six cross-sectional perinatal surveys carried out on a limited number of women between 1995 and 2021. A sharp decline in prevalence has been observed throughout the surveys, which may pose a problem of cost-effectiveness as the number of women testing negative increases. The aim of this study was to evaluate the toxoplasmosis seroprevalence in pregnant women and the incidence of seroconversion and congenital infection. We conducted a longitudinal retrospective cohort study that included all pregnancies with known *Toxoplasma* status that were followed at Lyon's public maternity hospitals between 2017 and 2022. We used a logistic regression model to identify factors (age group, population density of living area, and parity) associated with seropositivity.

A total of 65 021 pregnancies were included in the study. The seroprevalence of toxoplasmosis decreased consistently from 26.7% in 2017 to 22.3% in 2022 ($p = 0.013$), and the percentage of seroconversions remained constant at 0.1% or below. The seroprevalence increased linearly from 18.3% in women aged 20–24 years to 39.2% in women aged ≥ 40 years ($p < 0.001$). The seroprevalence was higher in pregnant women living in highly populated areas (adjusted prevalence ratio (aPR): 1.31; 95% confidence interval (CI): 1.22–1.41) and in women who had given birth two or more times before (aPR: 1.19; 95% CI: 1.14–1.24).

This study confirmed the ongoing decline in toxoplasmosis seroprevalence, while seroconversions remained stable, indicating a need for more tests in seronegative women in the future. These findings are crucial for assessing the cost-effectiveness of prenatal screening and guiding targeted interventions and improved surveillance programmes for *Toxoplasma*.

Role: Álvaro attended meetings to discuss the study design with the team, drafted the study protocol, and cleaned and analysed the data extracted from Lyon's public hospital database. The fellow was responsible for drafting the manuscript [4] under the guidance of his supervisors. The work will be presented as an online poster at ESCAIDE 2023 [12].

1.2.2. Resistant bacteria in patients with ventilator-associated pneumonia in intensive care units and association with influenza seasons in Lyon, France, 2004–2019

Supervisors: Cédric Dananché, Selilah Amour

Ventilator-associated pneumonia (VAP) is one of the major hospital-acquired infections in intensive care units (ICUs). Multidrug-resistant organisms (MDROs) are frequently associated with VAP: *Staphylococcus aureus* resistant to methicillin (MRSA), *Enterobacteriaceae* species resistant to third-generation cephalosporins (3GCE-E) and *Pseudomonas aeruginosa* resistant to ceftazidime (CRPA). Increases in antibiotic consumption and inappropriate prescription during influenza seasons might contribute to a higher selective pressure of resistant bacteria in ICUs. The main aim of this study was to assess the association between the incidence of VAP caused by the main resistant bacteria involved and the influenza season.

We conducted a retrospective surveillance-based study by extracting the data of ICUs of Lyon University Hospitals between 2004 and 2019 from the French National Healthcare-Associated Infections Surveillance Network of ICUs.

During the study period, 24 750 patients were intubated in the ICUs, of which 3 127 (7.9%) developed VAP. Among them, one or two MDROs were isolated in 1 126 (36.0%) patients. Overall, a decreasing trend was observed for MDRO-related VAP (p for trend <0.001 ; from 2010 for MRSA, from 2008 for 3GCE-E and from 2015 for CRPA). No evidence of seasonality of patients with MDRO-related VAP and no higher incidence rate of MRSA-, 3GCE-E- and CRPA-related VAP during the influenza seasons were observed.

In conclusion, a decreasing trend was observed in patients with MDRO-VAP in Lyon between 2004 and 2019, and no association was found between the influenza season and higher incidence of MRSA-, 3GCE-E- and CRPA-related VAP. However, these results need to be further evaluated in a larger number of patients and with precise data on antibiotic consumption. As antimicrobial resistance levels remain high for several bacterial species and antimicrobial groups, we recommend actively promoting antimicrobial stewardship programmes, strengthening training of healthcare staff, and improving biosafety measures and workflows in hospitals, to reduce cross-transmission and spread of antimicrobial-resistant bacteria.

Role: The fellow was in charge of cleaning and filtering the Lyon Réseau REA-Raisin database according to the objectives of the study. He was also in charge of analysing the data, presenting it to the research team and writing an internal report of the results [7].

Educational outcome

The fellow had the opportunity to engage in two different surveillance projects that were conducted at the local level but had national impact. Álvaro wrote a study protocol, gained practical skills in handling large datasets (including data cleaning and manipulation) and increased his proficiency in data analysis using R. He performed descriptive, stratified, univariate and multivariable analyses. These projects also enhanced his abilities in writing manuscripts and reports, as well as interpreting results to provide conclusions and recommendations on important public health matters.

2. Applied public health microbiology and laboratory investigations

2.1. The increasing age of respiratory syncytial virus-related hospitalisation during the COVID-19 pandemic in Lyon was associated with reduced hospitalisation costs

Supervisors: Jean-Sébastien Casalegno, Bruno Lina

Preventive measures applied during the COVID-19 pandemic have modified the age distribution, the clinical severity and the incidence of respiratory syncytial virus (RSV) hospitalisations during the 2020/21 RSV season. The aim of this study was to estimate the impact of these aspects on RSV-associated hospitalisation (RSVH) costs stratified by age group between pre-COVID-19 RSV seasons and the 2020/21 RSV season.

We compared the incidence, the median and the total RSVH costs from the national health insurance perspective in children below 24 months of age during the COVID-19 pandemic (2020/21 RSV season) with a pre-pandemic period (2014/15–2016/17 RSV seasons). Children included in the study were born and hospitalised in the Lyon metropolitan area. RSVH costs were extracted from the French medical information system (Programme de Médicalisation des Systèmes d'Information).

The RSVH incidence rate per 1 000 infants aged less than 3 months decreased significantly from 4.6 hospitalisations (95% CI: 4.1–5.2) to 3.1 hospitalisations (95% CI: 2.4–4.0) during the 2020/21 RSV season. In older infants and children up to 24 months of age, the number of hospitalisations increased during this period.

Overall, RSVH costs for hospitalised cases below 2 years old decreased by €201,770 (31%) during the 2020/21 RSV season compared to the mean pre-pandemic costs.

The sharp reduction in costs of RSVH in infants aged less than 3 months outweighed the modest increase in costs observed in the 3–24 months age group. Therefore, conferring temporal protection through passive immunisation of infants aged less than 3 months should have a major impact on RSVH costs, even if it results in an increase of RSVH in older children infected later in life. Nevertheless, stakeholders should be aware of this potential increase of RSVH in older age groups, presenting with a wider range of disease, to avoid any bias in estimating the cost-effectiveness of passive immunisation strategies.

Role: The fellow drafted the study protocol, assisted with obtaining permission from the ethics committee, prepared and sent consent letters to parents, collected and analysed epidemiological and costs data, and drafted the manuscript. The study was presented as a poster at the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 2023 [11] and published in the peer-reviewed journal *Vaccine* [1].

2.2. Determinants of protection against SARS-CoV-2 Omicron BA.1 and Delta infections in fully vaccinated outpatients

Supervisors: Antonin Bal, Bruno Lina

Understanding immune correlates of protection (CoP) against SARS-CoV-2 infections is crucial for predicting the risk of infection and could help to optimise vaccination strategies, accelerate the vaccine authorisation process and make large efficacy trials no longer necessary. We aimed to evaluate the association between the humoral and cellular immune responses and symptomatic SARS-CoV-2 infection with Delta or Omicron BA.1 variants in fully vaccinated outpatients.

Anti-RBD IgG levels and IFN- γ release were evaluated at PCR-diagnosis of SARS-CoV-2 in 636 samples from negative and positive patients during Delta and Omicron BA.1 periods. Median levels of anti-RBD IgG in positive patients were significantly lower than in negative patients for both variants ($p < 0.05$). The frequency of Omicron BA.1 infection in patients with anti-RBD IgG concentrations $\geq 1\ 000$ BAU/mL was 51.0% and decreased to 34.4% in patients with concentrations $\geq 3\ 000$ BAU/mL. For Delta infection, the frequency of infection was significantly lower when applying the same anti-RBD IgG thresholds (13.3% and 5.3% respectively; $p < 0.05$). In addition, individuals in the hybrid immunity group had 4.5 times lower risk of Delta infection compared to the homologous vaccination group (aOR = 0.22; 95% CI: 0.06–0.64). No significant decrease in the risk of Omicron BA.1 infection was observed in the hybrid group compared to the homologous group, but the risk decreased within the hybrid group as anti-RBD IgG titres increased (aOR = 0.08; 95% CI: 0.01–0.41); $p = 0.008$). IFN- γ release post-SARS-CoV-2 peptide stimulation was similar between samples whether patients were infected (either with Delta or Omicron BA.1 variant) or not ($p > 0.05$).

Our results showed that high circulating levels of anti-RBD IgG and hybrid immunity were independently associated with a lower risk of symptomatic SARS-CoV-2 infection in outpatients, with differences according to the infecting variant.

Role: The fellow was responsible for interpreting the laboratory results, performing all statistical analyses (including multivariable logistic regression analysis) and drafting the manuscript. The paper was presented in poster format at ECCMID 2023 [10] and published in the peer-reviewed *Journal of Medical Virology* [2].

2.3. Evaluation of different next-generation sequencing approaches for mpox virus whole genome characterisation

Supervisors: Laurence Josset, Quentin Semanas

Viral metagenomic next-generation sequencing (mNGS) enables early sequencing of the mpox (formerly named monkeypox) genome; however, this method lacks sensitivity and cannot produce complete genomes for low viral load samples. The objective of this study was to compare the performance of an in-house mNGS method (without enrichment) and a new hybridisation capture enrichment method for mpox virus in clinical samples.

A retrospective laboratory investigation was conducted in mpox-positive patients ≥ 18 years old, with a median age of 36 years (IQR: 30–40 years). Patients included in the study had available samples with low, medium and high Ct-values collected from skin lesions and were diagnosed in Lyon Public Hospitals between 7 June and 21 July 2022. Samples were sequenced using both hybrid capture-based target enrichment and in-house mNGS methods.

A total of 47 samples from skin lesions were sequenced. When we compared the samples with low Ct values ($n = 8$) sequenced with both NGS approaches, the median depth and coverage were 3 924 (IQR: 2 657–5 864) and 99.95 (IQR: 99.95–99.95) using the hybridisation capture, decreasing to 60.5 (IQR: 18.8–172) and 98.4 (IQR: 96.5–100), using the in-house mNGS ($p = 0.007$ for median depth; $p = 0.08$ for median coverage). Four complete genomes were obtained from samples with high Ct values (Ct values: 25–35.7) using the hybrid capture-based method and none using mNGS. Only samples with low Ct values showed a coverage proportion above 90% by mNGS (median: 98.4; IQR: 96.5–100).

The hybrid-capture target enrichment has a better median depth and coverage in samples with low or moderate viral load, increasing the sensitivity for variant calling and throughput capabilities. The hybrid-capture target enrichment method can improve the sensitivity and specificity of mpox characterisation, thereby improving surveillance by detecting new variants earlier in order to prevent or mitigate new outbreaks.

Role: The fellow followed and took part in the laboratory experiments and the technical activities, evaluated the performance parameters of both sequencing protocols, cleaned and analysed the data, and wrote a manuscript in the form of a short communication [5].

Educational outcome

The fellow gained experience working with DNA and RNA viruses of public health relevance, such as SARS-CoV-2, RSV and mpox. As the projects covered different virus types and completely different approaches, the fellow was able to work with diverse aspects of public health, such as immune correlates of protection against SARS-CoV-2, cost analyses from a health insurance perspective in relation to RSV hospitalisations before and after the COVID-19 pandemic, and the evaluation of two different sequencing approaches for mpox surveillance and outbreak investigation.

3. Biorisk management

3.1. Introductory training in Biosafety Level 3 laboratory at the Institut des Agents Infectieux, HCL

Supervisor: Alexandre Gaymard

The Institut des Agents Infectieux (IAI) is part of the HCL. The IAI has three independent BSL-3 laboratories: (i) a mycobacteria laboratory, (ii) a laboratory for multiple activities with two main components dedicated to respiratory and muco-cutaneous risks and (iii) the Sécurité Civile laboratory Biotox against bioterrorist threats. The training began with a two-hour theoretical lesson explaining the facilities, the pathogens they work with, the relevant legislation, the specifications of each laboratory, the protocols for entry and exit, the Safety Access System (SAS) for personnel and samples, the waste handling and disposal protocols, the cleaning and disinfection processes, the equipment maintenance and the disinfection and management of incidents in the BSL-3 (e.g. abnormal pressure, fire alarms, cabinet failures, accidents of operators, monitoring systems). After completing the theoretical part, the fellow went into the BSL-3 laboratory on a supervised visit and was trained on the checklist related to dressing and undressing protocols, the different laboratories and the pathogens they work with and the general methodologies, equipment and protocols of each of them. New automated and real-time cell culture equipment was demonstrated with mpox samples during the visit. Additional training specific to working in BSL-3 laboratories was done on a project basis and depending on the pathogens to be handled by the operator, for which the project supervisor was responsible.

Role: Álvaro successfully completed the BSL-3 training. He learned how to work in the BSL-3 laboratory of the training site while accompanied by other staff with supervisory levels of competence.

3.2. Simulation exercise as part of the biorisk assessment module

The fellow participated in a simulation exercise after completing the Biorisk and Quality Management module with other fellows. During the group exercise, participants conducted a biorisk assessment for a fictitious pathogen in a laboratory research project using the BioRAM software. The program considered several issues relating to the characteristics of the pathogen, such as its transmissibility or virulence, routes of infection and consequences in human and animal populations (e.g. morbidity, mortality, available treatments) and laboratory procedures for testing, containment and decontamination. The biorisk assessment results described the probability of direct exposure and the consequences at the individual level for laboratory staff and at the community level outside the laboratory, as well as the risk to animal populations. Ultimately, the comprehensive biosafety risk was assessed by integrating both the probability of infection and its corresponding consequences.

Role: The fellow completed the simulation exercise with other fellows and submitted it to the ECDC Virtual Academy (EVA) platform.

Educational outcome

The fellow gained experience in biosafety management and biosecurity in public health laboratories, complementing the experience he already had in a BSL-3 laboratory with animal infectious pathogens. The BSL-3 training gave him the opportunity to be trained in another BSL-3 laboratory with different protocols and focused on different pathogens, such as human respiratory viruses. It also provided him with the opportunity to compare biosafety procedures, facilities and workflows between different laboratories in different European countries. Álvaro also gained experience applying the concepts of biosecurity risk assessment and mitigation measures, as well as in performing risk assessments using tools such as BioRAM.

4. Quality management

4.1. External quality assessment for whole genome sequencing-based resistome profiling in antimicrobial-resistant *Salmonella* and *Campylobacter* at Instituto de Salud Carlos III (ISCIII)

Supervisor: Silvia Herrera León

Salmonellosis and campylobacteriosis were the first and second most-reported zoonoses in the EU/EEA in 2021. Antimicrobial resistance (AMR) in food-borne zoonotic bacteria remains a major public health problem, identified as one of the top three priority health threats in 2022 by the Commission and the Member States. Between 2020 and 2021, *Campylobacter Jejuni* and *C. coli* originating from both humans and animals revealed high to extremely high levels of resistance to fluoroquinolones; high levels of resistance to ampicillin, sulfonamides and tetracyclines were also observed in *Salmonella* spp. in humans.

In this context, the food- and waterborne diseases (FWD) AMR-RefLabCap project aims to support implementation and alignment of whole genome sequencing (WGS)-based analysis methods among national reference laboratories. In order to achieve this, the second EQA for WGS-based resistome profiling in antimicrobial-resistant *Salmonella* and *Campylobacter* was organised by the FWD AMR-RefLabCap project, with participation of the Reference Laboratory for FWD at the Instituto de Salud Carlos III (ISCIII).

DNA from three *Salmonella* and three *Campylobacter* strains was shipped from Statens Serum Institut to the ISCIII. A protocol for WGS-based analysis of *Salmonella* and *Campylobacter* was provided by the FWD AMR-RefLabCap. It covered the steps of DNA concentration and dilution, library preparation and DNA sequencing, WGS raw data retrieval and QC, genome assembly and bioinformatics analysis. ResFinder was used to search for antimicrobial resistance genes (ARGs) and chromosomal point mutations (PMs) in the samples. Results were compared with those obtained with AMRFinderPlus in SeqSphere+. Information on the agreed AMR results and on the applied methods and the selected quality control metrics was submitted through a web-based platform.

The laboratories participating in the EQA will be notified of the ring trial results. After discussion, if any deviations are identified, the protocols and techniques will undergo evaluation and enhancement.

Role: During the EQA, the fellow analysed raw reads and assembled genomes of *Salmonella* and *Campylobacter* using the online tools EnteroBase and PubMLST to obtain the sequence type (ST) and the serotype, even though it was optional to report the ST and serotype in this EQA. In addition, Álvaro used ResFinder to search for ARGs and PMs in *Salmonella* and *Campylobacter* samples, compared the results with those obtained with AMRFinderPlus in SeqSphere+, and participate in their submission through the dedicated web-based platform. He presented the results as an internal report [9].

4.2. Internal audit of point-of-care equipment implemented for the rapid diagnosis of SARS-CoV-2 in Lyon's public hospitals

Supervisor: Emilie Frobert

In November 2020, a rapid point-of-care and automated multiplex real-time RT-PCR assay for simultaneous qualitative detection and differentiation of SARS-CoV-2, influenza A virus and/or influenza B virus RNA (cobas® SARS-CoV-2 & Influenza A/B) from nasal and nasopharyngeal swab specimens was installed in the clinical laboratories of Lyon's public hospitals.

On 1 July 2022, three auditors (two quality service staff and a virologist, as well as the laboratory manager and a technician) conducted an internal audit in one of the clinical laboratories at one of Lyon's public hospitals. The cobas® SARS-CoV-2 & Influenza A/B technique and hematology and biochemistry devices were included in the audit. The risk analysis matrix was discussed (risk description, causes, consequences and impact, detection, action plan to be applied). Positive and invalid samples were randomly selected and all the accompanying documentation to these samples, entry in the IT system and traceability were audited. The training plan and the accompanying evaluation questionnaire were also assessed.

It was found that no preventive actions were taken to avoid potential causes of non-conformities previously. Deficiencies were noted in the training plan records for new technicians and missing data regarding the dates of the training courses and the name and signature of the trainer. The training plan was recorded in a separate software, but it is planned to be incorporated into internal software. A deviation was detected in the validation of one of the reports. The same report was found to be duplicated and validated by two different microbiologists. Corrective actions were opened with regard to the training plan, the validation of the results by the microbiologists and the avoidance of duplicate validations. It was recommended that the software supplier carry out tests to check traceability was accurate.

Role: The fellow observed the audit and participated in the discussion with the quality team, the virologist and the audited staff. Álvaro also wrote a reflective note including the audit results and his observations.

4.3. Quality management module homework: laboratory audit for the virology laboratory at the Hospices Civils de Lyon

Supervisor: Vinca Icard

On 31 March 2022, the fellow and the chief virologist carried out an audit on process management, quality control and documentation at the molecular virology laboratory. Different categories related to quality were assessed during the audit exercise: accommodation and environmental conditions, quality management, quality assurance, pre-analytical process and specimen management, analytical process, post analytical process, quality improvement, quality document and document control, technical record, control of non-conformities, equipment logbooks, biosafety documentation, personnel and stock documentation, standardised operating procedures, standardised report format, surveillance and outbreak response documentation, and transport documentation.

The audit showed a high level of compliance in process management and quality control, as well as for documentation. The techniques performed in the laboratory are annually assessed by an external quality assessment organisation (Quality Control for Molecular Diagnostics) and audited by the French accreditation body (Comité français d'accréditation). One of the few indicators that did not reach 100% was the reporting of laboratory results on a monthly basis, which is done on an annual basis. Similarly, the indicator 'review the method and reference value annually by the head of the lab' did not reach 100%, which is sometimes done every other year. Overall, the laboratory audit results were very satisfactory, with an overall score of 98%. It would be advisable to increase the frequency of the review of methods, reference values and summary reports. Regarding the documentation, it was detected that uncontrolled copies are sometimes found in the lab and that the personnel training files sometimes are not up to date. The use of controlled copies, as well as more regular monitoring of staff training records, was also recommended.

Role: The fellow conducted the audit with Vinca Icard using all the questions in the Excel file created for the Biorisk and Quality Management Module exercise. The fellow compiled the results and submitted the audit excel form to the ECDC Virtual Academy (EVA) platform.

Educational outcome

The fellow became familiar with the quality control system, quality assurance and accreditation procedures used at the HCL by observing and conducting internal audits. He also had the opportunity to participate in an EQA on WGS-based analysis of AMR of *Salmonella* and *Campylobacter* conducted at ISCIII as part of the FWD Reference Laboratory Network. In summary, the fellow improved his quality management skills in a new environment (a clinical laboratory in the field of public health) and understood the relevance of good quality management in national public health laboratories and medical diagnostic laboratories for the health system.

5. Public health microbiology management

5.1. Management and leadership of projects

The fellow was the lead investigator for the main projects he was involved in at the HCL. He worked with different hospital services and research teams within and outside of the hospitals, sometimes acting as the liaison between stakeholders. Álvaro led projects in different domains and was responsible for carrying out the analytical work and communicating the results. The fellow worked on all stages of the projects, including: designing and writing project proposals and study protocols, submitting documentation to the ethics committee, sending informed consent to patients, collecting and analysing data, attending follow-up meetings with stakeholders on the results, and writing the final manuscript/report or disseminating the results at conferences.

5.2. Translation of a study protocol on the identification of prodromal clinical signs of menstrual staphylococcal toxic shock syndrome from French into English and Spanish

Supervisor: Gerard Lina

The fellow assisted in the translation of a study protocol from French into English and Spanish. The protocol was for an ongoing case-control study on the identification of prodromal clinical signs of menstrual staphylococcal toxic shock syndrome. The protocol was translated so it could be sent to other international groups to extend the study to other EU countries, thereby increasing the study's sample size, as this is rare but serious disease.

5.3. Provide technical support to the Virus Respiratoire Syncytial study group in Lyon

Supervisor: Jean-Sébastien Casalegno

The Virus Respiratoire Syncytial (VRS) working group was founded in Lyon in 2014. It is an independent, non-profit multidisciplinary task force composed of national experts and field experts. The group pursues an ambitious research agenda that includes epidemiology, virology, clinical practice and qualitative research. It aims to estimate

the burden of RSV disease in the population, determinate the main drivers of RSV dynamics and predict severe RSV disease in the neonatal population, in order to implement a cost-effective prevention program.

The fellow attended group meetings and participated in the discussion of the different projects carried out by the group, encouraging interaction with medical doctors from different medical specialties. Álvaro provided technical assistance and advice on the analysis of temporal and seasonal trends of RSV and the spatial distribution of RSV disease burden in the Lyon metropolitan area in children aged less than 2 years and adults aged more than 65 years.

Educational outcome

The fellow was able to expand his knowledge in strategic planning, working with multidisciplinary teams, time management and effective communication (e.g. presenting epidemiological information to physicians from different medical specialties). As the fellow was involved in all phases of project development, he also gained experience in the ethical aspects of data collection and data protection. Time management is one of the pillars that the fellow has reinforced during the fellowship, as it was the fellow who managed his projects and the time dedicated to each of them, also learning to prioritise and work with different projects and study groups in parallel.

6. Teaching and pedagogy

6.1. Lectures on RSV in infants: virus, disease, immune response and strategies for active and passive paediatric RSV immunisation, academic courses, 2022–2023 and 2023–2024

Álvaro participated in preparing the material for lectures entitled 'RSV in infants: virus, disease and immune response' and 'Strategies for active and passive paediatric RSV immunisation' for the Master's degree course 3I: Immunology, Immunotherapy, Immunopathology at the Université Claude Bernard Lyon 1, delivered in the 2022 and 2023 academic years. The lectures were delivered face-to-face in collaboration with one of the project supervisors (Jean-Sébastien Casalegno). The lectures were three-hours long and were given alternately by the two presenters. Specific objectives were set at the beginning of the lecture and evaluated at the end. The audience was asked questions during each section (using the SLIDO application) to ensure active participation and to see if they understood the main messages. The lecture was evaluated at the end and the feedback from the students was very positive. The fellow wrote a reflection note. The lectures held in 2023 incorporated the lessons learned from 2022.

Educational outcome

These activities provided the fellow with a valuable opportunity to develop his teaching skills and to further deepen his knowledge of a disease through the development of teaching materials. Álvaro learned new teaching methods, including rotating speakers to engage the audience and maintain attention during long lectures or using mobile app questionnaires to increase audience participation.

7. Communication

7.1 Publications related to the EPIET fellowship

7.1.1. Manuscripts published in peer-reviewed journals

1. **Roy Á**, Polazzi S, Ploin D, Gillet Y, Javouhey E, Lina B; VRS study group in Lyon, et al. The increasing age of respiratory syncytial virus-related hospitalisation during COVID-19 pandemic in Lyon was associated with reduced hospitalisation costs. *Vaccine*. 2023;41:3796-3800. Available at: <https://doi.org/10.1016/j.vaccine.2023.05.021> (*published*)
2. **Roy Á**, Saade C, Laurence L, Clément B, Morfin F, Destras G, Valette M, et al. Determinants of protection against SARS-CoV-2 Omicron BA.1 and Delta infections in fully vaccinated outpatients. *Journal of Medical Virology*. 2023;e28984. Available at: <https://doi.org/10.1002/jmv.28984> (*published*)
3. **Roy Á**, Green MT, Gil Cuesta J, Rakesh A, Hermans V, Antierens A, Sunyoto T. Patients' characteristics and clinical presentations of an outbreak of skin ulcers with unknown origin in children in Musinga province, Burundi, 2021–2022. (*Manuscript in preparation*)
4. **Roy Á**, Gaucher L, Lina B, Wallon M. Monitoring epidemiological changes for toxoplasmosis: a six-year longitudinal study in pregnant women in Lyon, France, 2017–2022. (*Manuscript in preparation*)
5. **Roy Á**, Semanas Q, Bal A, Josset L. Evaluation of different next-generation sequencing approaches for mpox virus whole genome characterisation. (*Manuscript in preparation*)

7.1.2 Other reports

6. Outbreak of carbapenem-resistant *Enterobacteriaceae* in two wards of the Hepatogastroenterology Unit in a hospital in Lyon, France, 2022. (*internal report*)
7. Resistant bacteria in patients with ventilator-associated pneumonia (VAP) in ICUs and association with influenza seasons in Lyon, France, 2004–2019. (*internal report*)
8. Limited stay on next-generation sequencing of food- and water-borne bacteria and noroviruses at the Instituto de Salud Carlos III, Madrid, Spain. (*internal report*)
9. External quality assessment for whole genome sequencing-based resistome profiling in antimicrobial-resistant *Salmonella* and *Campylobacter* (EQA2-WGS-AMR), Instituto de Salud Carlos III, Madrid, Spain, 2023. (*internal report*)

7.2 Conference presentations

10. **Roy Á**, Saade C, Josset L, Clément B, Lina B, Morfin F, Destras G, et al. Determinants of protection against SARS-CoV-2 Omicron BA.1 or Delta infections in vaccinated individuals. In ECCMID 2023, Copenhagen. 15–18 April 2023. Poster presentation.
11. **Roy Á**, Polazzi S, Ploin D, Gillet Y, Javouhey E, Lina B, Myard-Dury AF, et al. The increasing age of respiratory syncytial virus-related hospitalisation during COVID-19 pandemic in Lyon was associated with reduced hospitalisation costs. In ECCMID 2023, Copenhagen. 15–18 April 2023. Poster presentation.
12. **Roy Á**, Gaucher L, Lina B, Wallon M. Rapid decrease in the seroprevalence of toxoplasmosis: a six-year study in pregnant women in Lyon, France, 2017–2022. In ESCAIDE 2023, Barcelona. 22–24 November 2023. Poster presentation.

8. EPIET/EUPHEM modules attended

1. Introductory Course part 1, 20/9/2021–16/10/2021, online.
2. Inject days on Operational Research, 27/10/2021–28/10/2021, online.
3. Inject days on Data Collection and Management, 10/11/2021–11/11/2021, online.
4. Outbreak Investigation Module, 6/12/2021–10/12/2021, online.
5. Biorisk and Quality Management Module, 17/01/2022–18/01/2022, online.
6. Multivariable Analysis Module, 14/03/2022–18/03/2022, online.
7. Multivariable Analysis inject day – Cox regression and multilevel analysis, 30/03/2022, online.
8. Project Review Module, 20/04/2022–22/04/2022, Spetses, Greece.
9. Introductory Course part 2, 25/04/2022–29/04/2022, Spetses, Greece.
10. Rapid Assessment and Survey methods Module, 06/06/2022–10/06/2022, Stockholm, Sweden.
11. Project Review Module, 29/08/2022–02/09/2022, Lisbon, Portugal.
12. Time Series Analysis Module, 07/11/2022–11/11/2022, Utrecht, the Netherlands.
13. Qualitative Research Virtual Inject Days, 31/01/2023 and 03/02/2023, online.
14. Vaccinology Module, 13/02/2023–17/02/2023, online.
15. Management, Leadership and Communication in Public Health Module, 08/05/2023–12/05/2023, Stockholm, Sweden.
16. Project Review Module, 28/08/2023–01/09/2023, Lisbon, Portugal.

9. Other training

1. Public Health Preparedness for Mass Gathering Events, World Health Organization (WHO), 23/05/2022, online.
2. BSAFE, United Nations security awareness training, 26/05/2022, online.
3. Epidemic Intelligence e-learning course, ECDC, 27/05/2022, online.
4. Introduction to Git/Github, Liza Coyer, 21/10/2022, online.
5. EAN Mini-Module on Media and Infodemic Management, 21/11/2022–22/11/2021, Stockholm, Sweden.

6. Training on next-generation sequencing of food- and waterborne bacteria and noroviruses, 12/06/2023–23/06/2023, Limited stay at Instituto de Salud Carlos III, Madrid, Spain.
7. Internal seminars on infectious diseases given by the residents every Friday (Les colloques de l'IAI) at the IAI, HCL, Lyon, France.
8. WHO Public Health Laboratories knowledge sharing webinar series: Risk Communication for Laboratory Leaders (14/09/2022); SARS-CoV-2 variants circulation and examples of genomic surveillance strategies (12/10/2022); Epidemiology and diagnostic testing for Leptospirosis, an emerging public health problem (22/03/2023).
9. Seminar on 'Mpox whole genome sequencing – from Illumina reads to phylogenetic analysis using Galaxy.org', Christina Merakou, 28/07/2023, online.

10. Missions

On 25 January 2023, the fellow signed the data sharing agreement to collaborate remotely with MSF in an outbreak of skin ulcers with unknown origin in children in Muyinga province, Burundi, analysing data from April 2021 to the end of January 2022. A detailed description of this project can be found in section 1.1.2.

11. Other activities

11.1. Manuscript revision

Álvaro reviewed a manuscript on SARS-CoV-2 for *npj Vaccines - Nature*.

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