

The title 'Summary of work activities' in a bold, blue, sans-serif font, followed by the author's name 'Kyriaki Tryfinopoulou' and the program name 'European Public Health Microbiology Training Programme (EUPHEM), 2014 cohort' in a white, sans-serif font, all set against a blue background.The section header 'Background' in a bold, blue, sans-serif font.

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'.

The ECDC Fellowship Training Programme therefore includes two distinct curricular pathways: Intervention Epidemiology Training (EPIET) and Public Health Microbiology Training (EUPHEM). After the two-year training EPIET and EUPHEM graduates are considered experts in applying epidemiological or microbiological methods to provide evidence to guide public health interventions for communicable disease prevention and control. Both paths that provide competency based training and practical experience using the 'learning by doing' approach in acknowledged training sites across European Union (EU) and European Economic Area (EEA) Member States.

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 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.

This report summarises the work activities undertaken by Kyriaki Tryfinopoulou, cohort 2014 of the European Public Health Microbiology Training Programme (EUPHEM) at the National School of Public Health, Hellenic Pasteur Institute, "Sotiria" General Hospital, Kapodistrian University of Athens, Hellenic Central Public Health Laboratory and the Hellenic Center for Disease Control and Prevention (KEELPNO) comprising the EUPHEM Consortium.

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

Kyriaki Tryfinopoulou is a Medical Microbiologist at the Central Public Health Laboratory (CPHL), National School of Public Health & Hellenic Centre for Disease Control and Prevention since 2009. She obtained the title of Medical Microbiologist in May 2005, and a Master of Science in Public Health in 2007. She completed her PhD thesis from the Department of Hygiene, Epidemiology and Medical Statistics of Medical School of the National and Kapodistrian University of Athens and graduated in 2013.

Before commencing EUPHEM she was working at the CPHL Antimicrobial Resistance and Hospital-Acquired Infections Laboratory combining her position with the responsibilities as deputy laboratory Biosafety Officer and as a trainer in Biorisk management, as Greek representative of the WHO "Biorisk Management Advanced Trainer Programme, Training trainers in Biorisk assessment, biorisk mitigation, performance and adult learning techniques: designing & delivering learning programmes".

Fellowship assignment: Public health Microbiology (EUPHEM) path

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 project or activity work 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 guide¹.

1. Epidemiological investigations

1.1. Outbreak investigations

A. Norovirus gastroenteritis outbreak investigation, Fourka, Kassandra, Chalkidiki, August 2015

Supervisors: Kassiani Mellou, Aggeliki Lambrou and Theano Georgakopoulou

On 11 August 2015, the Hellenic Center for Disease Control and Prevention (HCDCP) was notified by the Head of the Primary Care Unit of Kassandra, Chalkidiki, Northern Greece, regarding an increased number of cases with gastrointestinal symptoms (mainly vomiting and diarrhoea) since 9th of August and an outbreak investigation was initiated. The descriptive epidemiological data and the results from the laboratory testing of both human (faeces) and water samples indicated a common-source outbreak in Skala Fourkas followed by possible secondary person-to-person transmission. The cases increased on 9 August, peaked on 10 August and substantially decreased on 13 August, whereas since 16 August, a smaller increase of cases was reported (in total 108 recorded cases). Stool samples from five cases were found positive for Norovirus [Norovirus GI.P2 (n=3), Norovirus GII.P15 (n=2)], whereas in one water sample from a drilling (ground water well), high levels of enterococci and coliforms were found, indicating possible faecal contamination. The history of pipeline breakage before the onset of cases and

¹European Centre for Disease Prevention and Control. European public health training programme. Stockholm: ECDC; 2013. Available from: <http://ecdc.europa.eu/en/publications/Publications/microbiology-public-health-training-programme.pdf>

reports from tourists and residents that the water was turbid, coloured and with an unusual odour further supported this hypothesis. However, the possibility of the emergence of the outbreak due to contaminated water was not verified by water analyses, as water samples had not been analysed for viruses. Analytical epidemiology (case-control study) data showed that the odds of having consumed tap water from the public water supply network was 37 times higher in gastroenteritis cases than in controls (adjusted OR 36.9, $p=0.018$).

Recommendations were made for the immediate increase of the chlorine concentration in the water supply network. Furthermore, recommendations were given on the appropriate collection and storage of water samples during suspected waterborne disease outbreaks in order to assist on follow-up and verification of viral aetiological agents. Additionally, recommendations were given on keeping at least a hardcopy of the pipeline map of the water distribution network in every municipality, for the prompt and timely identification of any spatial association between cases and damages within the water distribution systems.

Kyriaki Tryfinopoulou participated in all stages of the outbreak investigation, was in charge of the design and the implementation of the analytical case-control study, created the questionnaires, and actively identified and interviewed cases and controls through telephone contacts. She created a database using EpiData, entered data and performed the statistical analysis with the use of Stata. Furthermore, she was in contact with the Regional Public Health Laboratory in which the specimens were analysed, as well as with the municipality of the affected area in order to identify any problems in the water supply network. She was actively involved in the formulation of conclusions and recommendations and she wrote the final report.

B. Focus investigation – re-active malaria case detection in the focus of two locally acquired malaria cases, Kato Souli, Marathon Municipality, Attica, October 2015

Supervisors: Danai Pervanidou, Agoritsa Baka

On the 9th of October 2015, two locally acquired *P. vivax* malaria cases were notified to HCDCP in patients from Greece. The cases were epidemiologically linked (family members, father and son). According to the case investigation, the patients had never travelled to a malaria endemic country and the estimated place of exposure was their house/field in the village of Kato Souli, Municipality of Marathon. As the risk of re-establishment of malaria in Greece exists in vulnerable and receptive areas, where the presence of adequate numbers of Anopheles mosquitoes is combined with the presence of migrant populations from malaria endemic countries, a focus investigation around the estimated area of exposure was initiated. The aim of this investigation was to timely assess and minimise the risk for further malaria local transmission. The focus area was defined as up to ~250 meters around the house/field of the cases. In this area, the investigation team visited a total of 36 households, including 12 migrant residencies. Demographic information and fever/symptoms history was recorded for each of the residents. Twenty persons (migrants) were tested by RDTs and 19 blood samples were also tested by microscopy and PCR. Total of 100 individuals were informed about the cases, malaria symptoms and personal protection measures against mosquitoes. Informative multi-lingual material regarding malaria and protective measures was also distributed.

No additional malaria cases were detected during this re-active case detection in the focus area or since then. The presence of large migrant populations from malaria endemic countries residing and working in the area was confirmed. Despite the fact that no additional malaria cases were identified in the particular area, further vigilance and enhanced response activities were recommended including enhanced surveillance, fever screening for the migrants from malaria endemic countries residing in the area, communication activities and intensified vector control. Finally, the need for interpreters/mediators in order to effectively communicate with migrants was emphasised.

Kyriaki Tryfinopoulou was involved in the field investigation as a member of the multidisciplinary team and participated within all the aforementioned activities of focus investigation which is a crucial component of the current Action Plan for the Management of Malaria 2012-2015 in Greece.

C. Invasive Meningococcal Disease (IMD) Outbreak investigation in a Roma population in the greater Athens area, Greece, March 2016

Supervisors: Georgina Tzanakaki, Konstantinos Kesanopoulos, Theano Georgakopoulou

A cluster of three IMD cases occurred in a Roma population camp in the Greater Athens Area (Menidi, Attica) on 27 and 28 March 2016. The patients were infants aged 16, 17 and 21 months old, and in two the outcome was fatal. Initially, blood samples from cases were sent to the National Meningitis Reference Laboratory and *Neisseria meningitidis* was identified within 4 hours. Twenty four hours later, the available meningococcal isolates were sent to the laboratory for further characterization. All three isolates were identified as *N. meningitidis* serogroup B and were identical by VNTR-analysis, confirming the presence of a single clone causing the outbreak. The isolates were further characterized as B: P1.18-1,3 : F1-5; for PorA and fetA respectively. WGS revealed that, only one peptide variant of the three included in the Men4CB vaccine was present, while MATS analysis revealed that, two of three antigens were expressed. An outbreak investigation team from HCDCP was immediately deployed to the Roma camp in order to proceed with the epidemiological investigation, contact tracing and chemoprophylaxis administration to all close contacts. During the field investigation, 128 contacts were recorded (71 children and 57

adults), and chemoprophylaxis was administered. No further IMD cases were reported after chemoprophylaxis was given.

In this outbreak investigation, the fellow had the great opportunity to be familiarised with a broad range of laboratory methods used during an IMD laboratory outbreak investigation and to understand the strengths and limitations of diagnostic and typing methods and their interpretation.

Training modules: The EPIET/EUPHEM introductory course familiarised participants with the methods and logistical aspects of outbreak investigations. The module "Outbreak investigation: from data analysis to communication of findings" provided fellows the opportunity to acquire skills and competencies regarding data management (entering, validating and cleaning data), data analysis and effective and appropriate data communication during outbreak investigations. The "Multivariable analysis module" provided a more comprehensive understanding of the principles of multivariable analysis and familiarised participants with the use and interpretation of different types of regression models.

Educational outcome: the fellow participated in multi-disciplinary outbreak teams, had hands-on involvement in outbreak investigations; case definitions, active case finding, questionnaire design, data collection, data analysis, field work, laboratory results interpretation and communication, formulation of recommendations and preparation of outbreak reports.

1.2. Surveillance

A. Laboratory surveillance of neuraminidase inhibitors (NAIs) susceptibility of influenza type A viruses circulating during the pandemic and post-pandemic seasons (2009-2015) in Greece

Supervisors: Athanasios Kossyvakis, Andreas Mentis

Neuraminidase inhibitors (NAIs) are used for the treatment of severely ill patients who are infected by seasonal influenza type A or B viruses. The severity and mortality rates of influenza cases were apparently higher during the post-pandemic than pandemic period in Greece leading to a consequential recent extended use of NAIs in the country. This extended use of NAIs may be associated with an increased risk for the emergence of resistant viruses, thus threatening the effectiveness of NAIs. In light of all these, the laboratory surveillance for NAI susceptibility of influenza viruses is crucial in both clinical and public health terms. The aim of the project was to determine the susceptibility to NAIs of influenza type A viruses circulating during the pandemic (2009-2010) and post-pandemic periods (2011-2015) in Greece. In parallel, molecular characterisation and concomitant phylogenetic analysis of NA and HA sequences were performed in order to decipher the resistance- conferring and permissive mutations. One hundred thirty four representative influenza A (H1N1)pdm09 and 95 influenza A (H3N2) viruses obtained and were tested for susceptibility to the neuraminidase inhibitors oseltamivir and zanamivir. Antiviral resistance was assessed by neuraminidase sequence analysis - as well as the fluorescence-based 50% inhibitory concentration (IC50) method. Only five influenza A(H1N1)pdm09 viruses (2,2%) exhibited highly reduced inhibition by oseltamivir, all isolated from immunocompromised patients. Zanamivir susceptibility was not affected. In all five cases, the H275Y oseltamivir resistance-conferring neuraminidase substitution emerged after oseltamivir treatment. No influenza A(H3N2)viruses with altered susceptibility to oseltamivir or zanamivir were detected. Based on phylogenetic analysis, oseltamivir-resistant viruses did not form a separate cluster. A pattern of permissive neuraminidase mutations was unearthed in both oseltamivir-resistant and -susceptible viruses in post-pandemic seasons. Several amino acid substitutions in the HA1 domain of the haemagglutinin gene of both post-pandemic A(H1N1)pdm09as well as A(H3N2) influenza viruses were discovered. The emergence of antiviral resistance in immunocompromised patients and the relation of neuraminidase and haemagglutinin mutations merit further investigation in terms of efficient infection control and evolutionary analysis of the virus, respectively.

In this project the fellow actively participated in several stages and learned by doing various aspects of influenza laboratory surveillance, including antiviral drug susceptibility monitoring, molecular characterization, phylogenetic analysis of seasonal influenza viruses and deposition of genetic and antigenic data to GISAID (genetic database), and TESSY, ECDC. The results of this study were presented at the 9th European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), Stockholm, November 2015. The fellow is a co-author of a manuscript accepted for publication.

B. Surveillance of resistance mechanisms to extended spectrum cephalosporins and carbapenems in human and animal *Salmonella enterica* isolates, Greece, 2008-2014

Supervisors: Georgia Mandilara, Panagiota Giakkoupi, Prof A. Vatopoulos

The monitoring in human *Salmonella* isolates of the prevalence of ESBL, plasmid-encoded Ambler class C β -lactamases (pAmpC) and carbapenemase phenotypes and the comparison with animal or food isolates is very useful

in national but also European and international level, as it can aid, as an epidemiological marker for the recognition of epidemic cross-border spread of multi-drug resistant *Salmonella* strains. During this project, 3,199 *Salmonella* isolates were screened retrospectively. Among them, 2556 were human isolates submitted to the National Reference Centre for *Salmonella* (NRCS) from public & private hospitals and diagnostic laboratories from all over Greece. The remaining 643 isolates recovered from food or food producing animals and were isolated at the National Reference Laboratory for *Salmonella* in animals (NRL-Vet) during official National monitoring programs and EU baseline studies. In all isolates with reduced susceptibility to ESC, the ECDC guidelines for presumptive and confirmed identification of ESBL, pAmpC and carbapenemases were performed. Only twelve *Salmonella enterica* isolates (0.37%), ten human and two of poultry origin, assigned to ten different serotypes were found ESCs resistant. In total three different ESBL (CTX-M-type, SHV-type, and GES/IBC-type) and 2AmpC-type enzymes (CMY-2 and DHA-1) families were identified. The results showed that the ESCs resistance of human and poultry *Salmonella enterica* isolates in Greece is still rare and sporadic while no co-resistance to both cefotaxime and ciprofloxacin observed. However, various ESC resistance genes incorporated in various serotypes, common and rare ones, indicating the multiplicity of the respective *bla* genes acquisition events and the evolving underlying situation, which favours for further dissemination. Recommendations for the necessity for close collaboration, communication and systematic monitoring in both clinical and veterinary contexts of ESCs resistance rates, mechanisms and plasmids were formulated, and at this time, a common database now exists in Greece for human and animal *Salmonella* isolates whilst comparative analysis of the respective resistant plasmids is in progress.

The fellow was the primary investigator and was actively involved in all stages of this project. Besides the design of this laboratory surveillance study and the laboratory work, the fellow had the opportunity to closely communicate with veterinarians and participate in multidisciplinary meetings as indicated by the One Health approach. The results of this study were presented at the 9th European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), Stockholm, November 2015.

C. Entomological surveillance for West Nile virus (WNV) detection in mosquito pools during the transmission period July-November 2015 in Greece

Supervisors: Elina Patsoula, Stavroula Beleri

In Greece, since the first WNV outbreak in 2010, large-scale entomological surveys are launched every year during the transmission period covering the majority of the country and a database has been constructed and is maintained in HCDCP. According to these findings, the circulation of WNV has been recorded in mosquitoes of the species *Culex pipiens*, which has been implicated as the West Nile virus vector in Greece, without excluding the involvement of other species in the transmission cycle, as *Ochlerotatus caspius*. From June to October 2015, mosquitoes sampling stations were located in different regions of Greece, where traps were placed in locations with a higher risk of WNV transmission (reported human cases over the previous years, large breeding sites of *Culex spp*). Mosquitoes were sent to the Department of Parasitology, Entomology and Tropical Diseases where they were systematically identified to species level and were pooled according to date, location and species. All mosquitoes of the species *Culex pipiens* and *Ochlerotatus caspius* were analysed for the presence of WNV RNA by real-time PCR. All insect surveillance data were communicated to the relevant authorities both in central and regional level for timely risk assessment and decision making for targeted vector control measures and for the evaluation of the effectiveness of mosquito control measures.

The fellow familiarized with the systematic mosquito identification to species level, using keys for morphological characteristics and also with the laboratory protocols for the detection of WNV RNA by real-time PCR. Communication with epidemiologists from the vector-borne diseases office of HCDCP in a regular basis helped the fellow to understand that the surveillance of a Mosquito-borne disease, such as WNV disease, requires the surveillance of human health and of intermediate hosts along with vector surveillance.

Training modules: The EPIET/EUPHEM introductory course familiarised participants with the main concepts of surveillance, including how to develop, validate, evaluate and operate a surveillance system. The Vaccinology module taught participants the main aspects of vaccine-preventable diseases surveillance, including vaccine coverage and efficacy. Finally, the rapid assessment and survey methods module provided fellows with knowledge on how to contribute to the multidisciplinary response to complex emergencies and apply their epidemiological skills to serve diseases surveillance.

Educational outcome: The fellow learned by doing several crucial aspects of surveillance, such as the need to integrate microbiological and epidemiological data in disease surveillance, the analysis of combined syndromic and laboratory surveillance data, the laboratory-based surveillance, the operation of microbiological support for surveillance systems, entomological surveillance and participation in disease-specific networks at national and European level.

2. Applied public health microbiology research

A. Detection, characterization and sequencing of ESBL and AmpC encoding plasmids isolated from human and poultry *Salmonella enterica* and poultry *E. coli* isolates in Greece from 2008 to 2014

Supervisors: Greece: Georgia Mandilara, Panagiota Giakkoupi, Prof Alkis Vatopoulos,
Norway: Umaer Naseer and Ulf R. Dahle

The present project was conducted in collaboration with Umaer Naseer, EUPHEM fellow in NPHI Oslo, Norway. Kyriaki Tryfinopoulou was ECDC funded for her travel and stay for 2 weeks in Oslo. Plasmids are the principal vehicles for the dissemination of Extended Spectrum Cephalosporin (ESC) resistance genes. The aim of this project was to detect, characterise and sequence plasmids identified in ESBL or AmpC producing *Salmonella enterica* and *E. coli* isolates from human and poultry sources isolated in Greece from 2008 to 2014, since the comparative studies of plasmids between different species and in different hosts will give us insight into zoonotic transmission of resistance. The first part of this project took place in Athens, at the National Reference Laboratory for *Salmonella*, *Shigella* and VTEC, where all the conjugation experiments and the initial characterisation of the number and size of the respective plasmids were performed. The plasmid sequencing part took place in Norwegian Public Health Institute in Oslo. Nine human and two poultry *Salmonella enterica* isolates as well as two *E. coli* poultry isolates were found to transfer their ESC-resistance markers in laboratory conjugation experiments. The plasmids size ranged between 78 kb to 250 kb. Four plasmids carried only *bla* genes, while the remaining were multiresistant plasmids, carrying genes for co-resistance to other antimicrobial classes. Among the 10 plasmids assigned to a known Incompatibility group, 6 belonged to IncI 1 group. Of note, we found two *bla*CMY- carrying plasmids of the same size and the same ST type in a human *S. Montevideo* and a poultry *S. Typhimurium* isolate. Comparative studies on the plasmid backbone and the accessory genes among sequenced plasmids are in progress.

The fellow, in close collaboration with the EUPHEM fellow in Norway were the principle investigators. Kyriaki was involved in all stages of a PHM research project, from identification of the public health problem, revising literature, designing the first part of the study, applying and learning new laboratory methods, such as Whole Genome Sequencing analysis, collecting and analysing data to writing a scientific paper. The first manuscript is under final preparation and both fellows are sharing first authorship.

Training modules: The EPIET/EUPHEM introductory course familiarized fellows with the development and presentation of study protocols. The Initial management in Public Health Microbiology module focused on other laboratory aspects of research, such as time and stress management, teamwork and effective communication of the laboratory results.

Educational outcome: Kyriaki conducted all stages of a PHM research project, from planning to write a scientific paper, identified the use and limitation of diagnostic and typing methods and their interpretation in surveillance and molecular epidemiology studies. She applied and learned new laboratory methods.

3. Applied public health microbiology and laboratory investigations

A. Meningococcal carriage in military recruits and university students during the pre MenB vaccination era in Greece (2014-2015)

Supervisor: Georgina Tzanakaki, Konstantinos Kesanopoulos, Athanasia Xirogianni

The relation between asymptomatic meningococcal carriage and invasive disease is not clearly understood. Carriage studies can provide valuable information on epidemiology, pathogenesis, serogroup distribution and possible transmission patterns, information which helps understanding the potential effects of control programs, such as vaccination. The main objectives of this study were to estimate the meningococcal carriage rate in healthy individuals aged between 18-26 years old, to identify the genogroups and compare those with previous carriage studies in similar populations in Greece, and to obtain baseline data on circulating genogroups and genotypes. This data may serve for future comparisons and estimations of the possible effect of immunization with the new multicomponent Men B vaccine (4CMenB), which is not yet broadly implemented in Greece, on the dynamics of meningococcal carriage. A total of 1420 oropharyngeal single swab samples were collected from military recruits and university students on voluntary basis. New York City Medium was used for culture and the suspected *N. meningitidis* colonies were identified by Gram stain, oxidase and rapid carbohydrate utilization tests. Further characterisation was carried out by molecular methods (multiplex PCR, MLST, WGS). The odds ratios for known factors associated with *N. meningitidis* carriage and the respective 95% confidence intervals were calculated. The overall carriage rate was of 12.7%; 15% and 10.4% for recruits and university students respectively. Meningococcal carriage rate in both groups was lower compared to our previous studies (25% and 18% respectively). MenB (39.4%) was the most prevalent followed by MenY (12.8%) and MenW (4.4%). Among the initial 76 Non Groupable (NG) isolates, Whole Genome Sequence Analysis (WGS) revealed that 8.3% belonged to MenE, 3.3% to MenX and 1.1% to MenZ, while, 53 strains (29.4%) were finally identified as capsule null (cni). Genetic diversity was found among the MenB isolates, with 41/44 cc and 35 cc predominating. These findings, have important implications on the epidemiology of meningococcus, pinpointing the public health importance

during the pre-MenB vaccination era in Greece, especially as the predominant clonal complexes within the MenB such as 41/44cc and 35cc are associated with meningococcal disease.

The fellow participated actively in all stages of the project, from the field carriage studies to writing a final manuscript as first author. The results of this project will be presented at the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), Stockholm, November 2016. The manuscript has been accepted for publication in PlosOne.

B. Bloodstream *Candida parapsilosis* molecular epidemiology and EUCAST susceptibility profiles to established antifungals and isavuconazole. Study in two Greek paediatric hospitals (2010-2014)

Supervisors: Aristeia Velegraki

Invasive candidiasis, most commonly represented by candidaemia, is a severe infection that causes high morbidity and mortality, whilst its incidence has been increasing throughout the world in recent years. The project aimed to provide, for the first time in Greece, retrospective data on the molecular epidemiology and antifungal susceptibility profiles of *C. parapsilosis* (sensu stricto-s.s) bloodstream isolates from two major paediatric hospitals in Athens in order to expand our understanding on the epidemiology of these invasive infections and on the transmission dynamics within the PICU and NICU environment. The study provided data for the molecular epidemiology of *Candida parapsilosis* bloodstream infections in critically ill children and neonates, a specific population at risk, not extensively nor systematically studied in comparison with adult patients in Greece thus far. During this laboratory investigation, Single Strand Polymorphism (SSCP) analysis of the Internal Transcribed Spacer (ITS) barcoding region was performed to certify its ability on rapid and accurate species identification within the species complex, and its results were confirmed by ITS sequencing. Furthermore, current yeast genotyping methods such as minisatellite DNA typing was used to attest its performance on rapid screening for *C. parapsilosis* hospital outbreak detection. Finally, the EUCAST reference microdilution method was used for susceptibility testing. SSCP analysis differentiated *C. parapsilosis* (s.s) within the *C. parapsilosis* species complex. M13 PCR fingerprinting using the strict 100% cut-off for genetic relatedness clustered the 37 *C. parapsilosis* isolates in 11 discrete genotypes with one being prevalent in both hospitals during the study period. The observation that one M13 profile remained in the respective hospital environments for years is an alert for the possible existence of specific reservoirs. All isolates were susceptible to amphotericin B, 14 isolates were intermediate susceptible to the echinocandins, 14 isolates were resistant to at least one azole. Low MICs were recorded for isavuconazole (range 0.01-0.12 mg/L). The obtained results lead to conclusions on the usefulness of the abovementioned methods as rapid screening methods for early detection and genotyping of *C. parapsilosis* which can be involved in outbreaks together with data on susceptibility profiles that can be used in future comparative studies.

Training modules: The introductory course and the initial management in public health microbiology module provided useful and necessary knowledge to the fellow helping her to complete her projects successfully.

Educational outcome: The fellow has deepened her knowledge in public health microbiology applying concepts of bacteriology and mycology to the public health disciplines, identifying the use and limitation of diagnostic and typing methods and their interpretation in patient diagnosis, outbreak investigations, surveillance and epidemiological studies in the community as well as in the hospital setting. She also familiarised with the preparation of study protocols, time management and team working.

4. Biorisk management

A. Procedural Biorisk Management Report for the National Reference Laboratory for Mycobacteria (NRLM_GR) "Sotiria" Chest Diseases Hospital of Athens (TB diagnostic services)

Supervisors: Dimitrios Papaentsis, Simona Karabela, Evangelos Vogiatzakis

During this project, procedural biosafety risk assessments were conducted using the BioRAM tool at the National Reference Laboratory for Mycobacteria (NRLM), Athens, Greece, on March and April 2016. The biorisk assessments were conducted by the fellow along with the Biosafety Officer and the Quality Manager of the lab. BioRAM software was used for the biosafety risk assessments in three different TB laboratory procedures; a) direct molecular testing with XpertMTB/Rif, b) clinical specimen handling for acid fast smear preparation and inoculation to LJ for solid culture, and c) manipulation of *M. tuberculosis* cultures for phenotypic Drug Susceptibility Testing. The NRLM_GR is by definition a high risk level TB laboratory (TB-containment laboratory), performing tests that cover the entire Mycobacteriology diagnostics and research range. All the implemented biorisk mitigation measures in the NRLM_GR, a BSL-2 laboratory with BSL-3 practices, have been taken into account through the biorisk assessment process. The relative biosafety risks as assessed with BioRAM software for the three different procedures have been judged as acceptable. However, there is a place for improvement and the need for financial support for the construction of a BSL-3 laboratory for the ideal safety of the laboratory personnel that virtually

provide high-quality TB diagnostics for the entire country.

During this project, the fellow was familiarized with the most recent biosafety guidelines concerning a TB laboratory and the factors that drive the risk governance in these settings.

B. Biosafety and Biosecurity Risk assessment at National Meningitis Reference Laboratory (NMRL)

Supervisor: Georgina Tzanakaki

Biorisk assessment at the NMRL using the BIORAM Biosafety and Biosecurity software was conducted by the fellow, in collaboration with the Head of the lab. The BIORAM assessment report was part of the whole Biorisk management program of the lab in the frame of the annual external accreditation process according to ISO 15189:2012 Medical Laboratories - Requirements for Quality and Competence by the Hellenic Accreditation Body (ESYD). The biosafety and biosecurity risks posed by the working microorganisms in different procedures were assessed as acceptable due to the personnel compliance to the biosafety guidelines and practices and the implemented biorisk mitigation measures.

Training modules: The Biorisk and Quality management module provided training on techniques for biorisk assessment and mitigation, including WHO recommendations on laboratory biorisk management. One day was dedicated to the international regulations for the transportation of dangerous goods and WHO certification was acquired on completion. A visit to the BSL4 laboratory as part of this module illustrated all aspects of biosafety management learned during the module.

Educational outcome: the fellow deepened her knowledge on biorisk management and had the opportunity to conduct Biorisk assessments in different laboratories. Also she practised safe laboratory procedures, decontamination and experienced different personal protective equipment when working on practical parts of her various projects.

5. Quality management

A. Annual EARS-NET External Quality Assessment (EQA) exercise through the scheme: UK NEQAS for special surveys, distribution 3628

Supervisor: Panagiota Giakkoupi, Prof Alkiviadis Vatopoulos

This project was part of ECDC network activities (EARS-Net) and the aim was to improve national diagnostic accuracy in the surveillance of Antimicrobial Resistance (AMR) in order to collect comparable and validated AMR data at the EU level. The main objective is to allow participants to monitor, evaluate and improve their own performance in antimicrobial susceptibility testing through the same educational tool. Unsatisfactory performance requires corrective and preventative actions. During this EQA exercise, distribution 3628, in total six specimens were sent for identification and susceptibility testing. The results of lab performance in this exercise were used for the annual laboratory accreditation inspection according to EN ISO:17025. The performance in all pathogen-antibiotic combinations were interpreted as satisfactory and no corrective or preventative action was needed.

B. WHO-Global Foodborne Infections Network External Quality Assurance System (EQAS) on serotyping and antimicrobial susceptibility testing of Salmonella and Shigella strains, 2014

Supervisor: Georgia Mandilara, Prof Alkiviadis Vatopoulos

The fellow was familiarised with the *Salmonella* and *Shigella* full antigenic typing (antigen, serogroup, serotype) EQA scheme. Overall, 8 *Salmonella* and 4 *Shigella* strains were sent for serotyping and antimicrobial susceptibility testing. The performance of the lab within the EQA was interpreted as satisfactory and no corrective or preventative action was needed.

C. Quality management audit at National Meningitis Reference Laboratory

Supervisors: Georgina Tzanakaki, Konstantinos Kesanopoulos, Athanasia Xirogianni

The fellow had the opportunity to become familiarised with the evaluation of the quality management of a laboratory using an evaluation tool provided by the Head of EUPHEM in the frame of the IPHMM module, as post module homework. The fellow conducted the quality management audit in the National Meningitis Reference Laboratory, which is a laboratory accredited according to ISO:15189. The quality manager and the Head of the laboratory responded to all the questions regarding the accommodation and environmental conditions, the quality management policy, the internal controls in use, the participation of the laboratory to an External Quality Assessment program, several critical steps during the pre-, analytical and post-analytical process, and procedures

in place for quality improvement. In addition, a thorough evaluation of the existing documentation in the laboratory was conducted. The quality assurance system of the laboratory was evaluated as efficient and satisfactory, up to 100%.

Training modules: In the Biorisk and Quality management module the fellows were familiarised with all aspects of laboratory quality management, including EQA and accreditation procedures.

Educational outcome: Throughout her various projects the fellow had the opportunity to become acquainted with the quality management in different laboratories and she practised in different EQA and accreditation procedures and standards, analysed results of EQAs and participated in EQA reports.

6. Teaching and pedagogy

A. Translation in Greek and facilitation of 3 case-studies to MSc (PH) students at National School of Public Health (NSPH)

The following case-studies were translated in Greek and delivered by the fellow, and were incorporated in the educational material provided to the MSc (PH) students in NSPH(core infectious diseases):

1. "Laboratory-confirmed cases of meningococcal disease in Saudi Arabia: effects of the Hajj vaccination policy according to the publication: Memish Z, Al Hakeem R, Al Neel O, Danis K, Jasir A, Eibach D. Laboratory-confirmed invasive meningococcal disease: effect of the Hajj vaccination policy, Saudi Arabia, 1995 to 2011. Euro Surveill.2013 Sep 12; 18(37).
2. "TB in Finland" based on the publication by Smit O, Vasankari T, Aaltonen H, Haanpera M, Casali N, Marttila H, Marttila J, Ojanen P, Ruohola A, Ruutu P, Drobniewski F, Lyytikainen O, and Soini H. Enhanced tuberculosis outbreak investigation using whole genome sequencing and IGRA. ERJ Express. Published on October 16, 2014 as doi:10.1183/09031936.00125914
3. "Salmonella in the Caribbean" (developed by a working group created by the World Health Organization (WHO), Last revision: Version - 24 September 2012, Aftab Jasir, Yvan Hutin with contributions from Androulla Efstratiou, Georgina Tzanakaki, Chris Williams and Pawel Stefanoff, Updated: September 5th, 2014: Aftab Jasir)

B. Design a Problem Based Learning (PBL) exercise to MSc (PH) students at NSPH (core infectious diseases) titled" Parapneumonic pleural effusions caused by *S.pneumoniae* serotype 3 in children immunised with 13valent pneumococcal vaccine "

Supervisor: Georgina Tzanakaki

The aim of this training activity was to introduce the MSc students of NSPH to basic problematic aspects of *S.pneumoniae* through a PBL exercise using a scenario about parapneumonic pleural effusions caused by *S.pneumoniae* serotype 3 in children immunised with 13valent pneumococcal vaccine. The learning objectives were: to acquire basic knowledge on the biology of *S.pneumoniae*, on the pathogenicity and the clinical significance of the microorganism and on pneumococcal vaccines. Also, to identify risk factors and populations at risk for severe pneumococcal disease and to interpret clinical and epidemiological information in relation to laboratory data. The exercise took place in two stages: stage 1-brain storming and stage 2-final presentations and discussion. During the second stage, fruitful discussions took place after each presentation and the students realized that, with all this actively gained knowledge, they were finally in position to discuss and find the solution to the problem.

C. Preparation and delivery lectures and practical exercises on Biorisk management

1. Preparation and delivery of a 4-hour lecture on "Biorisk management -the AMP module" to MSc students of NSPH (core: Public Health Microbiology)
2. Preparation and delivery of a 2.5 hour lecture on the components of Biorisk management focusing on Biorisk assessment to NSPH MSc students (core: Occupational & Environmental Health)
3. Preparation and delivery of a 2.5 hour practical exercise on Biorisk assessment with the use of BioRAM-lite software to NSPH MSc students (core: Occupational & Environmental Health)

D. Lecture during MediPIET Diseases-Oriented Training, Module 7: Food and Waterborne, Athens 18-20 April 2016 “Laboratory investigation of FWD outbreaks (clinical and food samples)”

Preparation and delivery of 1-hour lecture to 31 participants; MediPIET fellows from 2014-2016 cohort training sites (Albania, Fyrom, Lebanon and Tunisia) as well as public health professionals nominated by national authorities through the National MediPIET committee from various countries (Albania, Algeria, Armenia, Bosnia & Herzegovina, Egypt, Former Republic of Macedonia, Georgia, Israel, Kosovo, Lebanon, Libya, Jordan, Moldova, Montenegro, Morocco, Serbia, Palestine, Tunisia, Turkey and Ukraine). The content of the lecture included appropriate collection of clinical samples and food /water specimens and transportation, identification and characterization of the aetiological agents with focus in molecular typing techniques, interpretation of laboratory results and data flow and effective communication between public health microbiologists and epidemiologists during FWD outbreak investigations.

E. Lecture to laboratory staff, clinical doctors and members of the Infection Control committee “The role of bacterial molecular typing in the surveillance of MDR Gram-negative HAIs”

A one hour lecture was prepared and delivered by the fellow to health-care workers in the General Hospital of Athens “SOTIRIA” in the frame of the formal educational program of the laboratory. The purpose of the lecture was to familiarise both laboratory and clinical staff with the principles, techniques and interpretation of molecular typing along with epidemiological data. Also to demonstrate the value of molecular typing for both outbreak investigation and surveillance of multidrug Gram-negative hospital-acquired infections; a serious and difficult to battle Public Health problem in Greece.

Educational outcome: the fellow learned and gained experience on planning and organizing lectures, including defining learning objectives, preparing lecture material and exercises, and delivering/facilitating lectures and case studies to multidisciplinary audiences.

7. Public health microbiology management

A. National audit of Tuberculosis diagnostic services in Greece

Supervisors: Dimitrios Papaventsis, Panayiotis Ioannidis, Evangelos Vogiatzakis

The current financial situation in Greece along with the contemporary refugee crisis with thousands of refugees seeking asylum in Europe, the majority through Greece as the main point of entry, could likely affect both the functioning of the healthcare system and factors associated with TB epidemiology. Into this framework, the public health importance of the timely and accurate laboratory diagnosis of TB is considered a central component of any TB control program, as well as for the disease surveillance in Greece and in Europe. The aim of this project was to plan, develop and implement National Audit of TB diagnostic services in Greece through an electronic questionnaire using the most recent national, European and international standards and guidelines in order to evaluate the capacity and to what extent the quality and biosafety requirements are fulfilled in the national TB laboratory network. Data returned were evaluated in terms of response rate, current laboratories capacity and active networking, TB quality assurance indicators and compliance with basic biosafety guidelines. The gathered data were compared with a previous audit conducted during 2005, in order to have an estimation of the capacity trend over time. In addition, this audit was expected to function as a tool for the evaluation of the national TB laboratory network performance, for the identification of possible gaps and for the effective communication with Public Health stakeholders.

During this project, the fellow was familiarised with TB laboratory management, TB standards including ECDC and WHO diagnostic standards, guidelines, protocols and algorithms, the design and implementation of an audit scheme, the preparation of the questionnaire and data analysis and interpretation. The fellow also recognized the need for quality and biosafety management, as core elements of management of a public health microbiological laboratory. A report of the audit results is in final preparation and will be presented to all national PH stakeholders.

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

Public Health microbiology management was an integral component of all projects and activities during the fellowship. This included laboratory management, ethical and integrity considerations, team working, research collaboration, time management, and working in several multidisciplinary teams with microbiologists, biologists, physicians, veterinarians, epidemiologists, laboratory technicians, and entomologists.

Training modules: The one-week module on initial management in public health microbiology focused on principles, roles and responsibilities in public health management. Topics included time management, how to apply different management styles, team building and team work, tasks delegation, provision of structured feedback and

strategies for stress management.

Educational outcome: Kyriaki experienced working in a multidisciplinary public health team and understood the need of team management and close collaborations in planning, scheduling and organizing research projects.

8. Communication

A. Publications

1. Tryfinopoulou K, Kesanopoulos K, Xirogianni A, Marmaras N, Papandreou A, Papaevangelou V, Tsolia M, Jasir A, Tzanakaki G. Meningococcal carriage in military recruits and university students during the pre MenB vaccination era in Greece (2014-2015). (PlosOne, accepted)
2. Kossyvakis A, Mentis AF, Tryfinopoulou K, Pogka V, Kalliaropoulos A, Antalis E, Lytras T, Meijer A, Tsiodras S, Karakitsos P, Mentis AF. Antiviral susceptibility profile of influenza A viruses; keep an eye on immunocompromised patients under prolonged treatment. (European Journal of Clinical Microbiology & Infectious Diseases, accepted)
3. Georgakopoulou T, Mandilara G, Mellou K, Tryfinopoulou K, Chrisostomou A, Lillakou H, Hadjichristodoulou C, Vatopoulos A. Resistant Shigella strains in refugees, August-October 2015, Greece. *Epidemiol Infect.* 2016 May 16:1-5.
4. Lytras T, Kossyvakis A, Melidou A, Andreopoulou A, Exindari M, Gioula G, Kalliaropoulos A, Tryfinopoulou K, Pogka V, Spala G, Malisiovas N, Mentis A. Influenza Vaccine Effectiveness in preventing hospitalizations with laboratory-confirmed influenza in Greece during the 2014-2015 season: A test-negative study. *J Med Virol.* 2016 Apr 18. doi: 10.1002/jmv.24551
5. Tryfinopoulou K, Arabatzis M, Menounos P, Kritikou S, Polemis M, Velegraki A. Bloodstream Candida parapsilosis molecular epidemiology and EUCAST susceptibility profiles to established antifungals and isavuconazole. Study in two Greek paediatric hospitals (2010-2014) (in final preparation)
6. Kesanopoulos K, Tryfinopoulou K, Xirogianni A, Wong E, Papandreou A, Vassalos C, Georgakopoulou Th, Tsiodras S, Taha MK, Tzanakaki G. Investigation of an Invasive Meningococcal Disease outbreak in a Roma camp, Menidi, Attica, 27-29 March 2016 (in final preparation)
7. Tryfinopoulou K, Mellou K, Lambrou A, Kiritsi M, Katsiafliaka A, Potamiti-Comi M, Georgakopoulou T, Hadjichristodoulou C. A large waterborne Norovirus gastroenteritis outbreak in Northern Greece, August 2015: challenges for the investigation and management (in final preparation)
8. Tryfinopoulou K, Naseer U, Mandilara G, Valkanou H, Giakkoupi P, Dahle U, Vatopoulos A. Circulation of AmpC and ESBL producing epidemic IncI1 plasmids among *Salmonella enterica* isolates from human and poultry in Greece during 2008-2014 (in final preparation)

B. Reports

1. Summary of influenza activity in Southern Greece during the winter period 2014-2015 (weeks 40/2014 - 6/2015)
2. National Audit of TB diagnostic laboratories in Greece (in Greek and English, in final preparation)
3. A waterborne Norovirus outbreak investigation, Fourka, Chalkidiki, August 2015
4. Focus investigation – re-active malaria case detection in the focus of two locally acquired malaria cases, Kato Souli, Municipality of Marathon, Attica, October 2015
5. Invasive Meningococcal Disease outbreak in a Roma camp, Menidi, Attica, March 2016

C. Conference presentations

1. Tryfinopoulou K, Mandilara G, P.Giakkoupi, H. Kirikou, A. Charisiadou, A. Vatopoulos. *Salmonella enterica* diarizonae (50:z52:z35) carrying IBC-1 type extended-spectrum beta-lactamase (ESBL) isolated from a child, Greece 2013. European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), Stockholm, November 2014, poster presentation
2. K. Tryfinopoulou, G. Mandilara, H. Klonou, A.Vatopoulos. SHV-5-like extended-spectrum beta-lactamase (ESBL) in a Greek isolate belonging to the emerging monophasic *Salmonella enterica* subsp.*enterica* serovar Typhimurium 4,[5],12:i:- European clone. European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), Stockholm, November 2014, poster presentation

3. Tryfinopoulou K, on behalf of the project team. Retrospective study of resistance mechanisms to extended spectrum cephalosporins (ESCs) in human and food-producing animal *Salmonella enterica* isolates, Greece, 2008-2014. European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), Stockholm, November 2015, oral presentation
4. Tryfinopoulou K, Kossyvakis A, Pogka V, Kalliaropoulos A, Moutousi A, Tsiodras S, Karakitsos P, MentisAF. Neuraminidase inhibitor susceptibility profile of Greek seasonal influenza viruses during post pandemic seasons (2010 - 2014). European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), Stockholm, November 2015, poster presentation
5. Tryfinopoulou K, Kesanopoulos K, Xirogianni A, Marmaras N, Papandreou A, Papaevangelou V, Tsolia M, Jasir A, Tzanakaki G. Meningococcal carriage in military recruits and university students during the pre MenB vaccination era in Greece (2014-2015). Accepted at European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), Stockholm, November 2016 as oral presentation

D. Other presentations

1. Tryfinopoulou K. Ebola preparedness of Greece. Oral presentation to ECDC Director, Chief Microbiologist and Chief Scientist during the IPHMM, ECDC, Stockholm, February 2015
2. Tryfinopoulou K. Routine antimicrobial resistance surveillance systems-the example of WHO-Net. Workshop in the frame of 11th annual National Conference of Public Health, Athens, March 2016 (in Greek)
3. Tryfinopoulou K. The role of laboratory in Ebola and Complex Emergencies Situations (in collaboration with Horacio Gil-Gil, EUPHEM Fellow in Madrid, Spain). RAS module, Athens, June 2015
4. K. Tryfinopoulou. Retrospective study of resistance mechanisms to extended spectrum cephalosporins (ESCs) in human and food-producing animal *Salmonella enterica* isolates, Greece, 2008-2014 and Detection, characterization and sequencing of ESBL and AmpC encoding plasmids isolated from human and poultry *Salmonella enterica* and *E. coli* isolates in Greece from 2008 to 2014. Oral presentation at Norwegian Institute of Public Health, Oslo, February 2016

9. Training modules

A. EPIET/EUPHEM modules attended

1. EPIET/EUPHEM introductory course, Spetses, Greece, 2014 (three weeks)
2. Computer tools in outbreak investigations, Robert Koch Institute, Berlin, Germany, 2014 (one week)
3. Initial management in public health microbiology, ECDC, Stockholm, Sweden, 2015 (one week)
4. Biorisk and quality management module, ECDC, Stockholm, Sweden, 2015 (one week)
5. Multivariable analysis, AGES, Vienna, Austria, 2015 (one week)
6. Vaccinology, Public Health Institute, Krakow, Poland, 2015 (one week)
7. Rapid assessment module, NSPH, Athens, Greece, 2015 (one week)
8. Project review module, Lisbon, Portugal, 2015 (one week)
9. Bioinformatics and Phylogeny module, ECDC, Stockholm, Sweden, 2015 (3 days)
10. Project review module, Lisbon, Portugal, 2016 (one week)

B. Other training

1. Whole plasmid sequencing by Illumina MiSeq NGS technology, Norwegian Institute of Public health, Oslo, Norway (2 weeks, January-February 2016)
2. 19th EBSA conference and pre-conference course "Design and implementation of the biorisk management", 19-22 April 2016, Lille, France
3. Internship in the National Reference Laboratory for *N.gonorrhoeae* - laboratory surveillance of gonococcal resistance to antimicrobials at Hellenic Pasteur Institute (one week, November 2015)
4. Internship in medical parasitology (Dr E. Vassalou, Dept of Parasitology, Entomology and Tropical Diseases, NSPH) ; conventional laboratory methods for diagnosis of malaria (preparation and study of thin and thick films), in both routine and archive samples. Demonstration of concentration methods for diagnosis of filarial infections and for intestinal parasites identification and diagnosis (2 weeks, July-August 2015)

Discussion

Coordinator's conclusions

One of the main goals of the EUPHEM programme is to expose fellows to diverse and multidisciplinary public health experiences and activities, thus enabling them to work across different disciplines. This report summarises all activities and projects conducted by Kyriaki Tryfinopoulou during her two-year EUPHEM fellowship (cohort 2014) as a member state track fellow at the National School of Public Health, Athens, Greece. Kyriaki is the first appointed MS track EUPHEM fellow in Greece. The projects described in this portfolio demonstrate the breadth of public health microbiology. Outbreak and surveillance activities extended from regional to national and also international

outbreaks with excellent public health outputs in terms of formulation of recommendations, analysis of national databases and contribution towards disease specific networks at the national and European level. The outbreak investigations conducted incorporated all ten step steps' of an outbreak investigation and were diverse, ranging from foodborne, vaccine preventable to vector-borne disease investigations. The laboratory and epidemiologically based projects covered a range of disease programmes involving multidisciplinary working and teamwork on all levels such as physicians, laboratory technicians, epidemiologists, statisticians, government officials and public health officers, strengthening the fellow's ability to work within such an environment(s). 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. Projects had a clear outcome, with results communicated in many scientific journals and at conferences. The contributions made by this EUPHEM fellow towards public health in Greece and also within Europe as with all other fellows has highlighted the importance of developing a future critical mass of highly competent field public health microbiologists within Member States to contribute towards national preparedness as well as being available for international responses in the interest of the EU. The EUPHEM Coordinator Team concludes that the fellow has succeeded in performing all her objectives to a high standard and with a professional attitude. We wish the fellow every success in her future career as a public health microbiologist.

Supervisor's conclusions

Dr Kyriaki Tryfinopoulou was the first EUPHEM MS-track fellow trained among the Greek EUPHEM Consortium. Those two years of training turned out to be very successful for the fellow as she was involved on a great variety of Public Health activities as described in the core competences of the EUPHEM programme. Most importantly, the training programme was a great asset and an added value for the country as the fellow –through the training modules/projects- contributed in very important Public Health issues. During outbreak investigations, the fellow implemented analytical epidemiological investigation providing more evidence for the possible vehicle transmission and she gained experience on high throughput molecular techniques in laboratory outbreak investigation. The fellow's input on surveillance on resistance mechanisms to extended spectrum cephalosporins in human and animal *Salmonella* spp isolates was also an added value for the country since an important collaboration was strengthened among microbiologists and veterinarians resulting in a common database on resistance patterns on *Salmonella enterica* isolates of both human and animal origin. Most importantly, the detection, characterisation and sequencing of multi resistant common plasmids among human and animal *S. enterica* strains were carried out through the excellent collaboration initiated with the Norwegian Public Health Institute through the EUPHEM programme. Moreover, the results obtained from the fellow's research project on molecular epidemiology and anti-fungal susceptibility profile on *Candida parapsilosis* blood stream isolates, provided for the first time in Greece retrospective data in the paediatric population. Although Dr Tryfinopoulou was already trained on Biorisk management by WHO, through the additional training by EUPHEM programme, she was given the chance of implementing her expertise in the Reference laboratories/ members of the Greek EUPHEM Consortium. In addition, Quality management audit was implemented as a homework in one of the Reference Laboratories and this will eventually be expanded in many other laboratories. On teaching and pedagogy, the fellow has had the chance of implementing the methodology taught at the introductory course through case studies. This was achieved by translating and facilitating 3 case studies to our MSc Public Health students. Furthermore, the development of the Problem Based Learning study (PBL) on *S. pneumoniae* was an excellent example of actively gained knowledge to our MSc students for the first time in Greece.

Dr Tryfinopoulou's enthusiasm, high motivation and hard work gave us the joy of working with her during the past two years. Within a very short period of time, she was able to adjust herself rapidly in the multidiscipline and high demanding course not only focusing in the work she was given, but also involving herself willingly in all the activities of the host laboratory with a remarkable responsibility, accomplishing all assigned tasks in such a highly competent and professional manner. Additionally, Kiki was always pleasant and friendly, creating excellent relations with all members of the staff in each collaborative unit. I have no doubt that Kiki will continue her excellent work in the Public Health Microbiology area at both National and European Level.

Personal conclusions of fellow

The EUPHEM programme was for me a unique opportunity to work on diverse projects across various laboratories and departments covering the entire field of public health microbiology, in a continuous interaction with epidemiologists. I strongly consider this programme as the milestone of my carrier and I feel blessed for all wonderful people I have met and helped me broaden my perspectives in PH in general. I strongly believe that the structure of the programme, with all the modules and the projects provided by my training site, along with the "learning by doing" approach and multitasking everyday reality serves the objectives of EUPHEM in the most efficient way. In addition, the interaction with epidemiologists (facilitators, EPIET-fellows and local supervisors) during the modules, through case studies and simulation exercises, as well as during various projects was effective in building bridges between "epi" and "lab" colleagues. Because of this close collaboration, the added value in public health was shown in the most practical way. In general, I am proud to be part of the growing public health

microbiology community and I am trying to maintain and even strengthen my personal networks between European epidemiologists and PH microbiologists.

Acknowledgements of fellow

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Last but not least, my thoughts and gratitude go to my family for their love, understanding and support throughout my EUPHEM fellowship.