

The text "Summary of work activities" in a bold, white, sans-serif font, set against a blue background.The name "Adriana Cabal Rosel" in a white, sans-serif font, set against a blue background.The text "European Public Health Microbiology Training Programme (EUPHEM), 2017 cohort" in a white, sans-serif font, set against a blue background.The text "Background" in a bold, blue, sans-serif font.

According to the European Centre for Disease Prevention and Control (ECDC) Advisory Group on Public Health Microbiology ('national microbiology focal points'), public health microbiology is a cross-cutting area that spans the fields of human, animal, food, water, and environmental microbiology, with a focus on human population health and disease. Its primary function is to improve health in collaboration with other public health disciplines, in particular epidemiology. Public health microbiology laboratories play a central role in detection, monitoring, outbreak response and the provision of scientific evidence to prevent and control infectious diseases.

European preparedness for responding to new infectious disease threats requires a sustainable infrastructure capable of detecting, diagnosing, and controlling infectious disease problems, including the design of control strategies for the prevention and treatment of infections. A broad range of expertise, particularly in the fields of epidemiology and public health microbiology, is necessary to fulfil these requirements. Public health microbiology is required to provide access to experts in all relevant communicable diseases at the regional, national and international level in order to mount rapid responses to emerging health threats, plan appropriate prevention strategies, assess existing prevention disciplines, develop microbiological guidelines, evaluate/produce new diagnostic tools, arbitrate on risks from microbes or their products and provide pertinent information to policy makers from a microbiological perspective.

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.

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This report summarises the work activities undertaken by Adriana Cabal Rosel, cohort 2017 of the European Public Health Microbiology Training Programme (EUPHEM) at the Austrian Agency for Health and Food Safety.

All EUPHEM activities aim to address different aspects of public health microbiology and underline the various roles of public health laboratory scientists within public health systems.

## Pre-fellowship short biography

Adriana Cabal Rosel completed her bachelor in Veterinary science in Zaragoza, Spain, in 2009, and her passion for zoonoses and infectious diseases began after performing an internship in Lisbon on genotyping of *Mycobacterium bovis* strains. Right after, she worked in a University hospital in *M. tuberculosis* typing through SNP analysis while doing her Msc in molecular and cellular biology. Afterwards, she moved to Madrid to start her PhD on distribution, quantification and characterization of pathogenic *Escherichia coli* through a One Health approach, where she started in parallel a specialization course on Bioinformatics and Whole Genome Sequencing (WGS). She finished her PhD in 2016 and after working for few months as a genomics consultant, she moved to Vienna to join ECDC's fellowship programme in 2017, where she further developed her WGS-based typing skills in outbreak investigation and surveillance of communicable diseases.

## Methods

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

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

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

## Results

The objectives of these core competency domains were achieved partly through projects or activities (on-job services) and partly through participation in the training modules. Results are presented in accordance with the EUPHEM core competencies, as set out in the EUPHEM scientific guide<sup>1</sup>.

### 1. Epidemiological investigations

#### 1.1. Outbreak investigations

##### A. Listeriosis outbreak in Austria due to *L. monocytogenes* ST4-CT7652

Supervisors: Ariane Pietzka, Franz Allerberger, Werner Ruppitsch

By 21<sup>st</sup> December 2018, the local health authority in Styria, Austria, was informed about an outbreak of febrile gastroenteritis, including a culture-confirmed case of *L. monocytogenes* bacteremia, associated with the visit of a group of 32 people to a tavern (tavern A), in the province of Styria on 15<sup>th</sup> December 2018. In order to elucidate the outbreak source, stool samples were requested from visitors and staff; food and environmental specimens were collected at the tavern and at company X, a meat processor serving the tavern. Eleven stool samples and one blood culture originating from 19 cases, two samples of food collected at the tavern and a gully sample from company X, tested positive for the outbreak strain (serogroup IVb, multilocus sequence type ST4 and core genome multilocus sequence type CT7652). Active case finding identified two additional listeriosis cases unrelated to the tavern visit, suffering from fatal sepsis and endophthalmitis, respectively. Liver pâté from company X was identified as the highly likely source of the outbreak. Despite the outbreak occurred in a holiday period, the NRL acted promptly to control the listeriosis outbreak. Thanks to the available WGS data and its upload in Epidemic Intelligence Information System (EPIS), we could discard the presence of the outbreak strain in other countries, therefore confirming a single local source of the outbreak strain.

<sup>1</sup> European Centre for Disease Prevention and Control. European public health training programme. Stockholm: ECDC; 2017. Available from: <http://ecdc.europa.eu/en/publications/Publications/microbiology-public-health-training-programme.pdf>

The fellow was involved in the cgMLST analysis that allowed the confirmation of the presence of the outbreak strain in the case-patients, the food and gully samples from the incriminated company, analyzed the few descriptive data available from the cases and the company and drafted the manuscript that it has been published in Eurosurveillance.

## **B. Detection of a *Bordetella pertussis* outbreak through Whole Genome Sequencing, Austria, 2018-19**

Supervisors: Daniela Schmid, Werner Ruppitsch, Franz Allerberger, Markus Hell

*Bordetella pertussis* is the main bacterial species causing the respiratory disease known as whooping cough, reemerging worldwide during recent years. Evasion of the vaccine-induced immunity by strains that differ from the vaccine is a possible reason for its reemergence. The aim of the study was to investigate a possible explain the recent increase in the incidence of pertussis in Austria by comparison of the clinical strains from pertussis cases recovered through our newly implemented national pertussis isolate-based surveillance system consisting in 4 pilot laboratories (provinces A-D) among each other and with the vaccine strain in order to detect outbreaks and transmission chains through WGS-based typing. Isolates were whole genome (WG)-sequenced and typed using a new cgMLST scheme comprising 2.938 genes and a virulence gene (VGs) scheme encoding for vaccine targets including pertussis toxin (ptxP, ptxS1), pertactin (prn), filamentous hemagglutinin (fhaB) and fimbriae (fim2, fim3) genes. The sequence type (ST) was extracted from the WGS data and the genetic relatedness between the isolates assessed. We registered an incidence of 23.2 cases/100,000 population in Austria in 2018. The isolate-based surveillance system collected 75 *B. pertussis* isolates and 52 of those were from Province A. Overall, all the 75 isolates differed from the vaccine strain: all were ST2 and the closest match differed from 124 alleles. In addition, eight different genotypes were found based on the virulence genes variants and the STs. A group of isolates (n=20), which mostly belonged to Province A and carried the same frameshift deletion at the nucleotide 632 of the pertactin gene, seemed to cluster together. Interestingly, 15 out of those pertactin-deficient isolates were vaccinated and transmission was confirmed in three households. Our results show that the circulating strains in the Austrian province differed from the vaccine strain to detect *B. pertussis* transmission chains. To set up cgMLST cluster threshold, epidemiological data, virulence genes and cgMLST data are needed. Therefore, expansion of the surveillance system at national level is recommended to assess the impact of these strains on the vaccine effectiveness.

The fellow was involved all the 10-steps of the outbreak investigation. She the set-up of the whole surveillance system, the new cgMLST scheme, performed the microbiological analysis and analyzed the WGS data, communicated the results and drafted the manuscript. The article is being reviewed by the co-authors and its submission is planned this year 2019.

## **C. Outbreak of *Streptococcus pyogenes* in a maternal ward**

Supervisors: Werner Ruppitsch, Daniela Schmid, Markus Hell

In July 2018, an outbreak of postpartum *Streptococcus pyogenes* infections occurred among patients in the same obstetric ward of an Austrian hospital. The aim of the investigation was to identify the source and mode of transmission by descriptive epidemiology and Whole Genome Sequencing (WGS)-based typing. Four septic patients and the midwife attending their deliveries tested positive to the same *S. pyogenes* strain (0-4 alleles, ST52, emm28.0, RD2). The midwife had a paritium and while attending at the deliveries transmission of the infection could have occurred between patients. In order to prevent further infections, an intensified training programme in hand hygiene for health care workers, was immediately launched. This was the first time a nosocomial outbreak of *S. pyogenes*-caused puerperal sepsis was elucidated by use of WGS in Austria. The fellow was involved in the set-up of the new cgMLST scheme for *S. pyogenes*, the data analysis and drafted the manuscript. The article was published in Clinical Microbiology and Infection.

## **D. Hospital outbreak caused by linezolid resistant-*Enterococcus faecium* in Upper Austria**

Supervisors: Werner Ruppitsch, Heidrun Kerschner

*Enterococcus faecium* is part of the human gastrointestinal flora but may act as opportunistic pathogen. Environmental persistence, high colonization capability and diverse intrinsic and acquired resistance mechanisms make it especially successful in nosocomial high-risk settings. In March 2014, an outbreak of Linezolid-resistant *Enterococcus faecium* (LREfm) was observed at the haemato-oncology department of a tertiary care center in Upper Austria. We report on the outbreak investigation together with the whole genome sequencing (WGS)-based

typing results including also non-outbreak *LR<sub>Efm</sub>* and susceptible isolates. The 54 investigated isolates could be divided in six clusters based on cgMLST. Cluster one comprised *LR<sub>Efm</sub>* isolates of genotype ST117 and CT24, which was identified as the causative clone of the outbreak. In addition, the detection of four other clusters comprising isolates originating from haemato-oncology patients but also at other hospitals, pointed to *LR<sub>Efm</sub>* transmission between local healthcare facilities. *LR<sub>Efm</sub>* patients (n=36) were typically at risk for acquisition of nosocomial pathogens because of immunosuppression, frequent hospitalization and antibiotic therapies. Seven of these 36 patients developed *LR<sub>Efm</sub>* infection but were successfully treated. After termination of the initial outbreak, sporadic cases occurred despite a bundle of applied outbreak control interventions. WGS proved to be an effective tool to differentiate several *LR<sub>Efm</sub>* clusters in an outbreak. Active screening for *LR<sub>Efm</sub>* is important in a high-risk setting such as haemato-oncology, where multiple introductions are possible and occur despite intensified infection control measures. The fellow was involved in the all steps of the retrospective outbreak investigation that allowed to differentiate isolates obtained from patients at the same hospital that were part of the outbreak, from those that were not related. Also, the investigation identified a cluster that was not part of the outbreak, where inter-hospital spread was confirmed. A manuscript reporting these results was published in Antimicrobial Resistance and Infection Control.

## Training modules

At the Introductory course, the Outbreak module and the Multivariable analysis module fellows learned to describe the principles of outbreak investigation. In particular, they were taught on the steps of the investigation of an outbreak, including the development of case definitions, create questionnaires for data collection with Epidata software, generation and interpretation of epicurves, characterize cases by time, place and person, choosing the most suitable type of study according to the type of data and outbreak, calculate and interpret measures of association and formulate appropriate Public Health recommendations.

**Educational outcome:** Overall, the fellow participated in all steps of an outbreak investigation in collaboration with microbiologists, physicians and epidemiologists.

She applied microbiological techniques and the epidemiological knowledge acquired mainly at the Introductory course and at the Outbreak investigation module. She contributed to the generation of new questionnaires and the update of referral forms for data collection, to case-finding, conducted epidemiological analyses, communicated her results to primary laboratories and at scientific conferences, co-authored scientific articles and provided recommendations.

## 1.2. Surveillance

Supervisors: Werner Ruppitsch, Daniela Schmid

### A) Surveillance of *L. monocytogenes* by WGS-based typing of human and non-human isolates in Austria, 2017

*Listeria monocytogenes* (*L. monocytogenes*) is a ubiquitous bacterium, that can cause, usually in older age groups and immunocompromised persons invasive Listeriosis. From 2017 onwards, in Austria, any isolate of *L. monocytogenes* recovered from human and non-human samples undergoes whole genome sequencing (WGS) and core genome multilocus sequence typing (cgMLST) for cluster detection at the national reference laboratory (NRL). The aim of this study was to assess the usefulness of a WGS-based surveillance of *L. monocytogenes* in identifying the most likely sources of invasive Listeriosis in Austria and in detecting associations between sequence types (STs), extracted from the WGS data, and food matrices. In 2017, WGS data were available from 31 human and 1744 non-human isolates. A molecular cluster was defined as two or more isolates different from each other less than 10 alleles. An outbreak-cluster was defined as a cluster including human isolates from at least two patients, in addition to non-human isolates. We tested food matrices for associations with the ten most prevalent *L. monocytogenes* STs through calculating prevalence ratios (PR) with 95% confidence intervals (CI). A total of 1472 isolates were allocated to 132 clusters, including two outbreak-clusters, four single case-clusters and 126 clusters comprising non-human isolates. Out of the six clusters with human isolates, one could be traced back to an Austrian meat producing company, two to Austrian dairy producing companies and two further clusters to frozen vegetable producing companies, one based in Belgium, the other in Austria. We found ST21 associated with vegetables, ST121 and ST155 with fish and ST511, ST7 and ST451 associated with dairy products. The WGS-based surveillance of human and non-human isolates reliably and promptly identify the most likely origin of the clusters including human isolates. The routine WGS-based ST determination of non-human isolates facilitate the generation of hypothesis on the sources of invasive listeriosis.

The fellow participated in the improvement of the current referral form that is given to the food companies to collect data on the origin of *L. monocytogenes* isolates, curated the 2017 database containing more than 2000 isolates, analysed the genetic relatedness between human and non-human isolates to detect clusters and collaborated in the trace-back analysis of *L. monocytogenes* infection sources. Public Health recommendations were given to improve the surveillance system. In addition, the fellow wrote a manuscript as first author on the isolate-based surveillance of *L. monocytogenes* through WGS in Austria, which has been published in *Frontiers in Microbiology*.

## B) Mosquito screening for surveillance of pathogens at the point of entry: Vienna International Airport, 2018

Supervisors: Werner Ruppitsch, Karin Bakran-Lebl, Franz Allerberger, Peter Hufnagl

Invasive mosquito species have spread massively in Europe in recent years, which has been in part facilitated by climate change and globalization, thus giving mosquitoes the potential of acting as competent vectors of subtropical diseases. Surveillance data obtained in recent years indicates that exotic mosquito species are already present in Austria. The aim of the present project was to set up a surveillance system for invasive mosquito species at Vienna international airport in order to 1) comply with the International Health Regulations in terms of vector surveillance and control at airports 2) to investigate by molecular techniques such as RT-PCR, if airport mosquitos are carriers of exotic and/or autochthonous pathogens. Adult and egg traps were set up in June 2018, checked up in weekly intervals up to 18 times and finally removed in October 2018. The adult trap caught 2.072 adult mosquitoes. The largest proportion of mosquitoes belonged to the *C. pipiens* group (n=1.635, 93.5%), matching with the findings reported by Austria previously. 265 mosquito pools were created and further investigated for the presence of the exotic viruses. 14 mosquito pools yielded Usutu virus (USUV) cluster Europe 2 and 14 pools yielded West Nile virus (WNV) Lineage 2 with different genetic variants. Our findings are in accordance to those published by other groups and despite the number of infected individuals per 1000 mosquitoes examined was high (13.6), these results have to be carefully interpreted since the high number of positive mosquito pools found in our study suggests that vertical transmission (adults to eggs) is occurring and therefore the sampled mosquito fraction, mainly formed by *Culex spp.* was always part of the same mosquito population. Due to the recent autochthonous European cases of dengue fever and chikungunya virus infection and outbreaks of malaria, a continuous vector surveillance system that extends beyond a 2-year monitoring programme is advised, to track the transmission of these pathogens.

The outcome of the project was a surveillance report describing the set up of the surveillance system and main findings. The fellow participated in the field investigations, data collection and analysis of the surveillance data.

### Training modules

The EPIET/EUPHEM introductory course familiarized participants with the development, evaluation and analysis of surveillance systems.

**Educational outcome:** the fellow became familiar with the listeriosis surveillance system and analyzed the available WGS data to find new sources of infection

## 2. Applied public health microbiology research

Supervisors: Daniela Schmid, Werner Ruppitsch, Markus Hell

### A. An isolate-based surveillance system for *Bordetella pertussis* in Austria, 2018-2019

*Bordetella pertussis* is the main bacterial species causing the respiratory disease known as *whooping cough*, reemerging worldwide during recent years, since the introduction of the acellular vaccines in the 90's. Evasion of the vaccine-induced immunity by strains that differ from the vaccine is one of the reasons for its reemergence. The aim of the study was to set up an isolate-based surveillance system in to characterize and compare the circulating strains in Austria. In order to do so, the first objective was to set up the isolate-based

surveillance system and to develop a new core genome Multilocus Sequence Typing scheme (cgMLST) for this bacterium. From March 2018 onwards, hospitals, general practitioners and pediatricians servicing four pilot laboratories located in the Austrian provinces A, B, C and D were asked to collect two nasopharyngeal swabs (one for culture and one for PCR testing) from *B. pertussis* suspected cases at the catarrhal phase of the disease. Both were sent to pilot laboratories. When the PCR tests positive for *B. pertussis*, the other swab will be used for culturing, the isolate sent to the NRL for further DNA extraction and Whole Genome Sequencing. In addition, we will create an *ad hoc* cgMLST scheme using Ridom GmbH SeqSphere software using all complete genomes available at NCBI and the Tohama I vaccine strain as a seed genome. Validation of the scheme will be done by adding in a comparison table clinical strains from past outbreaks or clinical cases from and outside of Austria. We included several *Bordetella* species other than *B. pertussis*. Afterwards, all isolates collected within the surveillance system from May 2018 until August 2019 were typed with our newly implemented cgMLST and the genetic relatedness between the isolates assessed by generating a minimum spanning tree (MST). A cluster threshold of 6 allelic differences was defined. In addition, we extracted the classical MLST sequence types (STs) and the vaccine targets (virulence genes including pertussis toxin (*ptxP*, *ptxS1*), pertactin (*prn*), filamentous hemagglutinin (*fhaB*) and fimbriae (*fim2*, *fim3*) genes. We identified cgMLST clusters and transmission chains, when the epidata was available. WGS data on our isolates was compared with WGS data from *B. pertussis* strains recovered in different geographical regions and time periods. Results indicated that the clinical circulating strains in Austria differed from the vaccine strain in their MLST, cgMLST (120 alleles) and in the vaccine target genes. These data will be used to assess the impact on vaccine efficacy and to elaborate recommendation regarding the national vaccination calendar, if needed. The surveillance of the strains will be extended to other Austrian provinces.

The outcome of this project was a research protocol. The fellow coordinated the whole surveillance system including the set up, patient's data collection, laboratory analysis and epidemiological analysis. It is planned to continue after the fellowship, where the fellow will be also involved.

## Training modules

The fellow was trained at the EPIET/EUPHEM introductory course on how to develop a study protocol, how to present the results in an effective manner for drafting a manuscript afterwards.

**Educational outcome:** Development, validation and implementation of a new isolate-based surveillance system for pertussis, which include sampling design, core genome MLST scheme design, epidemiological and WGS data analysis. The fellow learnt to understand the limitations of the epidemiological data. She had several scientific presentations at several national and international conferences with different types of audiences. She is now writing a scientific article derived from the results of this Research project.

## 3. Applied public health microbiology and laboratory investigations

### A. Retrospective genomic characterization of linezolid-resistant *Enterococci*, 2012-2018

Supervisors: Werner Ruppitsch, Heidrun Kerschner

*Enterococcus faecium* is part of the human gastrointestinal flora but may act as opportunistic pathogen. Environmental persistence, high colonization capability and diverse intrinsic and acquired resistance mechanisms make it especially successful in nosocomial high-risk settings. In March 2014, an outbreak of Linezolid resistant *Enterococcus faecium* (LREfm) was observed at the hematooncology department of a tertiary care center in Upper Austria. We report on the outbreak investigation together with the whole genome sequencing (WGS)-based typing results including also non-outbreak LREfm and susceptible isolates. The 54 investigated isolates could be divided in six clusters based on cgMLST. Cluster one comprised LREfm isolates of genotype ST117 and CT24, which was identified as the causative clone of the outbreak. In addition, the detection of four other clusters comprising isolates originating from haemato-oncology patients but also at other hospitals, pointed to LREfm transmission between local healthcare facilities. LREfm patients (n=36) were typically at risk for acquisition of nosocomial pathogens because of immunosuppression, frequent hospitalization and antibiotic therapies. Seven of these 36 patients developed LREfm infection but were successfully treated. After termination of the initial outbreak, sporadic cases occurred despite a bundle of applied outbreak control interventions. WGS proved to be an effective tool to differentiate several LREfm clusters in an outbreak. Active screening for LREfm is important in a high-risk

setting such as haemato-oncology, where multiple introductions are possible and occur despite intensified infection control measures.

The fellow was the main responsible person together with another clinician for interpreting and analysing the epidemiological data, perform the genomic analysis and drafting the manuscript. The outcome of this competence is a published manuscript in Antimicrobial Resistance and Infection Control describing the retrospective characterization of all isolates and the outbreak.

## Training modules

The outbreak module included a section on phylogenetics showed the basics on how interpret phylogenetic trees in outbreak investigation. Also, a summary on microbiological techniques and when to use each of them was given to the fellows.

### Educational outcome:

The fellow applied concepts of bacteriology and immunology to the public health disciplines, she understood the use and limitation of diagnostic and typing methods and their interpretation; she gained more expertise in analysing WGS data and interpreting them.

## 4. Biorisk management

### A. Elaboration of an Instruction on how to work under BSL-2 conditions with *Candida auris*

Supervisors: Emilia Mellado, María José Buitrago

*Candida auris* is a nosocomial pathogenic fungus that can cause invasive infections. It is multidrug resistant (MDR) to the conventional antifungals used to treat fungal infections (Kean *et al.*, 2018). It can persist for prolonged periods in the environment and conventional disinfectants used for decontamination of surfaces (quaternary ammonium) do not eliminate it. Its manipulation is recommended *C. auris* in BSL-2 facilities although some prefer to handle it in BSL-2 facilities but applying BSL-3 containment procedures. The aim of this activity was to create an Instruction on how to work with *C. auris* in BSL-2 facilities that will be useful for the laboratory staff working at the Mycology Reference Laboratory at the Carlos III Health Institute, Madrid. This assignment was performed thanks to an ESCMID-funded Observership. The instruction describes the safety guidelines to be followed by the laboratory staff at the MRL when working with *C. auris*. It describes the personal protective equipment and the environmental disinfection guidelines to be followed and it is associated with the following internal Standard Operating Procedures (SOPs). We successfully created an instruction on how to work with *C. auris* in BSL-2 facilities, adapted for the MRL that will protect laboratory staff when dealing with this pathogen, thus avoiding its spread and minimizing the risk of infection for them.

### B. Training at the BSL-3: *Mycobacterium spp.* diagnostics workflow

During the 19.12.2018 the fellow received a one-hour demonstration at the BSL-3 about how *M. tuberculosis* samples are handled. In particular, she learnt about the workflow that is followed when suspicious samples of *Mycobacterium spp.* arrive at the laboratory. First, samples are cultured in three different media according to the AGES quality management system. Those that are positive enter into the BSL-3 for further species identification, resistance pattern and genetic characterization. There is an autoclave for the entry and exit of biological material and an entry/exit of personnel.

## Training modules

At the Biorisk Module the fellow learnt about the different techniques for biorisk assessment and mitigation, including the WHO recommendations on biosafety management in laboratories, international shipping and international regulations for the transportation of infectious substances and dangerous goods, a visit to the BSL-4 facility at the Public Health Agency of Sweden.

### **Educational outcome:**

To gain knowledge on the different biosafety levels and when to use which, depending of the pathogen. To understand the different personal protective equipment that is used at the BSL3 and BSL4 laboratories, the principles of biorisk management, its assessment and mitigation, and the international regulations for shipment of dangerous goods.

## 5. Quality management

### **A. Quality management module homework: laboratory audit**

Supervisor: Jutta Ressler

On the 3<sup>rd</sup> of May 2018, the fellow Adriana Cabal and the Quality Manager Jutta Ressler performed an audit on the process management, quality control and the documentation available at the IMED-WIEN laboratory (AGES Institut für Medizinische Mikrobiologie und Hygiene, Währinger strasse 25, 1090, Wien). Overall, it was concluded that the laboratory operated in a satisfactory manner, since the general indicator was equal to 99%. However, the evaluation showed that the laboratory could improve in some specific areas. For instance, the guidelines describing the biosafety procedures could be as part of a unique document compiling all of them in order to be more practical; the time of sample collection, time of sample receipt and time of release of the report by the laboratory were not available; usage of log sheets could be introduced in which each member of the staff can record its usage for other equipment such as PCR thermocyclers. In any case, it was stated that all planned actions should be followed up until completed.

The outcome was a report on the laboratory audit.

### **B. Audit on point number 6 of the ISO 17025:2017**

Supervisor: Jutta Ressler

On the 2<sup>nd</sup> of May 2019, the fellow Adriana Cabal and the Quality Manager of the Division for Public Health of AGES, located in Vienna, Austria, Jutta Ressler, conducted together an internal audit on the point 6 of the ISO/IEC 17025:2017. Subsections 6.1 to 6.6 were examined. Opportunities for improvement (OFI) as well as non-conformities were found in subsection 6.3 of the norm describing facilities and environmental conditions. Corrective actions were put in place for the non-conformities as well as recommendations.

The outcome was a report on the laboratory audit.

### **C. Quality assessment seminar for EUPHEM cohorts 2016 and 2017**

Supervisor: Aftab Jasir

The fellow prepared a seminar on Quality Audits for the Biorisk Module adapting the material provided by the World Health Organization. The lecture focused on the guidelines to be followed when preparing external audit, on how to plan and manage an internal audit, how to discuss the results from an audit and the importance of the corrective actions.

## Training modules

The fellow received training on quality management applied to laboratories and on ISO norms. She learnt to conduct an internal audit and understood the importance of working with validated techniques, SOPs and other documents part of the quality management system of the laboratory.



**Educational outcome:** She became acquainted with the concepts of quality assurance, external quality assessments, accreditation and audits. She saw the relationship between quality management and biorisk management.

## 6. Teaching and pedagogy

### A. Quality assessment seminar for EUPHEM cohorts 2016 and 2017

Supervisor: Aftab Jasir

The fellow prepared a seminar on Quality Audits for the Biorisk Module adapting the material provided by the World Health Organization. The lecture focused on the guidelines to be followed when preparing external audit, on how to plan and manage an internal audit, how to discuss the results from an audit and the importance of the corrective actions.

### B. 7<sup>th</sup> NGS workshop 8<sup>th</sup> NGS workshop

Supervisor: Werner Ruppitsch, Ariane Pieztko

The fellow facilitated at the 7<sup>th</sup> and 8<sup>th</sup> Next-Generation Sequencing workshops celebrated in Vienna, Austria, together with other experts in NGS. Both editions were hands-on for all participants. At the 7<sup>th</sup> edition, the genomic analyses were based on *Neisseria gonorrhoeae* and *Clostridium difficile* while at the 8<sup>th</sup> edition, those were about *Mycobacterium tuberculosis* and Shigatoxin-producing *Escherichia coli*.

### C. Teaching assignment: methods to detect carbapenemases

Supervisor: Aftab Jasir

The fellow organized a seminar on methods to detect carbapenemases together with other fellows at the Introductory Course. Each of them was responsible for a subsection on the mentioned topic. This included search on the literature for information, getting acquainted with the available techniques for carbapenemase detection and summarize the results found in a concise, clear and in an effective manner, so that the audience could follow.

### D. Vaccinology seminar at PRM 2019

At the PRM 2019, some fellows prepared seminars on vaccinology. Topics covered included principles of vaccinology, molecular and serological methods that are used, causes of vaccine failure, strain variation, etc. Case-studies were organized by the speakers.

The fellow prepared a reflective note on the teaching assignment. The topic of the assignment was: "Challenges among vaccinated populations: emergence of strains that differ from the vaccine. Example: pertussis" and it was delivered at PRM 2019 (August 26-30-2019).

## Training modules

The fellow learnt at the introductory course about Problem Based Learning (PBL) and the different teaching techniques, and how important it is to consider the audience.

### **Educational outcome:**

To identify the training needs of audiences with different backgrounds, to plan and organize a seminar and workshops, to prepare teaching material and case studies, to give lectures to persons from diverse backgrounds, and to evaluate a course.

## 7. Public health microbiology management

### A. Public Health management of pertussis and other projects/activities

The public health microbiology management competence was part of all projects carried out during the fellowship. The fellow communicated with high authorities (ECDC, Local Public Health Authority, etc.) in diverse situations during the Fellowship. For instance, at ECDC when giving a talk on the implementation of WGS in Austria or when requesting information on cases in outbreak investigations. She collaborated with multidisciplinary teams, coordinated all communications with the primary laboratories and lead the isolate-based surveillance system for *Bordetella pertussis* in Austria. She acted as the main link between epidemiologists and clinicians, providing results promptly and facilitating the exchange of information. Also, she communicated her public health findings related to her projects at different conferences. She has learnt to identify and apply best laboratory techniques for *B. pertussis* culture, to carry out and plan a study following ethical principles, to set up an isolate-based surveillance system for *B. pertussis* and to collect and analyze its data. Also, she was involved in the develop of partnerships between AGES and the primary laboratories to support epidemiological and microbiological investigations. She handled all communications during outbreak investigations (*B. pertussis*, *L. monocytogenes*, *S. pyogenes* and *E. faecium* outbreaks) with the primary laboratories or other stakeholders. In addition, she helped to improve the referral form sent to food providers for *L. monocytogenes* isolate data collection

#### Training modules

The fellow completed a one-week module on Leadership, Management and Communication in Public Health as part of the Fellowship programme where she learnt about Rapid Risk Assessment related to a Plague outbreak in Madagascar and how to take decisions in emergency situations, communicated with High Authorities in regards the implementation of WGS in Austria, received and gave constructive feedback, gave recommendations, learnt about team building and negotiation, ethics and integrity and learnt to provide scientific evidence to generate control measures.

#### Educational outcome:

To be able to work in multidisciplinary teams, to plan and organize research projects, to understand the different team roles, to motivate the team, to solve conflicts, to give feedback and to communicate with the media.

## 8. Communication

### Publications

**Adriana Cabal**, Franz Allerberger, Steliana Huhulescu, Christian Kornschöber, Burkhard Springer, Claudia Schlagenhaufen et al. Listeriosis outbreak in Austria due to contaminated pâté consumed in a tavern. *Eurosurveillance*, 24, (2019), <https://doi.org/10.2807/1560-7917.ES.2019.24.39.1900274>.

**Adriana Cabal**, Ariane Pietzka, Steliana Huhulescu, Franz Allerberger, Werner Ruppitsch, Daniela Schmid. Isolate-based surveillance of *Listeria monocytogenes* by Whole Genome Sequencing in Austria. *Frontiers in Microbiology* (2019), 10:2282. <https://doi.org/10.3389/fmicb.2019.02282>

Heidrun Kerschner\*, **Adriana Cabal**\*, Rainer Hartl, Werner Ruppitsch, Sigrid Machherndl-Spandl, Franz Allerberger et al. Hospital outbreak caused by linezolid resistant *Enterococcus faecium* in Upper Austria. *Antimicrobial resistance and infection control* (2019), 8:150. <https://doi.org/10.1186/s13756-019-0598-z>.

#### \*Equal contribution

**Cabal, A**, Schmid, D, Lepuschitz, A, Stöger, A, Blaschitz, M, Allerberger, F, et al. Nosocomial outbreak of group A *Streptococcus* puerperal sepsis. *Clinical Microbiology and Infection* (2018), 25: 521 – 523. <https://doi.org/10.1016/j.cmi.2018.11.028>.

Pietzka, A., F. Allerberger, A. Murer, A. Lennkh, A. Stöger, **A. Cabal Rosel**, et al. Whole Genome Sequencing Based Surveillance of *L. monocytogenes* for Early Detection and Investigations of Listeriosis Outbreaks. *Frontiers in Public Health* (2019), 7:139.

Hirk S, Lepuschitz S, **Cabal Rosel A**, Huhulescu S, Blaschitz M, Stöger A, et al. Draft genome sequences of interpatient and inpatient epidemiologically linked *Neisseria gonorrhoeae* isolates. *Genome Announcements* (2018), 6:e00319-18. <https://doi.org/10.1128/genomeA.00319-18>.

**In preparation:**

**Adriana Cabal**, Daniela Schmid, Markus Hell, Elisabeth M.Korninger, Johannes Möst, Franz Allerberger, Werner Ruppitsch. Genomic surveillance of *Bordetella pertussis* in Austria (in preparation).

Heidrun Kerschner\*, **Adriana Cabal Rosel\***, Rainer Hartl, Werner Ruppitsch, Franz Allerberger, Petra Apfalter. First report of *optxA* mediated oxazolidinone resistance in clinical *Enterococcus faecalis* isolates in Upper Austria (in preparation). \*Equal contribution

**Conference presentations**

Abstract accepted as poster at ESCAIDE 2019: An isolate-based surveillance system for *Bordetella pertussis* in an Austrian province, 2018-2019.

Oral presentation at DOSCH Symposium, *Pertussis, reemerging disease*, 13<sup>th</sup> May, Velden, Austria.

Oral presentation at the 13<sup>th</sup> OEGIT conference (Austrian Congress of Infection). An isolate-based surveillance system for *Bordetella pertussis* in Austria, 2018-2019, Saalfelden, Austria (2<sup>nd</sup> price for the best oral presentation).

E-poster presentation at ESCAIDE 2018, St Julian's, Malta. The benefit of WGS-based typing for Public Health Microbiology: retrospective analysis of human and food-associated isolates in Austria, 2017.

Oral presentation on "WGS-based surveillance of *B. pertussis* in Austria, 2018" at EUPert strain genomics, Copenhagen, Denmark, 13/09/2018

Oral presentation National on WGS-based characterization of *Bordetella pertussis* isolates obtained within a newly established isolate-based sentinel surveillance system for *B. pertussis* in Austria, 2018, Congress for Preventive Medicine and Hygiene, Graz, Austria, 05/06/2018.

Mini oral e-poster presentation at ECCMID on "Whole-genome sequencing based characterization of clinical *L. monocytogenes* isolates from Austria, 2010-2017 ", Madrid, Spain, 23/04/2018.

Oral presentation to ECDC Director, Chief Scientist, Chief Microbiologist and Head of Preparedness on WGS integration in Austria, ECDC, Stockholm, Sweden, 16/02/2018.

**Other**

1. Elaboration of a questionnaire for severe Influenza surveillance in EpiData.
2. Characterization through WGS-based typing *E. coli*, *K. pneumoniae*, *Enterobacter spp.* and *S. epidermidis* isolates to detect colistin-resistant genes and or linezolid-resistant genes.

**9. EPIET/EUPHEM modules attended**

1. Introductory Course, Spetses, Greece
2. Outbreak Investigation module, Berlin, Germany
3. Biorisk and Quality Management module, Stockholm, Sweden
4. Leadership and Communication Public Health Microbiology, Stockholm, Sweden
5. Multivariable Analysis, Nicosia, Cyprus
6. Rapid Assessment and survey methods, Athens, Greece
7. Project Review I, Lisbon, Portugal
8. Project Review II Module, Prague, Czech Republic

## 10. Other training

1. ESCMID Observership at the National Mycology laboratory, Health Institute Carlos III, Madrid, Spain (25/02-01/03/2019). The Observership was carried out with the aim of covering the Biorisk Management competence. The supervisors at the Centre were Emilia Mellado and María José Buitrago.
2. One-week training at Innsbruck Medical University on fungal diagnostics, Innsbruck, Austria (23/06-27/06/2019).

The training was carried out under the supervision of Dorothea Orth and the bacteriology team. It included training on direct and indirect detection methods for yeasts and micellar fungi. The fellow generated a small summary on the activities carried out during the training.

3. UNDSS online courses on Basic and Advance Security in the field I (07/05/2018).

## Discussion

### Coordinator's conclusions

As a fellow, Dr Adriana Cabal Rosel embodied the microbiology professional for whom the EUPHEM fellowship was created. Her solid scientific basis, her ability to transfer technology in particular with regards to Whole Genome Sequencing or capacity development at the hosting site, and her enthusiasm for disseminating the newly acquired knowledge are truly inspiring. Adriana has shown great ability in project leadership and competency in adapting to cultural diversity being able to bring unity to the team to achieve the best public health outcomes. Testimony to her abilities and to the excellent mentorship received from Dr Werner Ruppitsch and the site supervisory team at AGES are the numerous outputs delivered. She not only won awards for the excellent quality of her work, she was also awarded an ESCMID observership in Mycology at Instituto de Salud Carlos III in Madrid, Spain. In addition, she visited the Innsbruck laboratories as part of her training. With such variety of projects, activities and success, it has been therefore a pleasure to be Adriana's front-line coordinator over the past two years. The leadership skills developed through project management will be an asset wherever her career takes her, and I hope she will be actively involved as a fellowship alumna.

### Supervisor's conclusions

I am delighted to summarize our experiences with our first EUPHEM fellow Adriana Cabal Rosel. Adriana is among the best students/ young scientists I have ever instructed – so the selection process done by ECDC colleagues was great! In general, Adriana really impressed with her professionalism which include her broad scientific interest, her accurate, straightforward and rapid work style. These attributes allowed her to successfully complete this really challenging fellowship program. Adriana's project results represent significant contributions and improvements concerning our work as a public health institute:

Adriana's project on pertussis was the start for an isolate based *Bordetella pertussis* surveillance system in Austria and Adriana's successful work was the basis that our institute will become a national reference lab for pertussis. Her pertussis project was awarded with the second prize from the Austrian Society for Infection Control in 2019. A manuscript with Adriana being the first author is in preparation. Adriana's very detailed analysis of the Austrian Listeria WGS typing system provide useful recommendations for improvements of our system. The manuscript describing this work with Adriana as a first author has been published in Frontiers Microbiology. Adriana's involvement and contributions to the work of the institute resulted in two co-authorships (Frontiers Public Health, Genome Announcements). The investigation of two hospital outbreaks and one Listeria monocytogenes outbreak in the Austrian province Styria resulted in another three first authorships (published in CMI, Antimicrobial Resistance & Infection Control and EuroSurveillance). Adriana's work is an important contribution that help us to consolidate our institute as the leading center for outbreak investigation using WGS based tools in Austria. On an international level, Adriana is still involved in the genomic surveillance of *Bordetella pertussis* in Austria and in the development of a global WGS based typing scheme for *Neisseria gonorrhoeae* (together with colleagues from Germany, Denmark and under supervision of Prof. Magnus Unemo).

In conclusion, despite some problems as a small institute to fulfil all requirements, our participation in the EUPHEM fellowship program had a huge benefit for us. Adriana is a very enjoyable and outstanding colleague and I wish her the very best for all future challenges.

## Personal conclusions of fellow

The EUPHEM programme has provided me with the opportunity of applying my previous knowledge on traditional and molecular microbiology on outbreak and surveillance settings but also to gain new experience in Public Health Microbiology and Epidemiology. I had projects in different groups of disease, which make the daily work particularly interesting and dynamic. The main advantage of the EUPHEM programme over the EPIET one is the diversity of projects and activities to carry out, the additional knowledge gained at the training modules on epidemiology and Public Health Management and communication, which require a combination of skills acquired through the 2-years-fellowship. In addition, I have gained more experience in scientific communication, networking and in collaborating with multidisciplinary teams and I have built a strong network with other fellows, facilitators and supervisors within the EU that will benefit me and others in the future through collaborations and cascading of knowledge.

## Acknowledgements of fellow

In the first place, I would like to thank my EUPHEM supervisor on site, Werner Ruppitsch (AGES, Vienna) for his supervision, scientific support, flexibility and optimism but also for the freedom he provided me with during all the projects in which we worked together. Despite the challenges encountered, we always found a way to succeed. Secondly, I would like to thank my co-supervisor, Daniela Schmid (AGES, Vienna), for acting as a supervisor and all the scientific input provided, which has significantly improved my outcomes. To Franz Allerberger (AGES, Vienna), for his excellent scientific support and for his ability of solving problems when they aroused. In my department, I would like to thank Sarah Lepuschitz and Anna Stöger for their laboratory support, in particular in Whole Genome sequencing, and coaching. In the epidemiology department, I would like to thank my colleagues Lukas and Ziad but specially Ali, for their support and all good moments we shared. Also, I would like to thank other project supervisors such as Heidrun Kerschner for their scientific support and AGES bacteriology lab staff including Petra, Ernst and Silke. In AGES Graz, I would like to thank Ariane Pietzka for her scientific support and constant availability in the most urgent situations. Likewise, I would like to thank all primary laboratories that participated in the Pertussis project, especially Markus Hell, Johannes Möst and Eva Leitner. At the Veterinary Faculty, I would like to thank the virology lab, who helped me with the mosquito screening and in particular Jolanta and Karin.

I would particularly like to thank the EUPHEM Head Scientific Coordinator Aftab Jasir and my Frontline coordinator, Aura Andreasen, as well as all other coordinators, both EUPHEM and EPIET, and administrative staff at ECDC for all their support and constant efforts to improve the programme. Also, I would like to thank Emilia Mellado, María José Buitrago and Ana Alastruey, from the Carlos III Health Institute, who hosted me for carrying out my Observership visit. Similarly, I would like to thank the Mycology department at the Medical University of Innsbruck, in particular, Dorothea Orth and her team, for showing me all molecular techniques related to fungal infections.

Last, but not least, I would specially like to thank my cohort 2017 for the support provided and all the nice experiences we have lived together. I hope we continue these nice relationships in the future.