EUPHEM REPORT

Summary of work activities
Ani Ioana Cotar
European Public Health Microbiology Training
Programme (EUPHEM), 2013 cohort

Background

According to the European Centre for Disease Prevention and Control's (ECDC) Advisory Group on Public Health Microbiology, 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 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 Ani Ioana Cotar, cohort 2013 of the European Public Health Microbiology Training Programme (EUPHEM) at the 'Cantacuzino' National Institute for Research-Development in Microbiology and Immunology, and National Institute for Public Health, Bucharest, Romania. Ani Ioana Cotar is a senior microbiologist, scientific researcher from Romania. Before EUPHEM. She was working at National Institute for Research and Development in Microbiology and Immunology (NIRDMI) Cantacuzino from Bucharest in Vector-Borne Diseases Laboratory and Medical Entomology.

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.

Methods

This report accompanies a portfolio to demonstrate the competencies acquired during the EUPHEM fellowship by specific projects, activities and theoretical training modules. Specific 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 outputs 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

Objectives of these core competency domains were partly achieved through project/activity work and partly through participation in the 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

Supervisors: Florin Popovici, Adriana Pistol, Irina Codita

A. An outbreak of possible food poisoning after a christening, Bucharest, 26 July 2014

On 28 July 2014, the National Centre for Communicable Disease Surveillance and Control (NCCDSC) was informed by the Public Health Authority of Bucharest municipality (PHAB) and the Institute of Infectious Disease 'Matei Bals' about a possible food poisoning outbreak after a christening held on 26 July 2014 in a Bucharest restaurant, and started an investigation to identify the source of the outbreak. Together with an EPIET fellow we performed a retrospective cohort study to identify the food item/s as possible vehicle/s of the outbreak. Cases were defined as persons who attended and ate at the christening organised on 26 July 2014 in a Bucharest restaurant, presenting vomiting and one or more of the following symptoms: diarrhoea, nausea, fever, headache, chills or abdominal pains, within the next 48 hours. We applied a telephone questionnaire to 32 participants identified by contacting the party organiser. Faecal specimens from patients and kitchen employees and swabs from the throat, nose and hands of restaurant employees and kitchen surfaces were tested for foodborne pathogens. Toxigenicity testing of *Staphylococcus aureus* isolates was performed by PCR and their genetic relatedness was established by Pulsed Field Gel Electrophoresis (PFGE). Thirty two out of 44 participants responded to the questionnaire, and 18 met the case definition. The univariable analysis couldn't point to a specific food item related to developing an illness, but the clinical manifestation, the incubation period, duration of illness and laboratory results suggested as the most plausible cause of this outbreak a staphylococcal food poisoning. Environmental samples from the kitchen surfaces and samples from the kitchen employees, were positive for enterotoxin-producing *Staphylococcus aureus* harbouring the enterotoxin D gene and had PFGE profiles which were indistinguishable. Environmental investigations at the restaurant facilities showed deficiencies in food hygiene practices. We recommended training the food handlers and other personnel in the hygienic preparation and serving of food and the implementation of infection control measures.

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

B. Modules

The EPIET/EUPHEM introductory course familiarised participants with the methods and logistical aspects of outbreak investigations and the role of public health microbiology laboratories in an outbreak investigation.

The module 'Computer tools in outbreak investigations' taught essential data management skills (entering, validating and cleaning data), dataset management and how to perform descriptive and analytical studies (e.g. case-control and cohort studies, including stratified analyses).

The module 'Multivariable analysis' familiarised participants with basic definitions and concepts for the different types of regression models and provided the skills needed to perform and interpret multivariable analysis and to communicate the results.

Educational outcome: Participation in outbreak team meetings, involvement in outbreak investigations (case definitions, active case-finding, questionnaire design, data collection, data analysis, on-site visits), applying molecular typing techniques to provide outbreak investigations with microbiological support, writing of a report, communication of findings at a conference and writing a scientific article.

1.2. Surveillance

Supervisors: Adriana Pistol, Florin Popovici, Lavinia Zota, Denisa Janta, Vasilica Ungureanu, Maria Damian, Irina Codita

A. Introduction of scarlet fever surveillance system in Romania

In 2013, 1 887 cases of scarlet fever from the whole of Romania were reported to National Centre for Communicable Disease Surveillance and Control (NCCDSC). However it was unknown how the diagnosis of scarlet fever was established, and most cases were likely based only on clinical diagnosis. The incidence of reported scarlet fever in 2013 in Romania was 8.83/100 000. In Romania, there is no an established surveillance system for infections determined by Group A (β-haemolytic) Streptococcus (GAS), although cases are reported to a national statistic system.

The aim of this project was to introduce a surveillance system for scarlet fever in Romania to provide useful information about the number of cases, and to detect outbreaks of scarlet fever in order to implement prevention and control measures. At present, we do not have a national prevention policy on scarlet fever in Romania and there are national clinical guidelines for bacterial pharyngitis.

The specific objectives of the system were:

- the identification of scarlet fever cases to assess the magnitude of disease
- detection of scarlet fever outbreaks in the communities in order to implement control and preventive measures of outbreaks
- to acquire knowledge on the circulation of Group A (β-haemolytic) Streptococcus (GAS) strains isolated from scarlet fever outbreaks, and their phenotypic and genotypic characteristics.

The proposed case definition included as:

- clinical criteria: any person aged under 15 years who met the following three criteria: angina, fever and characteristic maculopapular rash
- laboratory criteria: isolation of GAS by culture from throat swab
- epidemiological criteria: an epidemiological link (having spent at least four hours in the same classroom/group/house with a confirmed scarlet fever case).

The case classification was:

- possible case: any person under 15 years of age which met clinical criteria
- probable case: any person under 15 years of age which met clinical criteria and have an epidemiological link with a confirmed case
- confirmed case: any person under 15 years of age which met clinical criteria and laboratory criterion.

An outbreak was defined as an occurrence of three (probable/confirmed) cases of scarlet fever in a community (school, kindergarten, etc.) reported at a maximum of 10 days distance to one another. In the proposed methodology, all components of scarlet fever surveillance systems in Romania are described (surveillance objectives, disease case definition, frequency of reports, target population under surveillance, data source, reporting flow chart throughout the system, type of surveillance system, sampling strategy, geographic coverage, type of information and database, reporting forms, information management, information flow, data protection, data access). Each County Department of Public Health will perform the epidemiological investigation for outbreak, collecting clinical specimens from suspected cases as well as from their contacts for laboratory diagnosis. Confirmed or probable cases will be isolated until the completion of 48 hours of antibiotic treatment.

In each unit (kindergarten, school, other childcare units) where a scarlet fever outbreak is detected, a disinfection with disinfectants active against GAS will be performed by the educational unit.

The methodology was implemented in May 2015 and future analysis of surveillance data will provide useful information about the magnitude of this disease in Romania and whether changes or improvements in the surveillance system must be introduced for a better surveillance in the next season of scarlet fever transmission. The fellow's task was to make the draft design of the surveillance system for scarlet fever in Romania. The fellow collaborated with the epidemiologist supervisors as well as with other epidemiologists and microbiologists working in the public health system to accomplish this task.

B. Analysis of surveillance of acute diarrheal disease with Salmonella performed in Arges county, Romania

In Romania, the epidemiological surveillance of acute diarrheal disease (ADD) with mandatory notification and reporting according to the Government Decision no. 589/2007, is passive and takes place yearly in all County Departments of Public Health and of Bucharest municipality between June-October. During the whole of 2013, 79 261 ADD cases were reported. Among these 7 450 ADD cases (9%) were with known bacterial aetiology, and 1 457 of these were Salmonella cases (19%). During the surveillance period (June–October 2013) 3 278 ADD cases were reported with known bacterial aetiology. 1 198 of these (37%) were Salmonella cases.

An analysis of surveillance data of ADD cases in the last five years (2009–2013) showed the absence of reporting of ADD with known aetiology in Arges county. The analysis of data at the national level (the whole year, not only the five months of surveillance, June–October) regarding the number of diarrheal cases with Salmonella, also showed that between 2009–2013, Arges county did not report any case. Arges county has a population of 636 643 people, which represent 3% from the country's population. The present study aimed to provide data on the situation of gastrointestinal infection with *Salmonella* (non-Typhi serotypes) in Arges county in 2013 and 2014. The analysis consisted of meetings with staff involved in the reporting and diagnosis of ADD cases from: Arges County Department of Public Health, and from two main hospitals from the county: Pitesti Emergency County Hospital and Paediatric Hospital. These institutions represent the healthcare units which are most frequented by the population of Arges county.

After discussions with the epidemiology staff, a suspected cause of the lack of *Salmonella* cases reported could be related to problems in laboratory diagnostic capacity of ADD cases in hospitals from the county. The appropriate clinical specimens collected from children with ADD who present to the Paediatric Hospital were sent and processed by Pitesti Emergency County Hospital. In this context, the specific questionnaires were administered only to the laboratory staff from Pitesti Emergency County Hospital and Arges County Department of Public Health to find out about: human resources (number, qualifications, training, etc.), financial resources (reagents), laboratory diagnostic tests used for detection and identification of *Salmonella* isolates (according with type of biological specimen), the capacity of sending the isolated strains to Cantacuzino Institute for confirmation, and further characterisation.

Analysis of data obtained after the administration of the questionnaire to laboratory staff involved in performing diagnosis of ADD in Arges County Direction of Public Health showed that this laboratory has the capacity of performing detection and identification of *Salmonella* isolates from clinical specimens. Nevertheless, a very small number of clinical specimens arrive to this laboratory from the whole county. The limited number of specimens received by this laboratory can explain the lack of detection of *Salmonella* isolates in faecal specimens or rectal swabs.

Data analysis of the questionnaires administered to laboratory staff involved in the diagnosis of ADD cases from Pitesti Emergency County Hospital showed several problems. The main issues were: the lack of appropriate selective media for isolation of *Salmonella* from faecal samples or rectal swabs, as well as of serotyping reagents. These issues can result in incapacity of detection, identification and serotyping of *Salmonella* isolates. The main recommendation was that the laboratory staff to be trained to perform laboratory diagnosis of ADD cases, especially for detection and identification of *Salmonella* as a possible etiological agent. The fellow had the task to design the standardised laboratory questionnaire, and to perform visits to Arges County Department of Public Health and Pitesti Emergency County Hospital for discussions with epidemiologists and microbiologists. Another task was to analyse obtained data from administered questionnaires and to draw conclusions to make public health recommendations.

D. Modules

The EPIET/EUPHEM introductory course familiarised participants with the development, evaluation and analysis of surveillance systems. Building on this course, the module on 'multivariable analysis' demonstrated the principles, application and interpretation of multivariable analysis and its role in field epidemiology.

Educational outcome: Participation in disease-specific networks at the national level; analysis of laboratory-based surveillance systems at hospital, county and national level; setting-up a surveillance system in a country; questionnaire design; the formulation of specific public health recommendations.

2. Applied public health microbiology research

Supervisor: Cornelia Ceianu

A. Molecular epidemiology study on West Nile virus in mosquito pools of *Culex pipiens* collected in 2011–2013 from south-eastern Romania

West Nile virus (WNV) is endemic in south-eastern Romania and after the unprecedented urban outbreak in Bucharest in 1996 caused by a lineage 1 WNV, cases of West Nile fever were reported each year. Furthermore, in 2010 a new epidemic occurred, this time produced by a lineage 2 WNV, Volgograd 2007–like strain.

After the first WNV outbreak, a passive surveillance system during risk of transmission of WNV for neuroinvasive cases was introduced in Romania, in which telephonic reporting to County Departments of Public Health is performed in 24 hours after case detection. Also, an active surveillance system for febrile syndromes of unknown aetiology (possible West Nile fevers) is performed only after confirmation of a case of meningitis/meningoencephalitis/encephalitis with WNV in the area where a confirmed case was exposed, if the area is newly affected and in the area where there have been reported cases of acute infection in horses.

Also, according to the EU Directive 2004/33/EC regarding donated blood and blood-components security, donation restriction is imposed for 28 days in the area where human cases of WNV infection occurred. After a second larger geographical outbreak, the Romanian Ministry of Health Order 1438/2011 approved a Plan of Action to ensure transfusion safety against the risk of post-transfusion transmission of WNV infection in humans. In general, both mosquito and seroconversion in bird-based surveillance of WNV provide evidence of WNV activity before human cases are recorded, but it was shown that mosquito surveillance performed better in terms of early WNV circulation detection. Surveillance of WNV infection of mosquito vectors provides information on the level of its amplification prior to the emergence of disease in humans.

The aims of this project were:

- to characterise WNV genotype circulating in mosquito populations collected in 2011–2013 from south-eastern Romania
- to establish the abundance and the maximum likelihood of the infection rate in *Culex spp.* pools collected from Danube Delta site
- to evaluate linkages between the temperature/precipitation and the mosquito population size.

In 2011 to 2013, we collected and analysed 30 855 mosquitoes. About 75% of these insects were captured in the Danube Delta, and 189 of the 1 185 mosquito pools tested were real-time RT-PCR-positive for WNV genome. The phylogenetic analysis based on NS5 partial sequences indicated that the viruses in circulation in Romania between 2011 and 2013 were very similar to a lineage 2 WNV isolated during the outbreak in Volgograd, Russia, in 2007. All our mosquito sequences were more than 99% similar to that isolate. These results represent a molecular evidence for the maintenance of the same isolates of Volgograd 2007-like lineage 2 WNV in south-eastern Romania between 2011 and 2013. In this research, significant positive linkages were found between temperature and mosquito population size as well as with the infection rate, while the highest temperature anomalies (in July 2012) were followed by the highest infection rate (40.74/1 000). Our results indicate a lag of 20–30 days from the temperature rise and the increased rate of WNV infection in mosquito vectors, a time which may be used to inform the possible exposed communities of the increased risk, and to intensify vector control operations. Under conditions of climate change, for better preparedness, any assessment of future WNV transmission should take into consideration the impacts of weather fluctuations. To the best of our knowledge, this is the first field study in Europe showing the weather impact on the WNV infection rate in mosquito vectors. The linkage between WNV outbreaks in Romania and severe heatwaves has been shown in a few studies. Paz et al. (2013) observed that the WNV outbreak of summer 2010 in Romania was preceded by extremely hot spells with deviations of 5°C above the 30-year mean average. Strong significant positive correlations were detected between the number of cases in humans in south-eastern Romania, at lags of 1–3 weeks between the temperature increase and the eruption of the disease in the city of Bucharest, an area with a continental climate, and a lag of 2–4 weeks in the Constanta area, on the Black Sea shore.

The fellow's tasks were:
- processing collected mosquitoes for the detection of WNV genome and screening tests by real-time commercial TaqMan assay
- performing the molecular identification of individual mosquito females for each period of capture using enzymatic restriction of fragment of mitochondrial cytochrome c oxidase subunit I (COI) gene
- calculation of the abundance of *Culex* spp. mosquitoes for each of the *Culex* species - *Cx. modestus* and *Cx. pipiens* for every interval of capture
- calculation of the maximum likelihood of the infection rate in mosquitoes using the PooledInfRate software

- analysing obtained data, writing a manuscript and the final report of the project as well as presenting the results to conferences.

B. Modules

While the EPIET/EUPHEM introductory course focused on the development and presentation of study protocols, the module 'Initial management in public health microbiology' focused on laboratory aspects, time management and collaboration as a team.

Educational outcome: Preparation of study protocol; multidisciplinary teamwork that combines microbiology, molecular epidemiology, entomology and statistics; data analysis; writing of scientific articles; scientific presentation at conferences.

3. Applied public health microbiology and laboratory investigations

Supervisors: Maria Damian, Aftab Jasir, Cornelia Ceianu, Dan Ionescu.

A. Molecular typing of carbapenemase-producing *Klebsiella pneumoniae*

Carbapenemase-producing Enterobacteriaceae (CPE), notably *Klebsiella pneumoniae*, produce serious infection (urinary tract infections, septicemia, pneumonia, and intra-abdominal infections) in debilitated and immunocompromised patients, in association with prolonged hospitalisation and increased fatality ranging from 24% to 70%, depending on the study population. CPE are spreading globally as multidrug-resistant pathogens for which there are only few treatment options available. In Romania, little is known about the distribution and spread of carbapenemase-producing *Klebsiella pneumoniae*, and the type of carbapenemases produced. This situation can be explained by the lack of a surveillance system for carbapenemase-producing *Klebsiella pneumoniae* isolates in Romania. Also the detection of such strains is not easy to perform and interpret by all hospitals of infectious diseases from the country. A structured survey was necessary to improve the knowledge about epidemiology, detection and characterisation of carbapenemase-producing isolates circulating in Romanian hospitals as well as for implementation of appropriate hospital infection control measures. The study was performed as a part of the European Survey on Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) projectto characterise the carbapenem-non-susceptible *Klebsiella pneumoniae* isolates collected from eight Romanian hospitals. The antimicrobial susceptibility testing of *K. pneumoniae* isolates was performed by disk diffusion method, whereas screening for carbapenemase production was done by a combination of Kirby-Bauer disk-diffusion methods, MastDisc ID inhibitor combination disks and the biochemical Carba NP II tests. Confirmation of carbapenemases was performed by amplification of the genes $bla_{OXA-48-like}$, bla_{NDM}, bla_{KPC} and bla_{VIM}. Sequencing of the PCR products was used for determination of a sub-type of the carbapenemase gene, whereas genetic relatedness among carbapenem non-susceptible isolates was evaluated using Pulsed Field Gel Electrophoresis (PFGE).

Among 75 investigated carbapenem non-susceptible *K. pneumoniae* isolates, 65 contained one of three Ambler classes of carbapenemase, A (KPC-type), B (metallo-beta-lactamase) or D (OXA-type), whereas for 10 isolates no carbapenemase activity was detected. PCR results showed that 51 strains harbored bla_{OXA-48} gene, 8 strains had the bla_{NDM} gene, and the bla_{KPC} and bla_{VIM} genes were present in 4 and 2 strains, respectively. The analysis of PFGE profiles of OXA-48 and NDM-1 producing *K. pneumoniae* suggests inter-hospital and regional transmission of epidemic clones.

This study presents the first detection of *K. pneumoniae* strains harbouring blaKPC-2 and blaVIM-1 genes in Romania. The PFGE results of this study are indicative of a clonal dissemination of *K. pneumoniae* harboring all of the four carbapenemase genes across the sample of Romanian hospitals enrolled. There is an urgent need for strengthening of hospital infection control measures to protect patients from these multi-resistant bacteria in Romanian hospitals. As part of the European network (project, EuSCAPE) this data will contribute to better understanding of the carbapenemase-producing *Klebsiella pneumoniae* in Europe and act as a piece of evidence for action from the EU as well.

B. Introduction of a molecular detection method of Usutu virus in vector population from south-eastern Romania

Usutu virus (USUV), a mosquito-borne flavivirus, belonging to the Japanese encephalitis antigenic complex, has recently been introduced to Europe and is spreading through Austria, Hungary, Italy, Spain and Switzerland, causing disease in birds and humans. Like West Nile virus (WNv), USUV may become a resident pathogen in Europe and the consequences for public health should be considered. In Europe the risk exists that potential emerging infectious diseases, such as those caused by WNv or USUV, will not be recognised in time by existing surveillance infrastructures of the various European countries. Usutu virus has not been detected yet in Romania, although it was recorded in Central Europe, including neighbouring country Hungary, and it has been involved in causing human disease in Italy. In this context, the aim of this project was to investigate if this virus is circulating in the vector population in south-eastern Romania for implementation of standard surveillance measures and an early warning system to detect USUV activity, and to assess the risk for public health.

We tested a total number of 229 mosquito pools collected from two study sites: Bucharest capital city and Danube Delta. 108 pools were collected in 2012 (53 from Bucharest and 55 from Danube Delta), and 121 pools in 2013 (56 from Bucharest, and 65 from Danube Delta), which tested negative for West Nile virus. The sensitivity of the test was evaluated as follows: viral RNA was extracted from a USUV strain received from CDC, reverse-transcribed and obtained cDNA was used as standard to perform a standard curve by real-time PCR specific for USUV using serial tenfold dilutions (in triplicate) of the standard. The amplification plots obtained for USUV standard shows that the assay detected dilution up to $1:10^6$. We did not find any RNA sample positive for Usutu virus among 229 samples tested, but the in house real-time RT-PCR test was validated, and can be used in future studies and be shared with other European countries laboratory which might be at risk of Usutu virus transmission in the population. The fellow was the main investigator in this study and validated the RT-PCR.

C. Introduction of molecular typing method for the characterisation of Romanian *Neisseria gonorrhoeae* strains

Molecular typing methods have not yet been used for molecular characterisation of *N. gonorrhoea* circulating in Romania. At the European level there is an accepted molecular typing method for this pathogen, namely *Neisseria-gonorrhoeae* Multi Antigen Sequence Typing (NG-MAST), which differentiates strains on the basis of sequence variation in fragments of two hypervariable genes, the *porB* gene and subunit B of the transferring-binding protein (*tbpB*) gene. NG-MAST is highly discriminatory, relatively easy to use and supported by an open-access, online database (www.ng-mast.net), enabling easy comparison of alleles and providing clear assignation of new alleles and types. NG-MAST is widely used to investigate specific gonococcal antimicrobial resistance phenotypes, to investigate treatment failures.

The project had the aim to introduce this molecular typing method to characterise strains of *N. gonorrhoeae* isolated from Romanian patients. Introduction of this typing method in laboratories for sexually transmitted infections will provide the capacity to identify possible associations between ST and AMR (antimicrobial resistance) phenotype and patient characteristics in the future. The role of the fellow was to establish the method in the labs in Romania.

Educational outcome: Application of laboratory methods to analyse and interpret resistance mechanisms; identification of the use and limitation of typing methods and their interpretation in outbreak investigations, surveillance and epidemiological studies; understand the limitations of laboratory methods; data analysis; make scientific presentations at conferences and write scientific articles.

4. Biorisk management

A. Biorisk management module, ECDC, Sweden

This five-day module provided techniques for biorisk/biosafety assessment and mitigation, including World Health Organization (WHO) recommendations on biosafety management in laboratories. One day focused on international regulations for the transportation of dangerous goods, as determined by ICAO (International Civil Aviation Organization).

Educational outcome: Understand processes associated with BSL3/BSL4 laboratories; experience different personal protective equipment; understand the principles and practices of biorisk management; biorisk assessment and biorisk mitigation.

5. Quality management

A. External quality assurance (EQA) of sequence-based typing of Legionella pneumophila

The proficiency panel was organised by the ESCMID Study Group for Legionella Infections (ESGLI). Sequence-based typing (SBT) is the current method used for epidemiological typing of clinical and environmental *Legionella pneumophila* strains. The method is used in the investigation of outbreaks of legionellosis caused by *L. pneumophila*. The fellow performed all the steps for SBT by amplifying and sequencing the standard seven genes: *flaA, pilE, asd, mip, mompS, proA* and *neuA* or *neuA_*and learned more about interpretation and validation of EQA schemes.

B. External quality assurance (EQA) of PCR of chikungunya virus

The EQA was organised by ENIVD (European Network for Diagnostics of 'Imported' Viral Diseases). The fellow performed the EQA test for detection by PCR of chikungunya virus from 12 samples. This EQA scheme was performed for the first time in our institute by optimising the protocol used for RNA purification and RT-PCR. Very good results obtained show that the developed diagnosis of chikungunya virus by PCR can be used in the laboratories.

Educational outcome: Understanding and applying the principles and practices of biorisk management, quality assurance and quality control.

6. Teaching and pedagogy

A. Molecular diagnosis of bacterial infections

Lecture for physicians performing residency in Laboratory Medicine at Bucharest University of Medicine and Pharmacy, Romania.

B. Laboratory investigations of legionellosis: detection, diagnostic and typing

Training organised by the Public Health Institute for medical personnel working in public health (epidemiologists and microbiologists)

C. Molecular typing methods for infectious disease outbreaks detection and epidemiological surveillance

Lecture for physicians performing residency in Laboratory Medicine at Bucharest University of Medicine and Pharmacy, Romania.

D. Molecular biology techniques (PCR, real-time PCR, RT-qPCR) used in Microbiology laboratory

Theoretical and practical presentations to microbiologists, who are working at the National Research and Development Institute for Industrial Ecology)

Educational outcome: Planning and organisation of lectures; defining learning objectives and teaching laboratory and microbiology topics to epidemiologists and future microbiologists.

7. Public health microbiology management

A. Initial management in public health microbiology, ECDC, Stockholm, Sweden

This one-week module focused on understanding roles and responsibilities in public health management. Topics included the identification of different management styles, team roles and team evolution, the delegation of tasks and the provision of structured feedback.

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 building and coordination, research collaboration, time management, and working in a multidisciplinary team with microbiologists, physicians, epidemiologists, laboratory technicians, entomologists.

Educational outcome: Working in a multidisciplinary public health team; understanding team management; planning, scheduling and organising research projects.

8. Communication

A. Publications

1. Dinu S, Cotar AI, Pănculescu-Gătej IR, Fălcuţă E, Prioteasa FL, Sîrbu A, Oprişan G, Bădescu D, Reiter P, Ceianu CS. West Nile virus circulation in south-eastern Romania, 2011 to 2013. Euro Surveill. 2015;20(20):pii=21130
2. Cotar AI, Zaharia A, Dragulescu EC, Pistol A, Popovici F, Codita I. The role of molecular typing methods in the outbreak investigation of a possible staphylococcal food poisoning. Romanian Biotechnological Letters (accepted December 2015).
3. Lixandru BE, Cotar AI, Straut M, Usein CR, Cristea D, Ciontea S, Tatu-Chitoiu D, Codita I, Rafila A, Nica M, Buzea N, Baicus A, Ghita C, Nistor I, Tuchiluş C, Indreas M, Antohe F, Glasner C, Grundmann H, Jasir A, Damian M. Carbapenemase-producing *Klebsiella pneumoniae* in Romania: A six-month survey. Submitted to PLOS ONE 2015 accepted after revision.
4. Cotar AI, Fălcuţă E, Prioteasa FL, Dinu S, Pănculescu-Gătej IR, Ceianu CS, Paz S. Transmission Dynamics of West Nile Virus in Mosquito Vectors Populations under the Influence of Weather Factors in the Danube Delta, Romania. Submitted to EcoHealth 2015.
5. Cotar AI, Dinu S, Pănculescu-Gătej IR, Fălcuţă E, Prioteasa FL, Bădescu D, Ceianu CS. Bloodmeal preference of urban Culex pipiens sl in a West Nile virus endemo-epidemic area in Romania, as revealed by DNA barcoding. (In preparation).

B. Reports

1. Cotar AI. Molecular epidemiology on West Nile virus in mosquito pools of Culex pipiens, 2011-2013, Romania.
2. Cotar AI, Zaharia A. An outbreak of possible food poisoning after christening party, Bucharest, 26 July 2014.
3. Cotar AI, Codita I. Analysis of surveillance of acute diarrheal disease with Salmonella performed in Arges county, Romania.
4. Cotar AI. Introduction of a molecular detection method of Usutu virus in vector population from south-eastern Romania.

C. Teaching materials

N/A.

D. Conference presentations

1. Cotar AI, Lixandru BE, Straut M, Usein CR, Cristea D, Ciontea S, Codita I, Jasir A, Damian M. Survey of carbapenemase-producing *Klebsiella pneumoniae* in Romania, 2014. ESCAIDE, Stockholm, Sweden, 2015.
2. Cotar AI, Dinu S, Falcuta E, Prioteasa L, Gătej R, Ceianu C. Very active circulation of lineage 2 West Nile virus in mosquito vectors in southeastern Romania in 2012-2013. ESCAIDE, Stockholm, Sweden, 2014.
3. Cotar AI, Zaharia A, Dragulescu EC, Pistol A, Popovici F, Codita I. Investigation of a possible outbreak of staphylococcal food poisoning, Bucharest 2014. ESCAIDE, Stockholm, Sweden, 2015.
4. Cotar AI, Dinu S, Falcuta E, Prioteasa L, Gătej R, Ceianu C. Continuous circulation of lineage 2 West Nile virus strain Volgograd 2007-like in south-eastern Romania, 2011-2013. Symposium of the Institute Pasteur International Network, Paris, 2014.
5. Cotar AI, Dinu S, Falcuta E, Prioteasa L, Gătej R, Ceianu C. West Nile Virus Transmission in Mosquitoes in Danube Delta in the Context of Weather Factors. Genes, Ecosystems and Risks of Infection (GERI) Conference, Crete, Greece, 2015.

E. Selection of other presentations

Cotar AI. 'West Nile virus circulation in south-eastern Romania, 2011-2013'. Annual meeting of EDENext project, Rovaniemi, Finland, 2014.

9. International missions

10. EPIET/EUPHEM modules attended

- EPIET/EUPHEM introductory course, Spetses, Greece (three weeks)
- Computer tools in outbreak investigations, Robert Koch Institute, Berlin, Germany (one week)
- Biorisk and quality management module, ECDC, Stockholm, Sweden, 2014 (one week)
- Initial management in public health microbiology, ECDC, Stockholm, Sweden, 2014 (one week)
- Multivariable analysis, AGES, Vienna, Austria, 2014 (one week)
- Vaccinology, Public Health England, London, United Kingdom, 2014 (one week)
- Rapid health assessment module, Athens, Greece, 2014 (one week)
- Project review module, ECDC, Stockholm, Sweden, 2014 (one week)
- ESCMID & ESCV Observers, EUPHEM fellows, Visit ECDC, ECDC, Stockholm, Sweden, 2014 (one week)
- Project review module, ECDC, Lisbon, Portugal, 2015 (one week)

11. Other courses

Discussion

Coordinator's conclusions

During the two year fellowship, the fellow, supervisors and training site have demonstrated the capability of addressing communicable disease threats of national and European concern in a structured joint approach between public health microbiology and epidemiology. It is often the combination of specialised diagnostics and epidemiology that produces credible evidence for decisions on interventions. This is particularly well illustrated by the examples of the outbreak investigation at the christening party and the field research on WNV in South-East Romania.

In addition, this portfolio includes many examples of public health microbiology studies on topics that have relevance for public health in other EU Member States, such as reliable characterisation of neisseria gonorrhoea, external quality assurance procedures for reference diagnostics, surveillance of streptococcal diseases and monitoring of pathogens in vector populations. The involvement of fellows and supervisors in such projects within the context of an EU-wide training programme offers valuable opportunities for sharing good practice, knowledge and experiences.

Activities were in line with the 'learning by doing' and 'in–service' approach of the EUPHEM programme and followed the core competency domains described professionals in mid-career and above. Activities were complimented by nine training modules providing theoretical knowledge. Projects had a clear educational outcome, with results communicated in scientific journals and at conferences.

The EUPHEM coordinator team concludes that the fellow and the training sites have succeeded in providing and performing all EUPHEM objectives to a high standard and with a professional attitude. This included good leadership abilities and networking.

Supervisor's conclusions (Irina Codita, Maria Domian Florin Popovici and Adriana Pistol on behalf of the supervision team)

Ani Ioana Cotar is the first Romanian fellow taking part in EUPHEM MS-track, the specialist pathway of ECDC for training public health microbiologists.

We learned together and experienced a difficult but beautiful journey, whose main targets were the collaboration and integration in the big European family of public health field workers, in particular in Romania.

Ani Ioana Cotar's personal achievements and progress in respect to her public health microbiology skills and knowledge were remarkable during the EUPHEM fellowship programme, despite first contact difficulties and limited resources. We were impressed by her tenacity and fight in both technical microbiology issues and public health integration of her laboratory work and epidemiological links.

During the two fellowship years she demonstrated a high capacity to take multiple and complex tasks, which she was able to solve in due time and she very much improved her communication skills.

Personal conclusions of fellow

The EUPHEM programme presents the unique opportunity to work in diverse projects across various departments, thus covering the entire field of public health microbiology in a two-year period. The fellowship successfully bridges the gap between microbiology and epidemiology by maintaining a close connection to the European Programme for Intervention Epidemiology Training (EPIET) network. EUPHEM fellows benefit from the fact that they are not restricted to conducting laboratory-based research, but instead learn to conduct field studies with the help of a multidisciplinary team. Based on the various courses that are given during the two years in combination with considerate supervision and guidance, essential public health skills are strengthened and knowledge of public health microbiology is increased. The EUPHEM programme strongly contributes to the growing public health microbiology community, enabling the fellows to establish personal networks between European public health laboratories.

Acknowledgements of fellow

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