Summary of work activities
Fátima Amaro
European Public Health Microbiology Training Programme (EUPHEM), 2015 cohort

Background

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.

This report summarises the work activities undertaken by Fátima, cohort 2015 of the European Public Health Microbiology Training Programme (EUPHEM) at the Centro Nacional de Microbiología, Instituto de Salud Carlos III (CNM-ISCIII), Majadahonda, Madrid, Spain.

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

Fátima Amaro is a Portuguese microbiologist specialised in vector-borne diseases. Her last years of investigation at the Centre for Vectors and Infectious Diseases from The National Health Institute in Portugal were dedicated to emerging vector-borne viruses and focused not only in human serological and molecular diagnostics but also in studies on vectors and reservoirs. Her PhD work was aimed at clarifying the relevance of phleboviruses in public health in Portugal. Fátima has also a strong background in ecology due to her Master in nature's conservation and management and before applying to EUPHEM she finished a post-doc within the project "New arboviruses isolated in Portugal. Risk assessment and public health application". She applied to the EUPHEM in order to increase her overall knowledge in infectious diseases, to widen her microbiology competencies and to be able to integrate the previous and the acquired knowledge to solve Public Health Microbiology problems.

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[1].

1. Epidemiological investigations

1.1. Outbreak investigations

A. An outbreak of *Candida auris* at La Fe hospital, Valencia, Spain, 2016-2017

Supervisors: Ana Alastruey and Laura Herrera

Candida auris is an ascomycetous yeast and an emergent pathogen which can cause fungemia and other deep seated infections in at risk populations including the critically ill and cancer patients exposed to broad spectrum antibiotics and invasive medical procedures. It is difficult to treat since it is resistant to the first-line antifungal fluconazole and exhibits variable susceptibility to other azoles, amphotericin B and echinocandins. *C. auris* has been reported in four continents and the first *C. auris* outbreak in a European hospital took place in a specialist centre for cardio-thoracic surgery hospital in London in 2016. In April 2016, an outbreak alert of *C. auris* occurring at the intensive care unit (SICU) of La Fe hospital, Valencia, in Spain, was raised. Immediate measures were taken but in January 2017 the hospital was still facing the outbreak. The fellow was invited to perform the descriptive

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[1] 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

epidemiological analysis of the outbreak and to participate in the process of controlling it. In this way, Fátima, together with an epidemiologist from the Instituto de Salud Carlos III (ISCIII), performed an onsite visit to get familiar with the outbreak situation, examined the prevention and control measures that had been taken so far and elaborated the descriptive analysis. Before and after the onsite visit, the fellow participated in teleconferences with the hospital staff, as well as with the various institutions involved in this investigation such as ISCIII, Public Health London, Centers for Disease Control and Prevention (CDC) and European Centre for Disease Prevention and Control (ECDC). The fellow helped in the design of the data base by choosing and describing the variables related to demographics, clinical and epidemiological data of the patients, including age, gender, underlying condition, previous exposition to antibiotics and antifungals and invasive medical procedures undertaken by the patients. A case was defined as an individual with *C. auris* isolation from a blood sample, at La Fe hospital, Valencia, Spain, from April 2016 to February 2017. A total of 41 cases occurred between April 2016 and February 2017. Males represented 63.4% of the cases (n=26) and were more likely to suffer candidemia (p=0.001, CI 95%). Seventeen of the total cases (41.5%) were older than 65 years and the median age was 60 (range 21-81 years). During that period, *C. auris* was also found in environmental samples and in 73 colonised patients who did not develop candidemia. Regarding risks factors, 100% of the patients have had previous treatment with antibiotics, 98% an invasive device of any kind and 88% had suffered previous surgery. These three factors proved to be associated with candidemia (p=0, CI 95%). The most common underlying condition was polytraumatism (32%) followed by cardiovascular disease (25%) and cancer (17%). In what concerns to the invasive procedures, the most represented ones were the insertion of urinary catheter (95%), parenteral nutrition (80%) and mechanical ventilation (73%) which were significantly associated with candidemia (p=0, CI 95%).

The final outcome of this investigation was a report with the descriptive analysis and the suggestions for the analytical study. We recommended to pursue a nested case-control study in order to determine the risk factors associated with the cases. All patients admitted to the SICU with candidemia or colonisation by *C. auris* from the beginning until the end of the outbreak period should be included in the study. To each patient (with candidemia or colonisation by C. *auris*) a randomly selected control patient should be assigned, chosen among those who did not develop either infection or colonisation in the same period and who meet the following criteria for matching: age, sex, admission unit and date of entry. For the comparison of the cases and the controls, a univariate analysis can be performed using Chi square, t- Student and U of Mann-Whitney. To study the association between a quantitative variable and a qualitative one of more than two categories, we suggest the analysis of variance (ANOVA).

B. Integration of molecular data in the study of the epidemiology of measles genotype B3 in Spain from 2006 to 2016

Supervisors: Aurora Fernández, Juan Emilio Echevarría and Josefa Masa

Measles virus (MeV) B3 genotype produced several outbreaks and sporadic cases in Spain from 2006 to 2016. Genotype B3 is endemic in many countries in Africa, and its importation into different continents has occurred in several different occasions. The objective of this project was to update the molecular epidemiology of MeV genotype B3 in Spain between 2006 and 2016, in order to clarify the origin of the outbreaks and the transmission chains. In this sense, the fellow used the MeaNS database and GenBank to find and download B3 genotype sequences and integrated all the molecular data with the epidemiological data obtained from the database of the National Centre for Epidemiology (CNE). Twenty eight haplotypes among the Spanish sequences were found, grouped in fifteen phylogenetic clusters. The most important outbreaks were caused by the haplotypes MVs/RioGrandedoSul.BRA/33.10 in 2010 and 2011 and MVs/Granada.ESP/43.10/ in 2010 and 2011 and by the variants MVi/Harare.ZWE/38.09 in 2011, and MVs/WesternAustralia.AUS/2.14 in 2014. Twenty sporadic cases were identified, 15 of them belonged to variants previously described. Six cases with unique sequences were also found, probably derived from imported haplotypes which produced outbreaks in Spain. The predominant haplotypes were identified as well as the variants and the origin of the genotype B3 causing outbreaks in Spain from 2006 to 2016. The variant analysis allowed to discriminate different transmission chains in the context of what was previously thought to be a single outbreak, based only on epidemiological data and, on the other hand, to join together what were thought to be different outbreaks. The integration of molecular data in the study of the epidemiology of measles is of utmost public health importance and recommended to verify the indicators of measles elimination programme status regionally, being the absence of endemic genotype(s) one of the criteria for verifying measles elimination in a country or region.

Within all the information gathered and analysed in this study, the fellow provided relevant information, namely, the transmission chains of the outbreaks caused in Spain by the B3 genotype during the last decade. Part of the results were presented as a poster at the XIV National Congress of Virology, Cádiz, Spain in June 2017 and the final results were accepted as a poster communication at the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), which will be held in Stockholm, Sweden in November 2017. A paper with the results is in preparation.

Training modules

During several lectures and case studies at the EPIET/EUPHEM "Introductory course", the fellows had the opportunity to get familiarised with the essential steps to follow in order to pursue an outbreak investigation. These concepts were further consolidated and completed during the "Outbreak Investigation and Management" module, with the usage of computers. In this latter module the fellows learned essential data management skills (entering, validating and cleaning data), dataset management, designing cohort and case-control studies and statistical analysis of the data using STATA. As a communication exercise, the fellows have written an outbreak report. During the "Initial Management in Public Health Microbiology Module" the fellows were exposed to an outbreak situation where they had to train working on a multidisciplinary team and reporting of an outbreak to different kinds of audience. The "Multivariable analysis" module provided the fellows the knowledge and tools to understand the use of this analytical process. Fellows were taught on how to recognise relevant variables to build up regression models and to identify and analyse third factors such as effect modification or confounding and how to interpret the final results of the created model.

Educational outcome: Working in cooperation with multidisciplinary teams such as microbiologists, epidemiologists and health care workers in the context of an outbreak; performing a site visit and conduct an onsite investigation; gathering epidemiological information; creating a database for an epidemiological investigation; integrating microbiological and epidemiological knowledge to investigate outbreaks; performing phylogenetic analysis; performing descriptive analysis through counting cases and orientating the data according to time, place and person; developing a report with formulated recommendations based on the analysis of epidemiological and microbiological data.

1.2. Surveillance

A. Evaluation of the surveillance system for antifungal drug resistance in Spain, 2014-2015

Supervisors: Ana Alastruey

In recent years evidence of resistance to antifungals has been increasingly reported, with variable prevalence levels, in different regions and countries of the world. Invasive fungal infections can cause morbidity and mortality in patients with weakened immune systems and with underlying diseases. They are costly and a common problem in healthcare settings. In this way, surveillance systems must be in place. Previous studies have shown that there are resistant *Candida* and *Aspergillus* strains in Spain and in June 2014 a laboratory surveillance system for antifungal resistance was established at the Mycology Reference laboratory from the National Centre of Microbiology (CNM), ISCIII in Madrid. Because a very limited number and non-systematic collected samples was arriving at the institute, an evaluation of the surveillance system was necessary. The aim of this work was to evaluate the system in order to provide recommendations for improvement. The fellow performed a descriptive study through the analysis of microbiological and epidemiological data, appraised the simplicity by analysing the structure and ease of operation (using online questionnaires), the representativeness (through the population coverage of the participating hospitals) and the data quality (calculating the completeness of data reported) and validity (proportion of cases complying with case definition). The antifungal surveillance system was found to be simple and presented good completeness but low validity. The representativeness was not optimal as the coverage was low.

At the end of project the fellow made recommendations to improve the surveillance system through the search for larger hospital coverage encouraging more hospitals to participate in educational campaigns and by dissemination of information about the system functioning and the importance of the surveillance system providing reliable data at a national level. Otherwise, a sentinel system, instead of a voluntary one, where a limited known number of hospitals would be fully engaged to participate in the surveillance system could be implemented. As essential measures it was suggested to make sure that all the participants have antifungal susceptibility tests available and that they record and provide the total number of isolates tested, in order to make possible to understand the proportion of resistant isolates. As a final outcome, the fellow wrote a report which will be presented to key stakeholders and her work is expected to be an important contribution for the improvement of the current surveillance system. The results were presented as a poster communication in ESCAIDE which took place in Stockholm, Sweden in November 2016.

B. Virological and epidemiological surveillance of influenza in Spain

Supervisors: Francisco Pozo, Inmaculada Casas and Amparo Larrauri

Influenza is an acute respiratory vaccine preventable disease. Annual influenza epidemics are associated with high morbidity and mortality and in Europe, influenza occurs in regular annual epidemics in the winter season. The Spanish Influenza Surveillance System comprises 17 networks of sentinel physicians (in 17 out of 19 autonomous communities) and 20 network-affiliated laboratories, including the National Influenza Reference Laboratory (CNM, WHO National Influenza Centre). In addition to the specimens taken by sentinel physicians, laboratories also collect specimens from non-sentinel sources (i.e. hospitals, collaborating laboratories). The main objective of this project was to provide relevant information about influenza, during the 2015-2016 influenza season, to the national health authorities and health professionals, in order to help to guide the measures for the control of the disease and contributing to reduce the burden of disease associated with influenza within the country. Every year the Spanish laboratories send the clinical samples or isolated strains to the National Influenza Reference Laboratory for genetic characterisation. The microbiological surveillance activities which take place in the CNM laboratory include not only diagnosis in clinical samples but also viruses' characterization. During the time of this project, in the influenza laboratory, the fellow identified and characterised types and subtypes of circulating strains of influenza viruses, assessed emergence of antiviral resistance and analysed strains. The generated data was submitted every week to the CNE. At the CNE, epidemiological and virological information was gathered and analysed in order to get a clear picture of the situation of influenza activity in Spain and to disseminate this information at local, national and international levels during the 2015-2016 season. At CNE the fellow analysed weekly epidemiological data for reporting in the National Weekly Surveillance. The National weekly influenza reports were systematically sent to local and regional health authorities and to all the members of the surveillance system every week. This information was then publicly released in the surveillance system's webpage and uploaded on The European Surveillance System managed and maintained by ECDC. After that, the information flowed to the European and worldwide levels (ECDC, Euro WHO and WHO). Besides contributing to the weekly analysis of the influenza season, the fellow also analysed data and described the regional heterogeneity of the influenza epidemic in Spain in the season 2015-2016. In this season, the influenza epidemic started in the week 3/2016, two weeks later than the previous season. At the national level, there was a low level of intensity of influenza activity, although the influenza incidence remained above the threshold baseline for 11 weeks, with an atypical epidemic wave occurring with a stable incidence from week 4/2016 through week 12/2016. This unusual epidemic development at the national level was related to a marked geographical heterogeneity in the way the influenza activity occurred throughout the season. The maximum incidence of influenza was recorded between weeks 4/2016 and 11/2016, depending on the sentinel network. At the national level, there was an intense viral circulation (> 40% of positivity) for 14 consecutive weeks, although a heterogeneity was also observed between the different national networks. Regarding the geographical distribution of the influenza activity, there was an increase of incidence in the north-western peninsular region, which moved to the northeast and southeast throughout the season. Since the beginning of the season, the flu activity was associated with a prevalence of virus A (H1N1) pdm09, with an increasing contribution of virus B as the season went though. These data were published in the online Weekly Epidemiological Bulletin (Boletín Epidemiológico Semanal) of ISCIII describing the regional heterogeneity of the influenza epidemic evolution in Spain in the season 2015-2016 (available at: http://revista.isciii.es/index.php/bes/article/view/974/1190).

With her activities in this project the fellow contributed to the surveillance system which leads to the annual interpretation of the current trends on influenza. This surveillance system is very important for the identification of high-risk persons, the determination of the effectiveness of current prevention, antiviral treatment and recommendation strategies, and refinement of vaccines.

C. Evaluation of WHO's quality indicators for measles laboratory surveillance in 2015

Supervisors: Josefa Masa and María de Viarce

The "Measles Elimination Plan" in Spain was established in 2001. Subsequently, in 2004, the "Regional Advisory Group on Vaccination" of the "European Technical Advisory Group on Experts on Immunization" recommended the inclusion of rubella in the elimination strategy. Surveillance of measles, rubella and congenital rubella syndrome are included into the Spanish Surveillance System and countries with elimination goals should monitor the quality of their surveillance systems by periodically calculating indicators of performance. The objective of this project was to evaluate quality indicators for measles laboratory surveillance. For the concretization of this objective, the fellow worked at the CNE with the management of laboratory infection markers and genotype data to be included in the national report (2015), and calculated and analysed indicators of measles laboratory surveillance for Spain in 2015 according to WHO and ECDC recommendations.

With her work, the fellow contributed to the "Annual update on Measles and Rubella Elimination Status in Spain for 2015" requested by the "WHO Regional Office for Europe" which is available online

(http://www.isciii.es/ISCIII/es/contenidos/fd-servicios-cientifico-tecnicos/fd-vigilancias-alertas/fd-enfermedades/fd-enfermedades-prevenibles-vacunacion/pdf_2016/Vigilancia_SAR_RUB_2015web.pdf). In addition she presented and discussed the main results of the report in an oral session at CNE in June 2016.

D. Assessment of the laboratory capacity for the surveillance of measles and rubella in Spain

Supervisors: Juan Emilio Echevarría and Aurora Fernández

The objective of this project was to assess the laboratory capacity for the surveillance of measles and rubella in Spain. In order to update the information about the national laboratory network, the National Reference Laboratory for measles and rubella have sent e-mail surveys to the public health officers in charge of the surveillance of measles and rubella in the different autonomous regions. The questions sought to obtain data on the laboratories responsible for surveillance, the techniques used and in the absence of these, where samples were sent for analysis and/or confirmation of results. The fellow gathered all the information sent by the different laboratories and elaborated another questionnaire that was sent to the epidemiologists in order to fill some information gaps. With these data the fellow elaborated a database and proceeded to the respective analysis. A total of 15 out of 19 autonomous regions have answered reporting data on 54 different laboratories. The main conclusions of this project were 1) the capacities of the regional laboratories are very different but all of them have the support of the National Reference Laboratory to complete the panel of techniques; 2) the confirmation of rubella IgM with further avidity tests is not usually performed, except for pregnant women; 3) additional surveys are needed to clarify the internal flow of samples to Regional or National reference Laboratories in some autonomous regions and 4) some autonomous regions have not named a regional reference laboratory.

The final outcome of the fellow's work was a report which results will be presented at the next meeting of the "National Committee for Measles and Rubella Elimination" in Spain. Hopefully her work will contribute to help the competent authorities to identify possible areas for action and to engage activities for the improvement of the current surveillance system.

Training modules

During the EPIET/EUPHEM "Introductory course", the fellows got acquainted with the concepts of developing, evaluating and analysing a surveillance system. The module on "Bioinformatics and phylogenetics" provided essential tools to interpret microbiological data. In the "Rapid assessment and survey methods" module, by performing a case study, the fellows were introduced on how to set up a surveillance system, to interpret surveillance data and to set an alert and response system in complex emergency situations.

Educational outcome: Becoming acquainted with three different surveillance systems in Spain (antifungal drug resistance, inlfuenza and measles) and their functioning at national and international levels; developing a framework to evaluate the surveillance system using standard criteria; describing the resources used to operate the system; describing and evaluating a surveillance system using attributes defined by CDC and ECDC such as simplicity, flexibility, acceptability, data quality and representativeness; applying combined microbiological and epidemiological knowledge in surveillance; performing phylogenetic analysis; analysing surveillance data; listing conclusions and recommendations and providing a report with the recommendations for improving or discontinuing a surveillance system.

2. Applied public health microbiology research

Is it possible to predict antimicrobial resistance in *Neisseria gonorrhoeae* through the knowledge of its genome?

Supervisors: Raquel Abad and Julio Vázquez

According to WHO, *Neisseria gonorrhoeae*, the aetiological agent of the sexually transmitted disease gonorrhoea, annually causes an estimated 106 million new cases globally. The treatment options have diminished rapidly because of the emergence and worldwide spread of antimicrobial resistance. The emergence, *in N. gonorrhoeae*, of decreased susceptibility and resistance to the extended-spectrum cephalosporins (ESC), such as ceftriaxone and cefixime, together with the longstanding high prevalence of resistance to penicillins, sulphonamides, tetracyclines and, more recently, quinolones and macrolides, is cause of major concern. The Spanish Reference Laboratory for *Neisseria*, *Listeria* and *Bordetella* (SRLN) carries out antimicrobial susceptibility testing and molecular epidemiology of *N. gonorrhoeae* by multi-antigen sequence typing on all *N. gonorrhoeae* strains received as part of its support

functions at the National Health System. As a result of the data generated until now, Spain appears as one of the most affected European countries with strains resistant to cefixime. Although ceftriaxone resistance has not been detected yet, Minimum Inhibitory Concentrations have been increasing steadily during the last years. Whole Genome Sequencing (WGS) has evolved and become more cost-effective and accessible than before but it is not yet implemented in the SRLN. Additionally the data about genes conferring antimicrobial resistance to *N. gonorrhoeae* are scarce in Spain. The aim of this project was to contribute to elucidate the genome-based epidemiology and molecular mechanisms of ESC resistant *N. gonorrhoeae* isolates received at SRNL during the last years, in order to provide information that can be useful for future diagnostic or treatment tools. The strains were primarily selected for decreased or moderate susceptibility to cefixime and/or ceftriaxone.

The fellow developed the research protocol, performed all the laboratory procedures for the WGS of 82 isolates of *N. gonorrhoeae* selected at the SRLN from samples received between 2013 and February 2017 and analysed the genes for antimicrobial resistance. A preliminary analysis of the known decreased susceptibility to ESC molecular determinants that have been reported to influence cephalosporin susceptibility, including *penA*, *mtrR*, *ponA* and *porB* mutations was carried out in all the selected strains. Mutations in *penA* were identified in 65/81 (80%) of the isolates. Adenosine deletion in the 24bp efflux pump *mtrR* promoter region was found in 55/81 isolates. Most isolates, 76/81 (94%) also presented the *ponA* L421P mutation, associated with resistance to beta-lactam compounds. Mutations in *porB* were also found. The factors having the greatest influence on susceptibility of the analysed ESC isolates were the *penA* mosaic allele, the *porB* A121N and G120K mutations, and the *mtrR* -24 A deletion. WGS is positioned to become an essential tool, by improving understanding of the molecular epidemiology of *N. gonorrhoeae* and genetic prediction of gonococcal antimicrobial resistance.

This project has added value at national and EU level through the implementation of a WGS protocol for drug-resistant *N. gonorrhoeae* molecular surveillance. The data generated by the fellow provides important insights, into the genome-based epidemiology and molecular mechanisms of ESC resistance in *N. gonorrhoeae* at national and international levels and can be used to provide more reliable evidence for policy makers. The knowledge about the genetic basis of resistance may be used, in the future, for the design of molecular tests which could contribute to rapidly and accurately identify patients at risk for treatment failure, and also to try to develop novel therapeutics for more appropriate treatments. A paper with the results is in preparation.

Training modules

In the EPIET/EUPHEM "Introductory course", fellows had lectures about how to organise and write a research protocol and as, a practical exercise, they had to write one, following a template with the sections that must be included. The module "Initial management in public health microbiology" focused, among other things, on time management and working as a team during the research projects. The "Phylogenetic and bioinformatics" module showed some informatics tools that can be used for analysing molecular data in research projects. The "Outbreak investigation and management" and "Multivariable analysis" modules provided further insights and practical lessons on how to write and communicate the results of the research.

Educational outcome: Conducting all stages of a public health microbiology research project, from the preparation of study protocol to writing a manuscript; reviewing literature; identifying limitations; integrating microbiological and epidemiological data in a public health research project; performing laboratory work to conduct WGS of *N. gonorrhoeae* from the culture of the strains to the library construction; conducting phylogenetic analysis and interpreting the results; managing databases and analysing data.

3. Applied public health microbiology and laboratory investigations

A. Zika virus: development of new diagnostic tests

Supervisors: Fernando de Ory, María Paz Sánchez-Seco, Ana Vázquez and Laura Herrero

Zika virus (ZIKV) is a vector-borne flavivirus transmitted by *Aedes* mosquitoes. Prior to 2015, ZIKV outbreaks occurred in areas of Africa, Southeast Asia, and the Pacific Islands but in May 2015, the Pan American Health Organization issued an alert regarding the first confirmed ZIKV infections in Brazil and currently, outbreaks are still occurring in many countries and territories. Despite the main vector of ZIKV, *A. aegypti*, is only confirmed to be established in a few European countries (Portugal- Madeira Island, Russia, Turkey and Georgia), potential vectors such as *A. albopictus* have a more generalised distribution. Moreover, ZIKV is a public health concern in this region especially because imported cases of ZIKV are often reported, as well as sexual transmission among travelers returning from affected areas. During the first week after onset of symptoms, ZIKV disease can often be diagnosed by performing real-time reverse transcription-polymerase chain reaction (rRT-PCR) on serum or urine samples. At CNM laboratory, which is the Spanish reference laboratory for ZIKV diagnostics, it is available an in house

conventional reverse transcription PCR assay, which is time consuming, and a commercial rRT-PCR which is not cost effective. On the other hand, ZIKV and dengue (DENV) viruses share clinical, epidemiological and antigenic features making difficult the serological diagnosis. The overall goal of this project was to improve the ZIKV diagnostics at CNM. In this way, one of the objectives was to develop and validate an rRT-PCR for the detection of ZIKV virus in order to make the detection of the virus more affordable to the Spanish National Health System. The second objective was to evaluate ZIKV-IgA and ZIKV-IgG avidity assays in order to improve the serological diagnosis of ZIKV when viremia falls to undetectable levels. For the accomplishment of the first objective, with the use of different bioinformatics tools, the fellow designed primers and probes in order to set up a new rRT-PCR and then optimised the limit of sensitivity and the specificity of the technique with RNA from ZIKV and other flaviviruses available at the CNM. For the second objective, the fellow organised a collaboration between the Spanish and Portuguese national reference centres for ZIKV diagnostics and the transportation of biological samples from Portugal to Spain. She gathered the available serological and molecular information and studied patients from both centres with travelling history to/from ZIKV endemic areas with confirmed or suspected ZIKV infections. As controls, patients with known recent or past DENV infection were also included in the study. The fellow performed ZIKV-IgG avidity and ZIKV-IgA with commercial ELISA and calculated both sensibilities and specificities. In this way, simultaneous ZIKV IgM, low avidity IgG and IgA positive results seem to clearly corroborate a recent ZIKV infection. However, the serological diagnosis of ZIKV in symptomatic patients should be supported by careful inquiry at the time of admission. The date of the beginning of the symptoms together with the date of sample collection thus provides useful information and may help interpret ZIKV low avidity IgG and IgA together. At the initial and active stages of the infection, IgA tests seem to be more valuable while low avidity IgG appears to be more useful at the active and later stages. The combined use of both techniques seems preferable in order to avoid false negative results.

The implementation of the new rRT-PCR and the ZIKV IgG avidity and IgA tests will help the CNM in performing its functions as a national reference laboratory for this virus and to strengthen laboratory capacity not only to confirm ZIKV infections but also to differentiate ZIKV infections from other arboviral infections by serological methods. In addition, this project was useful to reinforce the cooperation between Portuguese and Spanish reference laboratories. Part of the results were presented as a poster at the XIV National Congress of Virology, Cádiz, Spain in June 2017 and the final results were accepted as a poster communication at the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), which will be held in Stockholm, Sweden in November 2017. A paper with the results has been submitted.

B. Protozoa, enteric viruses and *Escherichia coli* as a potential cause of gastrointestinal disease in patients with diarrhoea of unknown origin

Supervisors: Silvia Herrera, David Carmena and Maria Cabrerizo

The clinical manifestations of gastroenteritis do not allow the distinction between viral, bacterial or parasitic gastroenteritis. In Spain, the basic diagnostic at the regional level is mainly focused on the detection of the different bacteria causing diarrhoea. When the diagnosis at that level leads to no etiological cause, a request is made to specific units of CNM and usually focuses on the identification of one or two pathogens according to the suspicion of clinical symptoms, meaning that sometimes the cases may remain undiagnosed. The objective of this project was to obtain information on the role of the different pathotypes of *E. coli,* the most common protozoa (*Cryptosporidium*, *Blastocystis* and *Giardia*), and viruses (noroviruses and rotaviruses) as potential causes of diarrhoea in order to evaluate the possibility of implementation of routine syndromic diagnosis at CNM using a panel of the pathogens. In order to do that, the fellow studied 148 stool samples from patients belonging to different age groups, with clinical signs of diarrhoea from eleven Spanish provinces whose samples, with an initial negative diagnosis to the main bacterial species causing diarrhoea, were submitted for the study of *E. coli* from 2015 to 2017. Detection and characterisation of *Giardia duodenalis*, *Cryptosporidium* spp. and *Blastocystis* spp by PCR and sequencing of the SSU rRNA gene and norovirus and rotavirus by PCR and sequencing of the RNA polymerase gene and VP7 capsid gene, respectively were performed by the fellow*. G. duodenalis* was confirmed in one sample (0.7%), but it was not possible to determine the assemblage to which it belonged. *Cryptosporidium* was found in 4% of the analysed samples, and two species were identified: *C. parvum* (n= 2) and *C. hominis* (n= 4). *Blastocystis* was found in 4.7% of the investigated samples and the assigned subtypes were 1 (n= 4), 3 (n= 1) and 4 (n= 2). Norovirus was detected in 20% of the samples, 18% belonging to genogroup II and 2% to genogroup I. Rotavirus (G9.P [8]) was detected in 1.4% of the samples. A statistically significant association was found between the presence of norovirus and the age group <5 years, as expected (p=0.0086), and between *Cryptosporidium* and the female genus (p= 0.012). Data provided by the enterobacteriacea research group showed that 38.5% of the samples were positive for one of the 5 *E. coli* pathotypes investigated, with 42.1% being enteroaggregative *E. coli*. Although a limited number of samples was studied, infections with *E. coli*, protozoa and enteropathogenic viruses seem to be relatively frequent in patients with primary non-affiliated diarrhoea.

During the project the fellow facilitated the communication between three different laboratories at CNM and provided evidence to support the idea that the diagnosis of patients with gastrointestinal conditions should be

approached from a holistic point of view which considers all the etiological agents potentially involved. The results of this work will be presented as a poster communication in the X National Congress of the Spanish Society of Tropical Medicine and International Health which be held in Bilbao, in October 2017 and a paper is in preparation.

Training modules

In the "Biorisk & Quality Management "module the fellows learned how to safely handle and send biological samples according to international regulations. In the "Bioinformatics and Phylogeny" the fellows were in contact with some informatic tools to perform genomic analysis. At the "Initial Management in Public Health Microbiology" module the fellows acquired communication and time management skills.

Educational outcome: Integrating microbiological and epidemiological data, facilitating communication between different laboratories at national and international levels; constructing databases; performing database analysis; learning new laboratory techniques; applying concepts of bacteriology, virology and parasitology to Public Health disciplines; communicating with multidisciplinary teams and different personalities, managing time and stress.

4. Biorisk management

Before EUPHEM, the fellow has attended to courses on "Working on BSL2 and BSL3 laboratories" and "Transport of infectious substances" and has participated in the "Biosafety: design, construction and decontamination of biocontainment laboratories" workshop. She had worked for twelve years in BSL2 environment and for a couple of years in BSL3 facilities. During the EUPHEM programme she worked in BSL2 conditions with Zika virus, enteric pathogens and *N. gonorrhoeae* and organised a shipment of samples from Portugal to Spain.

A. Laboratory preparedness in Spain

The fellow has prepared and presented a short oral communication ("Preparedness for emerging and re-emerging diseases in Spain") for the "Initial Management in Public Health Microbiology", in Stockholm, February 2016. The presentation was focused on the existing surveillance systems and the Spanish laboratory capacity for emerging and re-emerging diseases. For the preparation of the communication the fellow gathered information from interviewing different researchers at the ISCIII.

Training modules

In the EUPHEM module "Biorisk management" fellows were trained on techniques used for both biorisk and biosafety assessment as well as mitigation, including WHO recommendations on biorisk management in laboratories. The fellows had theoretical and practical lessons and in the end a formal assessment and certificate was provided for international regulations on the transport of dangerous goods according to the International Civil Aviation Organization (ICAO). There was also the opportunity to visit a BSL4 facility at the Public Health Agency of Sweden, Stockholm. In addition, the fellows had to analyse two different scenarios and to deliver reports about biosecurity and biosafety assessments, focusing on mitigation measures, regarding biosafety issues.

Educational outcome: Consolidating the training in BSL2 and BSL3 practices and learning the functioning of a BSL4 facility; applying the obtained knowledge for safely working in laboratory research including the use of personal protective equipment and decontamination and waste control strategies; applying international regulations for the shipment of infectious substances and getting familiarised with the principles and practices of biorisk management, assessment and mitigation.

5. Quality management

Supervisor: Silvia Herrera

A. *Salmonella, Shigella* and *Campylobacter*: External Quality Assessment (EQA)

The increased number of isolates of *Salmonella, Campylobacter* and *Shigella* which are resistant to antimicrobials is of major concern since these isolates are associated with infections characterised by increased morbidity and mortality. Although new typing technologies such WGS are becoming available, we cannot forget that most of the worldwide microbiological surveillance on those pathogens is based on serotyping, antimicrobial resistances profiles and/or pulsed field gel electrophoresis (PFGE), being the last one the gold-standard method for *Salmonella*

subtyping. In this context, an invitation was sent to the CNM by the Department of Microbiology & Infection Control from the Statens Serum Institute, on behalf of ECDC, to participate in the Seventh External Quality Assessment (EQA) on *Salmonella* (further EQA-7), which is focused on PFGE on this specific pathogen. On the other hand, the CNM signed up to participate in the annual Global Foodborne Network External Quality Assurance for *Salmonella* and *Shigella* serogrouping, serotyping and antimicrobial susceptibility testing (further WHO GFN EQA 2015). The aim of this project, englobing the two different EQA's, was to perform the serotyping and identification of the provided specimens of *Enterobacteriacea*, to analyse the obtained results, to submit them according to the EQA´s specifications and to make the final reports. In the EQA7, the fellow was responsible for performing the PFGE for *Salmonella* typing and for the analysis of the gel. Regarding the WHO GFN EQA 2015, the fellow was responsible for the serotyping of *Salmonella* and *Shigella* O or H antigens, the identification of the *Campylobacter* species and the unknown Enterobacteriaceae by phenotypical/genotypical methods and performing the antibiograms and phenotypical/genotypical determination of ESBLs, AmpC-enzymes and carbapenemases.

The capacity to detect and respond to foodborne disease outbreaks by conducting laboratory-based surveillance of *Salmonella* and other foodborne pathogens is essential nowadays. In this sense the EQAS are of major importance to evaluate and assure the diagnostic quality of the reference laboratories and it is fundamental that these laboratories are able to show a standardised capacity and ability to perform the necessary microbiological diagnosis. At the end of the project the fellow provided data for the online submission of the results and wrote reports. When the organisers released the EQA reports, the fellow followed up the nonconformities and discussed the recommendations with the head of the unit.

Training modules

In the "Biorisk and quality" module the fellows were exposed to different aspects concerning quality management in both internal and external quality controls according to the ISO 15189 standards. They were taught about norms and accreditation processes, assessments and audits, good practices for documentation and record keeping, sample and equipment management, among others that contribute to the quality in laboratories. As a homework, the fellow performed a quality assessment of the Mycology reference laboratory at CNM. The overall scoring of the laboratory quality was excellent and only small issues could be addressed in order to improve it.

Educational outcome: Becoming acquainted with the different methods for typing and serotyping enterobacteria and the phenotypical/genotypical determination of ESBLs, AmpC-enzymes and carbapenemases; applying the concept of EQA; performing, evaluating and analysing results of an EQA; understanding the principles and practices of quality assurance according to those outlined by international and EUA directives; writing reports; make conclusions and recommendations.

6. Teaching and pedagogy

A. Lectures in the Master "Applied Microbiology in Public Health and Infectious Disease Research"

Supervisors: Silvia Herrera, Ana Alastruey and Horacio Gil Gil

During the last years the "Master in Applied Microbiology in Public Health and Infectious Disease Research" was organised by the ISCIII and the Universidad de Alcalá de Henares and held at the National School of Public Health in Madrid, Spain. In this master the fellow:

- Facilitated, in December 2015, the Kalundborg norovirus outbreak case study, which belongs to the ECDC training material.
- Facilitated, in December 2015, the case study "*Salmonella* in the Caribbean" which belongs to the ECDC training material.
- Facilitated, in November 2016, the Kalundborg norovirus outbreak case study, which belongs to the ECDC training material.
- Revised a practical session on "Bioinformatics tools for bacterial identification and characterisation" prepared in 2016 by the previous EUPHEM, Horacio Gil Gil (MS-EUPHEM fellow, cohort 2014), and facilitated one session in March 2017. Participants had to solve different scenarios and interpret the results and identify the consequences in Public Health. The teaching material included exercises using 16S rRNA for bacterial identification, Multi-Locus Variable Number tandem Repeats Analysis (MLVA) for characterisation of *Bartonella* and using New Generation Sequencing data to investigate a *Streptococcus pyogenes* outbreak.

B. Organisation of the course REVIVE-Sandflies

The programme REVIVE (Vector Surveillance Network), coordinated by the Portuguese National Health Institute (INSA), through its Department of Infectious Diseases, aims to monitor the activity of hematophagous arthropods and to characterize the species and their seasonal occurrence in Portugal. The network also aims to detect and identify important pathogens for Public Health, the incidence of vector's infection and the introduction of exotic/invasive species, in order to make it possible for the health authorities to take prompt adequate control measures in case of need. The first REVIVE Protocol (2008-2010) was created as a network between the General Directorate of Health Administration of different Portuguese regions and the Institute Ricardo Jorge. The second protocol (2011-2015) extended the surveillance to include tick vectors with public health importance in Portugal. The third protocol (2016-2020) is being implemented and, for the first time, phlebotomine sandflies are also contemplated in the surveillance system.

As an expert in sandflies and in sandfly-borne viruses, the fellow was invited to organise a course and give training to the personnel involved in the programme. The main objective of this course, with the duration of one day, in May 2016, was to teach the participants concepts about the biology of sandflies, to enlighten them about laboratory methods for detection of sandfly-borne pathogens, to train them on sandfly surveillance methods, and to teach control and prevention measures for sandfly transmitted diseases. The fellow organised the course, prepared three theoretical lectures and two practical sessions and elaborated a 32 pages notebook with practical information and guidelines to be followed in the national sandfly surveillance.

C. Organisation of the course "Epidemiology for microbiologists"

Supervisors: Silvia Herrera, Ana Alastruey and Horacio Gil Gil

The CNM has an internal training program with different courses covering a wide range of topics. Every year this programme is reviewed and new courses are proposed. In 2016 Horacio Gil Gil and Leonidas Georgalis (EU-EPIET fellow, cohort 2014) proposed, for the first time, the course Epidemiology for microbiologists (Epi4micro) which received many applications that had to be refused due to logistic constraints. Looking at this high demand, it was decided to organise the same course in April 2017. The aim of the course was to increase the knowledge of the microbiologists in the field of epidemiology in addition to promote and to strengthen the collaboration between microbiologists and epidemiologists at ISCIII. In this way, Fátima Amaro and Patricia Ndumbi (EU-EPIET fellow, cohort 2015), planned and organised the course, and identified teachers for the theoretical and practical sessions where basic epidemiology concepts were reviewed and consolidated. Fátima Amaro was also responsible for the lecture "Outbreak investigation" and facilitated the case study "Oswego Mazowszanka: an outbreak of gastrointestinal illness following a christening party" (ECDC material).

Training modules

In the "Introductory course" the fellows had contact with several pedagogical and teaching concepts through different theoretical lectures. The fellows also participated in different case studies having the opportunity to get acquainted with this way of teaching.

Educational outcome: Identifying training needs, planning, organising and evaluating courses; moderating a case study guiding participants to answers and explaining epidemiological/microbiological/clinical concepts surrounding a disease or an outbreak; practicing new pedagogical methods; preparing teaching material; delivering a seminar to a multidisciplinary audience (MSc students, microbiologists, health care workers); adopting new training tools; assessing own performance through feedback assessments; time management.

7. Public health microbiology management

A. Public Health management during an outbreak

During the outbreak investigation, Fátima Amaro was involved in public health management and communication with clinicians and other health care staff and epidemiologists onsite. She also participated in teleconferences with staff from CNM, Public Health London, ECDC and CDC.

B. Public Health microbiology management components as part of regular projects

Public health microbiology management was present in all projects and activities during the two years of the fellowship. Having to work in different laboratories, the fellow had to improve her capacity of integration into the diverse teams and to be able to work in multidisciplinary environments. The ability to multitask and to do serious time management was also used. In several occasions the fellow served as a communication facilitator between different microbiology laboratories at national (e.g. "Enteric viruses and protozoa as potential causes of non-bacterial diarrhoea") and international levels (e.g. "Zika virus: development of new diagnostic tests"), and also between microbiologists and epidemiologists at the host site. (e.g. "Virological and epidemiological surveillance of influenza in Spain"; "Integration of molecular parameters in the study of the epidemiology of measles genotype B3 in Spain from 2006 to 2016"; "Assessment of laboratory capacity for the surveillance of measles and rubella in Spain").

Training modules

The EUPHEM module "Initial management in public health microbiology" focused on the understanding of roles and responsibilities in public health management. Through theoretical lectures and challenging exercises, the fellows among other things, had the opportunity to be introduced to different management styles, to participate in role plays and engage in emergency situations, to play as a team and to learn how to be a team leader delegating tasks. The fellows were also introduced to several tools for effective time and stress management, and practiced real time communication with higher authorities after working under pressure in simulated complex emergency situation.

Educational outcome: Working in multidisciplinary teams; mediating scientific discussions; establishing national and international collaborations; understanding the principles of public health management, learning to multitask and manage stress and time more effectively; exercising and applying communication skills with different audiences; understanding laboratory management.

8. Communication

Publications

1.   Amaro F, Sanchéz-Seco MP, Vázquez A, Alves MJ, Zé-Zé L, Luz MT, Minguito T, De La Fuente J, De Ory F. The application of Zika virus IgG avidity and IgA ELISA tests to characterize past and recent infections. (submitted)
2.   Amaro F*, Fernández-Garcia A*, Bangert M, Mosquera MM, Antón A, Castellanos A, Hoyas C, De Ory F, Masa J, López-Perea N, Torres de Mier M, Echevarria JE. Integration of molecular parameters in the study of the epidemiology of measles genotype B3 in Spain from 2006 to 2016. (in preparation)
3.   Amaro F, Vázquez JA, Navarro C, Martín E, Abad R. Whole genome sequence analysis of *Neisseria gonorrhoeae* with decreased susceptibility to cephalosporins isolated in Spain from 2013 to 2017. (in preparation)
4.   Amaro F, Robinson E, Arce P, Cabrerizo M, Carmena D, Herrera-León S. Diarrheagenic *Escherichia coli*, viruses, and protozoa as potential cause of gastrointestinal disease in patients with diarrhoea of unknown etiology. (in preparation)

*authors contributed equally

Reports

1.   Descriptive epidemiology of an outbreak of *Candida auris* at La Fe hospital, Valencia, Spain, 2016-2017.
2.   Report on the Seventh international External Quality Assessment (EQA-7) for *Salmonella enterica* ssp. *Enterica*.
3.   Report on the WHO GFN EQA 2015: Antimicrobial susceptibility testing of *Salmonella*, *Shigella* and *Campylobacter* and optional genotypic characterization of AmpC-, ESBL- and carbapenemase-producing test strains.
4.   Evaluation of the microbiological surveillance system for antifungal drug resistance in Spain, 2014-2015.
5.   Annual update on "Measles and Rubella Elimination Status in Spain for 2015" requested by the WHO Regional Office for Europe (participation on the report).
6.   Laboratory capacity for the surveillance of measles and rubella in Spain, 2017.

7.   REVIVE- Sandflies. (Teaching report, 2017).
8.   Epidemiology for microbiologists. (Teaching report, 2017).

Conference presentations

1.   Amaro F, Herrera S, Alastruey A. Evaluation of the microbiological surveillance system for antifungal drug resistance in Spain, 2014-2015. ESCAIDE 2016, 28-30 November 2016, Stockholm, Sweden. (Poster presentation)
2.   Amaro F, Fernández-García A, Bangert M, Mosquera MM, Antón A, Castellanos A, Hoyas C, De Ory F, Masa J, López-Perea N, Torres De Mier MV, Echevarría JE. Molecular epidemiology of measles virus genotype B3 in Spain (2006-2016). XIV National Congress of Virology, Cádiz, Spain, 11-14 July 2017. (Poster presentation).
3.   Amaro F, Sánchez-Seco MP, Vázquez A, Alves MJ, Zé-Zé L, Luz MT, Minguito T, De La Fuente J, De Ory F. XIV National Congress of Virology, Cádiz, Spain, 11-14 July 2017. (Poster presentation)
4.   Amaro F, Robinson E, Arce P, Cabrerizo M, Herrera-León S, Carmena C. Protozoa, enteric viruses and *Escherichia coli* as a potential cause of gastrointestinal disease in patients with diarrhoea of unknown origin. Relevance of syndromic diagnostics. X National Congress of the Spanish Society of Tropical Medicine and International Health, Bilbao, Spain, 23-25 October 2017 (Poster presentation)
5.   Amaro F, Sánchez-Seco MP, Vázquez, Alves MJ, Zé-Zé L, Luz MT (3), Minguito T, De La Fuente J, De Ory F. Potential use of Zika virus IgG avidity and IgA ELISA tests to discriminate past and recent infections. ESCAIDE 2016, 6-8 November 2017, Stockholm, Sweden. (Poster presentation)
6.   Amaro F, Fernández-Garcia A, Bangert M, Mosquera MM, Antón A, Castellanos A, Hoyas C, De Ory F, Masa J, López-Perea N, Torres de Mier M, Echevarria JE. Integration of molecular parameters in the study of the epidemiology of measles genotype B3 in Spain from 2006 to 2016. ESCAIDE 2016, 6-8 November 2017, Stockholm, Sweden. (Poster presentation)

Other presentations

1.   Preparedness for emerging and re-emerging diseases in Spain. Initial Management in Public Health Microbiology Module. ECDC, Stockholm, Sweden, 12 February 2016.
2.   Measles surveillance in Spain: some data for the 2015 annual report and WHO indicators. ISCIII, Centro Nacional de Epidemiología, Madrid, Spain, 2 June 2016

Other

1.   Amaro F, Oliva J, Delgado-Sanz C, Pozo F, Gómez-Barroso D, León-Gómez I, Gherasim A, Casas I, Mateo S, Larrauri y el Sistema de Vigilancia de Gripe en España. Heterogeneidad en la evolución geográfica de la onda epidémica gripal en España. Temporada 2015-16 Available at: http://revista.isciii.es/index.php/bes/article/view/974/1190

Training modules

In the Introductory course the fellows had to deliver a communication and received advice from other fellows and coordinators for improvement in future communications. Additionally EUPHEM fellows had a problem based learning (PBL) exercise were they had to deliver a final presentation.

Educational outcome: Working in multidisciplinary teams; communicating with people with different backgrounds, managing stress and emotions.

9. International missions

A. Rapid needs assessment at Elliniko refugee camps in Athens, Greece

Supervisor: Kostas Danis

In early June 2016, Médecins Sans Frontières planned a measles-mumps-rubella (MMR) vaccination campaign in three sections of a refugee camp in Elliniko, Athens, Greece: Elliniko I (Hockey stadium), Elliniko II (Olympic arrivals) and Elliniko III (baseball stadium). In order to assess the health and sanitary needs of refugees and to estimate baseline vaccination coverage among people less than 15 year old, a previous rapid assessment survey was conducted on 24th June 2016.

Fátima Amaro was randomly selected between the attendants of the "Rapid Assessment and Survey methods" module to participate in the survey and was assigned to Elliniko III section. The fellow collected information on demographics, access to health care, health conditions (infections, chronic diseases, trauma, injuries), vaccination status for children <15 years, hygiene conditions and non-food items available in the camp. For data collection, the fellow teamed up with a translator and proceeded to interview nineteen participants face-to-face, using a structured questionnaire and Epicollect +(a mobile data collection application). Data was further analysed and a report was elaborated.

Training modules

The training for pursuing this task was given by Kostas Danis and Alex Spina during the "Rapid Assessment and Survey methods".

10. EPIET/EUPHEM modules attended

1. EPIET&EUPHEM Introductory Course 2015, Spetses, Greece (three weeks)
2. Bioinformatics and Phylogeny module, Stockholm, Sweden (three days)
3. Outbreak Investigation and Management module 2015, Berlin, Germany (one week)
4. Biorisk & Quality Management Module 2016, Stockholm, Sweden (one week)
5. Initial Management in Public Health Microbiology Module 2016, Stockholm, Sweden (one week)
6. Multivariable Analysis Module 2016, Vienna, Austria (one week)
7. Rapid Assessment and Survey methods module 2016, Athens, Greece (one week)
8. Project Review Module 2016, Lisbon, Portugal (one week)
9. Project Review Module 2017, Lisbon, Portugal (one week)

10. Other training and scientific meetings attended

1. Seminar: High throughput sequencing analysis of respiratory clinical specimens from Madrid. CNM, ISCIII, Madrid, Spain, 21rst January 2016.
2. Workshop: Zika virus, an international emergency. CNE, ISCIII, Madrid, Spain, 18th February 2016.
3. Course: Scientific writing abstract course, 1rst Edition (online course January-Februaruy 2016)
4. Seminar: Interoperability and standardization of the clinical records as a tool for the management of knowledge. CNE, ISCIII, Madrid, Spain, 7th April 2016.
5. Seminar: Health assistance in conflict zones. CNE, ISCIII, Madrid, Spain, 14th April 2016.
6. Seminar: Pneumonia mortality risk factors in children from medium to low income countries. CNE, ISCIII, Madrid, Spain, 21rst April 2016.
7. Course: Advanced Security in the field. United Nations Department of safety and Security (online, mandatory for the requested for the Rapid assessment and survey methods module)
8. Course: Basic Security in the field. United Nations Department of safety and Security (online, mandatory for the completion Rapid assessmente and survey methods module).
9. First EVD LabNet (Emerging Viral Diseases-Expert Laboratory Network) meeting. Madrid, Spain, 21-23 November 2016.

Coordinator's conclusions

One of the main goals of the EUPHEM is to expose fellows to diverse and multidisciplinary public health experiences and activities, thus enabling them to work across different disciplines. The projects described in this portfolio demonstrate the breath of the public health microbiology work of Fátima. Epidemiological studies included participation in local and national outbreak investigations with clear public health outcomes while surveillance activities extended from evaluation of the surveillance system for antifungal drug resistance in Spain to work on influenza surveillance and assessment of the laboratory capacity for the surveillance of measles and rubella in Spain. The laboratory and epidemiologically based projects covered a diverse range of disease programmes involving multidisciplinary team work with colleagues in other roles and specialities; clinicians, statisticians, epidemiologists, laboratory technicians, public health officers and government officials and colleagues in different member states, which demonstrates how the fellow has developed strengths in adapting to different environments and contexts.

Activities were in line with the "learning by doing" and "on the job training" ethos of the EUPHEM programme and fulfilled the core competency domains described for professionals in their mid-career and beyond. Activities were

complemented by nine training modules providing theoretical knowledge. Projects had a clear outcome, with results communicated in scientific journals and at conferences. Fátima has been active in contributing to training of others during her fellowship with the development of new training materials as well as direct training and facilitation activities which highlights the contribution that fellows can make to capacity building beyond the programme.

The contributions made by this EUPHEM fellow towards public health in Spain and also within Europe indicate the importance of developing and maintaining a critical mass of highly skilled field public health microbiologists within Member States to contribute towards national preparedness as well as being available for responses in the interest of the EU. The EUPHEM Coordinator Team concludes that the fellow has succeeded in performing all her tasks to a very high standard and has conducted herself in a highly professional and effective manner throughout. We wish the fellow every success in her future career as a public health microbiologist.

Supervisor's conclusions

The EUPHEM programme was a great opportunity for Fátima and has provided her with the unique tools to future work in the field of public health microbiology and epidemiology whilst working with colleagues from very diverse disciplines, both nationally and internationally. Moreover the projects allowed her to gain confidence in other fields of public health microbiology different from vector-borne diseases, her background. At the same time they allowed local supervisors to carry out projects that would otherwise have been hard to achieve. For the host institution, it has provided the opportunity to build new bridges and strengthen collaboration between the different sectors within public health on both a national and international level. Thus, this two years training programme has turned out to be very successful for both the fellow and the training sites as well as an added value for the country as the fellow –through the training modules/projects– contributed in very important Public Health issues. During the two-year fellowship, Fátima developed both personally and professionally and gained new skills through her involvement in a variety of public health activities both in microbiology and epidemiology. Her projects covered all core domains within the programme and showed that she was able to work on these projects independently, occasionally consulting colleagues and peers for advice. Her commitment with the programme, and her excellent team-work has been very well welcome for all project supervisors. It was a great pleasure to have Fátima as a EUPHEM fellow within ISCIII and we highly appreciate her contribution and achievements within the fellowship programme. We wish Fátima every success for her career in Public Health Microbiology.

Personal conclusions of fellow

Having worked previously only with vector-borne diseases, the EUPHEM programme gave me the opportunity to contact with a very broad spectrum of infectious diseases groups. I have gained a whole new perspective on Public Health and learned about the essential integration of the epidemiological and microbiological fields.

The modules were often a starting point and/or an important complement for the projects and were a valuable help to accomplish the fellowship objectives. The programme helped me to fill some important knowledge gaps that I believe it would not be possible to fill in any other way. It gave me new tools to face a variety of Public Health challenges and opened my mind to another ways of learning and teaching. My communication skills were seriously improved as well as my ability to multitask and manage different kinds of situations.

Besides forming people, the programme also intends to create a European network of public health specialists. As far as I am concerned, and if I am giving the right opportunity, I expect to contribute to this network, especially because during this two years of fellowship I was able to create contacts not only at the host institute, but also with my dear co-fellows from all over Europe who I look forward to cooperate in the future.

Acknowledgements of fellow

I would like to thank Loredana Ingrosso (ISS), my frontline coordinator during the first year, for making me work hard and teaching me how to improve my way of work, paying attention to every detail. A very special thank you to Aftab Jasir (ECDC), for very affectionate guidance and enlightenment through my fellowship, for the constructive criticism that made me greatly improve my projects making me grow professionally… and for all her the passion for this big family. I also would like to thank all the EUPHEM and EPIET team, from the coordinators to the administrative staff, for their great work in this fellowship which made it so enriching and enjoyable.

I am deeply grateful to my local supervisors at ISCIII, Silvia Herrera and Ana Alastruey. Their extended knowledge about the research groups at ISCIII gave me the opportunity to work in very interesting projects. They were always available for me, even from distance, and ready to help and to offer advice, giving me, at the same time, the right amount of independence and encouragement to enhance my skills and perform my tasks. I would not

think twice if I was offered the opportunity to work with them again. At a personal level, they always supported me and offered their sincere friendship that I will take home with me.

I also have to thank all the project supervisors at ISCIII which offered me the projects and let me work in their laboratories, as well as their staff, which received me with open arms.

To my all my co-fellows from cohort 2015: we made it!!! We went through this fellowship with a smashing amount of amazing moments. We have built a great team that I expect to be very fruitful not only at a professional level but also very lasting on a personal perspective. I also have to leave a special word for Horacio Gil Gil from the previous cohort. His friendship and his kind words during all the fellowship were priceless (and will always be).

Finally, I would like to thank my family, including the four-pawed ones, who were deprived of my company for such a long time. Specially my husband, my safe haven and heaven, who always supported me and was expecting me every time I went home, with the same passionate and joyful heart.