EUPHEM

EUPHEM REPORT

Summary of work activities

Ákos Tóth

European Public Health Microbiology Training Programme (EUPHEM), 2014 cohort

Background

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 Ákos Tóth, cohort 2014 of the European Public Health Microbiology Training Programme (EUPHEM) at the National Center for Epidemiology (NCE), Budapest, Hungary. Ákos Tóth is a clinical microbiologist from Hungary.

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

Ákos Tóth graduated as a molecular biologist, microbiologist in 2002 and received a PhD degree in 2011. Before the fellowship he worked at the NCE as head of the National Reference Laboratory for multidrug resistant Gram-negative and Gram-positive bacteria. He was responsible for detection and confirmation of antibiotic resistance mechanisms in multidrug resistant nosocomial bacterial pathogens. He applied for an MS-Track fellowship position in The European Programme for Public Health Microbiology Training (EUPHEM) because he wanted to acquire new knowledge and experiences in field of epidemiology, infection prevention and public health management that would enable him to contribute even further towards the protection of the population against the threat of multidrug resistant pathogens.

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 guide[1].

1. Epidemiological investigations

1.1. Outbreak investigations

A. A norovirus outbreak on a student coach trip from Greece to Hungary, December 2014

Supervisors: Ágnes Fehér, Ágnes Csohán

On 15 December 2014, a local health authority informed the National Center for Epidemiology of a gastroenteritis outbreak amongst a Greek student group who travelled by two buses from Thessaloniki, Greece on 13 December 2014 to Budapest, Hungary via Belgrade, Serbia. We initiated an outbreak investigation to identify possible vehicles and modes of transmission. The passengers completed a questionnaire on their symptoms, consumed foods and travel details. We defined a case as a person who attended the coach trip with gastroenteric symptoms after 13 December. Stool specimens from cases were investigated for norovirus using an ELISA technique. Food safety officers inspected the kitchen that had prepared the dinner and collected samples from left-over food at the hotel where they stayed in Budapest Overall, 111/124 (89.5%) passengers responded; 56 (51.4%) were female, 104 (95.4%) were aged 17 years. Forty-six cases (42%) occurred within a 24h period. Four of eight stool samples provided were norovirus positive. The risk of illness was highest amongst those who had consumed salami from the Belgrade-breakfast (RR=2.2; 95%CI=1.5-3.3) and pork meatloaf from the Budapest-dinner on the same day (RR=1.9; 95%CI=1.3-2.9) and travelled on bus No1 (RR=1.9; 95%CI=1.2-3.0). No other guests or kitchen staff in the Budapest hotel reported illness during that period. We did not find any strong associations between the outcome and possible exposures due to insufficient information collected. Epidemiological investigation suggested that exposure occurred during the coach trip and the virus spread by contact transmission. For communication during the outbreak investigation, knowledge of international relevant languages, especially English was required along with the development of an improved English trawling questionnaire was recommended to the local authorities in order to reduce the recall biases. The fellow participated in the ten steps of the outbreak investigation and worked together with the epidemiologists from the NCE and local authorities.

[1] European 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

B. A Clostridium perfringens outbreak in an elementary school in Budapest, April 2015: the key role of microbiological tests in the investigation

Supervisor: Ágnes Fehér

On 15th April 2015, an elementary school located in Budapest, Hungary reported an elevated number of gastrointestinal illnesses amongst schoolchildren and an outbreak investigation was initiated. A case-control study was conducted using a standardized questionnaire. We defined a case as a person who was in the school on 14th April 2015 and developed diarrhoea or abdominal cramps during the following 48 hours. We randomly recruited controls by email amongst asymptomatic persons who were in the school on 14th April. Left-over food from the kitchen and the canteen and stool specimens were investigated for possible causative agents. Amongst the 467 exposed persons, 43 cases were interviewed, but only 23 of the recruited 120 controls responded to the questionnaire. Overall, 25 (58%) of the cases were female, 37 (86%) were ≤14 years. The median incubation time was 19 hours and all cases occurred during the first 27 hours after the lunch. Meatball consumption showed association with gastrointestinal illness (OR=8.8; 95%CI: 0.9-84.5). *C. perfringens* was isolated from the faecal samples of all investigated cases and some left-over food (bone soup, meatball and black pepper). *C. perfringens* was the most likely cause of the outbreak, the bone soup and the meatballs were the vehicles and black pepper was suspected as a potential source. The microbiological investigation strongly supported these findings and played a key role in this investigation. The low core temperature and lack of cooling of freshly cooked food contributed to the outbreak, therefore thorough review of cooking standard procedures and education were recommended to canteen and kitchen staff. The fellow was involved in the descriptive and analytical study, and interpretation of the epidemiological and microbiological findings.

C. Training modules

The EPIET/EUPHEM introductory course, the Outbreak module and Multivariable Analysis module trained the participants on the major steps of outbreak investigations, descriptive (time, place and person) and analytical epidemiological methods (e.g. study design, effect modification, bias, confounding, causality), univariable analysis, stratified analysis (STATA), principles of multivariable analysis, use of multivariable analysis (i.e., third factors – effect modification and confounding), type of multivariable analysis: linear, logistic, Poisson and Cox regression – development of an optimal regression model, interpretation of the results from the regression model, managing a third factor in a multivariable analysis, how to make recommendations, communication of findings, writing of outbreak reports and scientific manuscripts.

Educational outcome: Involvement in all steps of outbreak investigations (case definitions, active case finding, data collection, descriptive and analytical data analysis, laboratory investigation), participation in a multidisciplinary outbreak team and European collaboration for outbreak investigation via EWRS, interpretation of findings, writing of reports, implementation of prevention measures and recommendations.

1.2. Surveillance

A. Description and evaluation of the influenza sentinel surveillance system in Hungary

Supervisors: Zsuzsanna Molnár, István Jankovics, Gyula Csabai, Zoltán Kis, Ágnes Csohán

In Hungary, the current surveillance system has been operating since 2005 and had not yet been evaluated. The project aimed to describe the current sentinel influenza surveillance system based on three consecutive influenza seasons (2012/2013, 2013/2014 and 2014/2015), and to evaluate three attributes (representativeness, acceptability and timeliness) for the two surveillance components; Influenza-Like-Illness (ILI) sentinel surveillance and sentinel virological surveillance system.

The Hungarian influenza surveillance system meets the recommended criteria for an ILI sentinel site surveillance. The clinical data are reported by a nationally organized network of general practitioners (GPs). A subset of specimens from ILI patients is routinely tested for influenza and its subtypes at the National Influenza Reference Laboratory (NIRL). Both parts of the influenza surveillance system operate during the influenza surveillance season (week 40 to week 20 of the following year). This surveillance provides national data to the national and international stakeholders on a weekly basis during the influenza surveillance season.

The ILI sentinel surveillance observes more than 20% of the general population in each investigated season. Very strong correlation was found between age and geographical distribution of general population and the observed population (r^2=0.98 and r^2=0.99 for each of the three season, respectively). In the virological surveillance the correlation between number of consultations for ILI cases and tested virological samples was very strong regarding age-group distribution (r^2= 0.82-0.9) and strong or moderate for geographical distribution (r^2=0.5-0.77).

In the ILI sentinel surveillance the willingness of participating GPs was very high in each investigated season (99.53%-99.98%). In the virological surveillance 15%, 17% and 11% of the 100 invited GPs did not sent samples for virological investigation in the corresponding seasons.

The timeliness of ILI surveillance system was three days from collection of clinical data to publication of results to all stakeholders for each season. In the virological surveillance the laboratory-time (time between sample received and report of the result) was significantly longer than the expected 1 day ($p<0.001$) during the weeks when influenza activity was above the seasonal threshold.

In conclusion, all three investigated attributes of ILI sentinel surveillance were excellent and this finding suggested that the surveillance provides high quality data for national and international requirements. Regarding virological surveillance two parts of the system could be improved. The willingness of taking samples is not appropriate in several regions of Hungary. To improve the representativeness of virological data we recommended interviewing of those GPs who provided a lower number of samples in order to establish any potential issues. Furthermore, laboratory-time increased during the epidemic period and reflected the intensity of influenza activity. This finding could be explained due to insufficient resources (staff, consumables or equipments) for laboratory investigation. To improve the laboratory-time we recommended surveillance of the lab-capacity in order to identify and provide additional resources. The fellow worked together with epidemiologists and virologists in this project to combine laboratory and epidemiological data in order to create a complete picture of the system. The fellow was responsible for whole evaluation of epidemiological and virological parts of surveillance system and as a EUPHEM fellow had a notable role to perceive the improvable areas in virological sample proceeding.

B. Training modules

The EPIET/EUPHEM introductory course familiarised participants with many aspects and concepts associated with surveillance, including the principles of surveillance and how to develop, validate, evaluate and operate a surveillance system. In addition to this course, the Vaccinology module taught participants how to evaluate vaccination programmes using data on surveillance, vaccine coverage, vaccine safety, seroepidemiology, vaccine effectiveness and outbreak investigation. The rapid assessment module introduced techniques for surveillance in complex emergency situation, including morbidity and mortality surveys.
Educational outcome: Participation in disease-specific networks at the national level; understanding analysis of laboratory-based surveillance systems at hospital, country and European level; understanding of the need to integrate microbiological and epidemiological data in disease surveillance; evaluation of surveillance systems, identification of common goals; understanding ethical principles on data protection and confidentiality regarding information in databases; writing of report.

2. Applied public health microbiology research

A. Molecular epidemiology of Neisseria gonorrhoeae in Budapest, Hungary, 2012-2014

Supervisors: Eszter Balla, Mária Dudás, Tímea Erdősi

Gonorrhoea is still a global public health problem. The European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) survey in 2009-2010 revealed that there were prevalent genogroups of *Neisseria gonorrhoeae* (NG) (e.g. G1407, G225, G387) in Europe and amongst these G1407 and G225 were associated with antibiotic resistance. We aimed to investigate the clonality of Hungarian NG isolates and associations between genogroups, antimicrobial resistance and epidemiological variables. Non-duplicate NG isolates from patients that had visited any of six sentinel Dermatology and Venereology clinics in Budapest, Hungary in 2012-2014 were included and typed by NG multi-antigen sequence typing (NG-MAST) and genogroups (GG) based on sequence similarity of *porB* and *tbpB* alleles. We tested associations between genogroups, antimicrobial susceptibilities and patient characteristics (gender, age, sexual behaviours), and expressed results as odds ratio (OR). We investigated 114 isolates from corresponding patients (median age 30 years, range 14-61y; males 82%). Of these, 43% were men having contact with sexworkers and 11% were men having sex with men. Of the 50 identified sequence types 16 were newly described and seven were unique. Eighty-five isolates could be assigned to eight main GGs, with G1407, G387, G225 and G5333 being the most prevalent. G1407 was significantly associated with decreased susceptibility to cefixime (OR:12.5;$p<0.0001$) and azithromycin (OR:8.85;$p=0.001$). All isolates belonging to G1407, G225 and G5333 were resistant to ciprofloxacin ($p<0.0001$) and were associated with men having contact with sexworker (OR:2.46;$p<0.05$). Amongst the isolates analysed we found association between the main European genogroups, antibiotic resistance, and men having contact with sexworkers. Sexworkers likely serve as a reservoir of important NG genogroups in Hungary, thus their regular screening and awareness training is recommended in order to prevent spread of gonorrhoea. During the project the fellow was responsible for design of this study, implementation of NG-MAST typing method at the NCE, writing scientific abstract and manuscript, and collaborated with experts of Euro-GASP.

B. Training modules

The EPIET/EUPHEM introductory course trained the participants on how to develop a study protocol and to present results and write a scientific manuscript. 'Initial management in public health microbiology' module focused on other aspects of research, including time and stress management, communication and team work.
Educational outcome: Preparation of study protocol, management, implementation of new typing method, analysis of data, interpretation of typing results, gaining expertise on data analysis with STATA, writing of scientific manuscript, adherence to ethical principles, delivering a scientific presentation at a conference. Interactions and collaborations within European networks.

3. Applied public health microbiology and laboratory investigations

A. First description of a rifampicin resistant Neisseria meninigitidis Y serogroup strain causing recurrent invasive meningococcal disease in Hungary

Supervisors: Tamás Tirczka, Tímea Erdősi, Zsófia Bognár

A Hungarian soldier previously immunized against *N. meningitidis* by polysaccharide vaccine (ACYW serogroups) was twice infected with meningococci within six weeks. The patient was treated with ceftriaxone during both episodes and he successfully recovered. His close contacts received rifampicin prophylaxis. An investigation was performed to characterize the genetic background of the pathogens in order to ascertain if the recurrent invasive meningococcal disease (IMD) was caused by the same strain and to attempt to find out the possible reason for reinfection. In the first episode, the pathogen was non-culturable and the presence of meningococcal DNA in CSF was determined by Real Time and conventional PCRs using species (target gene *ctrA*) and serogroup specific (target genes $siaD_{WY}$, $siaD_{B}$, $siaD_{C}$) primers. The second episode occurred six weeks later and the strain was phenotyped using slide agglutination with monoclonal antisera. Antimicrobial susceptibility to penicillin, ceftriaxone, ciprofloxacin and rifampicin was determined by gradient MIC test and interpreted using EUCAST guidelines. In both episodes the pathogens were characterized by multilocus sequence typing (MLST) and sequencing of variable regions of *por*A and *fet*A genes according to the recommendation of the European Meningococcal Disease Society. The presence of mutations associated with rifampicin resistance in the specific region of *rpoB* gene, encoding the β subunit of bacterial RNA polymerase was investigated by sequencing. Both meningococci belonged to the Y serogroup, sequence type ST-23 (ST-23 complex/Cluster A3) and had identical fine type (Y:P1.5-2,10-1:F4-1). In the first episode we found wild-type *rpoB* allele implying the susceptibility to rifampicin of the pathogen. The culturable isolate from the second episode proved resistant to rifampicin (MIC value >32 mg/L) and a point mutation at amino acid residue S557 (S557F) in the *rpoB* gene associated with high level rifampicin resistance was identified. The patient was screened for terminal complement component defects, and C8 component absence which significantly increases the susceptibility to IMD was revealed. Test for HIV antibodies was negative. This is the first description of a meningococcus strain that belonged to the Europe-wide prevalent *N. meningitidis* Y serogroup in Hungary. Previous immunization of the patient with polysaccharide vaccine was ineffective due to the absence of the C8, thus immunization with conjugate vaccine was proposed. Considering that in the last five years no rifampicin resistance was observed amongst culturable meningococci isolates in Hungary the emergence of rifampicin resistance probably evolved during the prophylactic treatment of contacts which is of serious concern. Therefore, we have proposed the implementation of centralized rifampicin susceptibility testing of *N. meningitidis* strains within a defined timeframe in order to intervene and administer appropriate prophylaxis to close contacts if a resistant strain is detected. The implementation of the proposed modifications could improve the effectiveness of the meningococcal public health management.

B. Malaria in Europe: Detection and typing of imported and outbreak cases

Supervisor: José Miguel Rubio Múñoz
Servicio de parasitología, Instituto de Salud Carlos III (ISCIII), Madrid, Spain

Malaria, together with tuberculosis and HIV, is an important cause of morbidity and mortality, especially amongst children globally. The disease is caused by the protozoan parasite Plasmodium, and is transmitted by the *Anopheles* mosquito vector. Five Plasmodia species affecting humans and amongst them, *P. falciparum* is the most prevalent malaria species worldwide, especially in Africa, causing the most severe form of the disease and being responsible for over 90% of the deaths. Each year, 219 million cases of malaria are estimated to occur around the world.
The objectives of the training were to teach the EUPHEM fellow on the surveillance of imported cases of malaria in Spain, to learn the microscopic and molecular methodology as a powerful research tool for malaria surveillance and to characterize possible autochthonous malaria outbreaks or follow-up the effectiveness of antimalarian therapy. Molecular tools allow 1) more sensitive estimations of prevalence and incidence that include subclinical cases of parasitemia; 2) differentiation between recrudescent, relapsing, and new infections; 3) outbreak and transmission route investigation. The methods learned were: microscopic examination of blood films for detection of *Plasmodium* spp.; antigen detection test; detection of *Plasmodium* spp. in blood samples by PCR methods (multiplex, semi-nested PCR for detection of *Plasmodium* spp., real-time PCR); and genotyping of *Plasmodium falciparum* by nested PCR method.
The fellow genotyped four *P. falciparum* positive samples using *msp1* and *msp2* nested PCRs. Three were taken from the same patient who had already been treated with an antimalarial drug and the samples were sent to evaluate the efficacy of drug therapy. The results suggested that both patients had a polyclonal infection of *P. falciparum* because three of four detected alleles were identical from the samples of patient A taken on day 0 and on day eight, therefore, the infection was considered as recrudescence and suggested a treatment failure. Molecular detection is important to detect low level parasitaemia (or sub microscopic malaria) what could be the hidden source of local transmission and is recommended for routinely use in Hungary. Furthermore implementation of genotyping of malaria is also recommended in order to confirm a possible outbreak or follow-up the efficacy of

drug therapy. With the knowledge gained from this study trip the fellow can contribute to implement these molecular methods in the NCE.

C. Prevalence of the anti-hepatitis delta (HDV) antibodies amongst pregnant HbsAg carriers living in the middle part of Hungary

Supervisor: Erzsébet Rusvai

Hepatitis B virus may cause a potentially life-threatening liver disease. More than 200 million people are chronic carriers of hepatitis B virus, whose blood or other body fluids can transmit the infection to susceptible persons. Hepatitis delta virus (HDV) requires the presence of hepatitis B virus (HBV) surface protein (HBsAg) to be able to infect hepatocytes. The HDV-HBV co-infection may result in a more severe acute disease and HDV super-infection of chronic HBV carriers commonly causes cirrhosis.
An average 5% of HBsAg carriers globally are infected also by HDV, but a relative high (up to 20%) rate of HDV was observed amongst HBV infected carriers in certain parts of eastern, southern and central Europe.
There is limited information about the prevalence of HDV amongst patients with hepatitis B (HBV) in Hungary. A survey conducted in 1992 found 13% HDV prevalence amongst patients with chronic liver disease due to HBV. However, there is no information about the prevalence of the HDV amongst symptomless Hungarian pregnant women, one of the most vulnerable populations. Our main objectives were to examine the presence of antibodies to hepatitis delta virus in HbsAg positive serum samples and to detect the delta virus genome in the anti-delta virus positive samples.
During the period 15 November 2012 to 07 March 2014, 37 958 serum samples of pregnant women were investigated for the presence of the hepatitis B virus surface antigen. The serum samples were collected from the middle part of Hungary, e.g. from Budapest, and the counties of Pest, Nógrád and Szolnok (36% of the Hungarian population). Ninety (0.24%) of the investigated individuals were found to be carriers of the HBsAg. All the positive samples were tested for the presence of antibodies to HDV using commercially available ELISA test and reverse-transcriptase PCR was used for HDV confirmation. Four (4.44%) of the tested individuals were found HDV positive. The most important outcome of our work was to prove the presence of the hepatitis delta virus in the young, symptomless population of the HBsAg carrying pregnant women living in Hungary and to highlight the importance of early detection and provision of care to prevent severe liver disease caused by dual infection.

D. Feasibility of two commercial ELISA kits for detection of measles-specific IgG in immunocompromised individuals

Supervisor: Zita Rigó

In immunocompromised patients, measles can appear in a more severe form and can cause serious complications. Therefore, special attention is required to assess the immunity status (measles-specific antibody (IgG)) of immunocompromised patients.
Enzyme-linked immunosorbent assays (ELISA) are widespread for the detection of morbilli antibodies. These systems are more easily applied for mass screening and evaluation of immunity status is faster and easier compared to immunofluorescence microscopy technique (IFA) which is the gold standard method. The aim of the study was to assess the applicability of two ELISA methods for detection of immunity status (protected or susceptible) of immunocompromised patients.
Morbilli IgG level was measured within two groups: a group of healthy individuals (n=20) and a group of immunocompromised individuals (n=44). Two commercially available ELISA tests (Anti-measles IgG EIA EUROIMMUN, Anti-measles IgG EIA SIEMENS) based on different operating principles were compared with each other and with one immunofluorescence kit (Anti-measles IgG IF MASTAFLUOR). The correlation between the IFA and the ELISA OD results were assessed within and between the two individual groups using Pearson's correlation coefficient. The agreement of each ELISA system was determined by comparing the interpreted ELISA result categories to the IFA result categories (positive, equivocal or negative).
In the group of healthy individuals strong correlation was found between the EIA EUROIMMUN and IFA assays (r^2=0.907, P<0.0001), and between the EIA SIEMENS and IFA assays (r^2=0.699, P<0.0001) also, and an agreement of 100% were calculated for IFA compared to both ELISA tests. In contrast, in the group of immunocompromised individuals weak correlation was found between the two ELISA and IFA assays (r^2=0.086, r^2=0.1322), respectively and a calculated agreement of 27.3% and 29.5% were found for the IFA compared to EIA EUROIMMUN and EIA SIEMENS, respectively. Comparing the agreements of the two ELISA kits for IFA assay a significant association was observed (P<0.001).
We found a weak correlation and low level agreement between both ELISA tests and the IFA assay in the group of immunocompromised individuals. Most of the disagreements were false positive results in both ELISA tests probably due to the presence of non-specific antibodies. Our results suggested that the two ELISA tests could not be applied for determination of immune status to measles amongst immunocompromised individuals and the IFA assay is recommended.
Educational outcome: Application of virology, bacteriology, parasitology and immunology concepts to public health disciplines; understanding limitations of laboratory methods and the appropriate use of diagnostic algorithms; scientific presentation at a conference; writing of a scientific article.

4. Biorisk management

A. Ebola virus training

The fellow had a five-day training period in the National Biosafety Laboratory (NBL) at the National Center for Epidemiology, Hungary. During the training the fellow had an overview of the local, national and international biosafety and biosecurity regulations, along with theoretical and practical training.
Educational outcome: The fellow had a tour in the technological part of the NBL guided by the Engineer–in-Chief. The fellow was involved in the following procedure in the containment area: cell culture inoculation with Ebola virus and Lassa virus, preparation of Ebola virus IFA slides, Ebola virus infective titration using Fluorescent Immunofocus Unit assay, and inactivation for PCR and isolation of viruses from different clinical specimens.

B. Training module on Biorisk and Quality Management, ECDC, Sweden

The module provided theoretical and practical education concerning biorisk and biosafety management: e.g. biorisk assessment (BioRAM Lite), mitigation and performance; international transport of infectious substances (categorization, packaging, documentation – certificate on achieved knowledge). As part of the module the BSL4 laboratory at the Smittskyddinstitutet, Stockholm, was visited.
Educational outcome: Understanding the processes associated with BSL3/BSL4 laboratories, experience of different types of personal protective equipment, understanding the principles and practices of biorisk management; biorisk assessment and biorisk mitigation.

5. Quality management

A. Sixth External Quality Assessment scheme for typing of verocytotoxin-producing Escherichia coli (VTEC)

Supervisors: Tünde Mag, Ivelina Damjanova

As ECDC contact point for food and waterborne diseases at the National Center for Epidemiology, the Department of 2nd Bacteriology and the Department of Phage and molecular typing were asked to participate in the Sixth External Quality Assessment scheme for typing of verocytotoxin-producing *Escherichia coli* (VTEC) to determine their current laboratory capabilities for diagnostics and the methods used for VTEC identification and characterisation. The fellow was responsible for: 1) reception of the EQA simulated specimen panel and cultivation; 2) performing pulsed field gel electrophoresis (PFGE) on ten isolates; 3) serotyping of VTEC starins; 4) using PCR for detection of the VTEC toxin genes and other virulence determinants; 5) reporting of results (problems encountered); 6) evaluation of results and recommendations for future improvements.

B. External quality assessment scheme for influenza virus detection, culture and antiviral susceptibility for the European Reference Laboratory Network for Human Influenza in 2015

Supervisors: István Jankovics, Mónika Rózsa, Zoltán Kis

European Reference Laboratory Network for Human Influenza (ERLI-Net) is required to undertake a regular EQA exercise in order to assess the capability of the member laboratories including Influenza Reference Laboratory (IRL) at the National Center for Epidemiology in Hungary to accurately detect and characterize influenza viruses and to test antiviral susceptibility.
The EQA panels consisted of eight and ten simulated clinical samples containing influenza viruses from subtypes that are circulating, or have recently circulated, in humans, The samples were prepared by Public Health England (PHE) and were distributed to participant laboratories.
The fellow was involved in 1) rapid detection of influenza virus; 2) virus isolation and culture (on tissue and embrionated chicken eggs); 3) virus characterization (subtyping and lineage determination using PCR and haemagglutination); 4) determination of antiviral susceptibility (detection of mutations conferring antiviral resistance in individual panel samples, and determination of Osteltamivir and Zanamivir susceptibility status for each sample); 5) reporting and evaluation of results.

C. Participation in external audit for the Bacteriology Division at National Center for Epidemiology

Supervisors: Tamás Tirczka, Judit Pászti

Laboratories at the National Center for Epidemiology are accredited under the EN ISO 15189 by the National Accreditation Body. The fellow was responsible for compiling and supervision of Standard Operation Protocols (for antibiotic susceptibility testing methods, conventional PCR methods), for appropriately calibrated and maintained laboratory equipments, tools at the Bacteriology Department and was involved in preparation for external audit. The fellow participated in the laboratory meeting to evaluate and provide feedback based on the results of the audits.

D. Design and evaluation of national, clinical bacteriological ring trials organized by the National Center for Epidemiology

Supervisors: Tamás Tirczka, Ildikó Visontai

Objective of the ring trial was to assess the quality of work of participating laboratories regarding culturing, identification, and antibiotic susceptibility test of aerobic pathogenic bacteria and interpretation of the results. The ring trial was organized and coordinated by the National Center for Epidemiology. Participation in the ring trial is mandatory for Hungarian laboratories by law. The role of the fellow was to characterize the test strains, compile the ring trial tests, compose the expected results and evaluate the results of participating laboratories.

E. Training modules

The training module on Biorisk and Quality Management, Stockholm, Sweden provided an overview of quality management concepts in diagnostic laboratories, according to the ISO 15189 standard. Topics covered included factors influencing quality in laboratories, internal and external quality control, norms and accreditation, assessments and audits, documentation and record keeping, sample management, management of equipment and temperature-controlled devices, process improvement, customer service and international health regulations.

Educational outcome: Understand the principles and practices of quality assurance; analyse and summarise results of an external quality assessment; participation on external accreditation audit; understand accreditation procedures; understand and learn about the design and performance of the EQA; evaluation of results and making recommendation.

6. Teaching and pedagogy

A. Outbreak investigation training course at the National Center for Epidemiology

Supervisors: Ágnes Fehér, Ágnes Csohán, Ivelina Damjanova, Judit Pászti
Contribution to organisation and implementation of a three day epidemiological training targeted specialists in field epidemiology, general practitioners, infectologists and public health specialists working in administrative bodies of government offices. Lectures on 'Identification and typing of pathogens in outbreak situations (virology and bacteriology)', facilitating case studies in group practice of outbreak investigations.

B. Supervision of PhD and University students

The fellow was the main supervisor of an university student (Thesis: Characterization of carbapenemase-producing *Enterobacteriaceae* isolates in Hungary) and co-supervisor of a PhD student (Thesis: Survival capability of high risk, multiresistant bacteria on cotton swatches treated with commercially available antimicrobial agents).

C. Lecture on 'EUCAST Day' Scientific meeting, Budapest, Hungary

A lecture was given by the fellow for general practitioners, infectologist, clinicians, clinical and public health microbiologists about the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and current topics of its activities.

D. Lectures on 'Infections caused by multidrug resistant pathogens and *Clostridium difficile* in the Hungarian healthcare system: new challenges and pratical solutions' roadshow

Lectures were given by the fellow for specialists in field epidemiology, general practitioners, infectologists and public health specialists. The lectures were: ‚Microbiology of multidrug resistant Gram-positive pathogens: Methicillin resistant *Staphylococcus aureus*', ‚Microbiology of multidrug resistant Gram-positive pathogens: vancomycin resistant *Enterococcus faecium*' and ‚Microbiology of multidrug resistant Gram-negative pathogens: *Acinetobacter baumannii*'.
Educational outcome: Planning and organization of lectures and training course, facilitating case studies, defining learning objectives and teaching laboratory and microbiology topics.

7. Public health microbiology management

A. Public health microbiology management components as part of regular projects

Managerial components of public health microbiology management were part of all projects and activities throughout the fellowship.
This included: Communication with people from different backgrounds and with different nationalities, working in a multidisciplinary team (microbiologists, physicians, laboratory technicians, epidemiologists), scientific presentations (poster, oral), scientific writing, quality management knowledge, laboratory management, project and time management, organisation of and participation in meetings.

B. Training modules

The training module on Initial Management in Public Health Microbiology, Stockholm, Sweden provided an overview of EU decision making (how the EU make decisions, role of ECDC), management in public health microbiology: management vs leadership, wheel of success, management styles, art of motivation, prioritising workload using Covey's Matrix, communication and learning styles, 'ESP' communication, crisis management - 'TCUP'. Through practical educational part of the module there were excellent opportunities to know different situations in public health management:

- simulation exercise: set up a crisis management team, solving a theoretical problem (report on ebola situation in Guinea)
- communication exercises: report to WHO, briefing of the Minister of Health, simulated interviews with journalist, debriefing the EU delegation
- presentation from fellows to the ECDC Director and Chief Microbiologist and Chief Scientist, as high level authorities

Educational outcome: Obtaining experience of working in a multidisciplinary public health team; understanding team management; understanding roles and formal responsibilities in public health microbiology; planning, scheduling and organising research projects.

8. Communication

A. Publications

1. Kádár B, Kocsis B, Kristof K, Tóth Á, Szabó D. Effect of antimicrobial peptides on colistin-susceptible and colistin-resistant strains of *Klebsiella pneumoniae* and Enterobacter asburiae. Acta Microbiol Immunol Hung. 2015 62:501-8.
2. Kis Z, Tóth Á, Jánvári L, Damjanova I. Countrywide dissemination of a DHA-1-type β-lactamase-producing *Klebsiella pneumoniae* ST11 international high-risk clone in Hungary, 2009-2013. Accepted for publication in the Journal of Medical Microbiology (Kis Z and Tóth Á contributed equally to this article)
3. Tóth Á, Berta B, Tirczka T, Jekkel Cs, Ábrahám A, Prohászka Z, Krisztalovics K, Bognár Zs, Erdősi T. First description of a rifampicin resistant *Neisseria meninigitidis* Y serogroup strain causing recurrent invasive meningococcal disease in Hungary (in final preparation)
4. Tóth Á, Dudás M, Erdősi T, Balla E. Molecular epidemiology of *Neisseria gonorrhoeae* in Budapest, Hungary, 2012-2014. (in preparation)
5. Kovács K, Nyul A, Mestyán Gy, Melegh Sz, Fenyvesi H, Jakab G, Szabó H, Jánvári L, Damjanova I, Tóth Á. Emergence and interhospital spread of OXA-48-producing *Klebsiella pneumoniae* ST395 clone in Western Hungary. Infectious Diseases. 2016, DOI: 10.1080/23744235.2016.1207252
6. Kocsis B, Kádár B, Tóth Á, Fullár A, Szabó D. MgrB variants in colistin susceptible and colistin resistant *Klebsiella pneumoniae* ST258. Accepted for publication in Journal of Microbiology, Immunology and Infection

B. Reports

1. Tóth Á, Mag T, Damjanova I. Sixth External Quality Assessment scheme for typing of verocytotoxin-producing *Escherichia coli* (VTEC).
2. Tóth Á, Rubio Munoz JM. Malaria in Europe: Detection and typing of imported and outbreak cases.
3. Tóth Á, Fehér Á, Csohán Á. Juhász G, Farkas Á. A norovirus outbreak on a student coach trip from Greece to Hungary, December 2014.
4. Tóth Á, Papp E, Bodzai Zs, Bényi M, Popovics É, Fehér Á. A *Clostridium perfringens* outbreak in an elementary school in Hungary, April 2015.
5. Tóth Á, Molnár Zs, Jankovics I, Csabai Gy, Kis Z, Csohán Á. Description and evaluation of sentinel influenza surveillance in Hungary

C. Conference presentations

1. Hanczvikkel A, Tóth Á. Silver-susceptibility of multidrug resistant nosocomial Gram-positive and Gram-negative pathogens. 17th International Congress, Hungarian Society for Microbiology, July 2015, Budapest, Hungary
2. Tóth Á, Juhász G, Fehér Á. A norovirus outbreak on a student coach trip from Greece to Hungary, December 2014" 10th Congress of Hungarian Association of Hygienic Physicians, October 2015, Eger, Hungary.
3. Kis Z, Tóth Á, Tirczka T, Jánvári L, Pászti J, Damjanova I. Molecular epidemiology of plasmid-mediated AmpC β-lactamase producing *Klebsiella pneumoniae* in Hungary, 2009-2013. European Scientific Conference on Applied Infectious Disease Epidemiology 2015, November 2015, Stockholm, Sweden.
4. Hanczvikkel A, Tóth Á. Survival capability of high risk, multiresistant bacteria on cotton swatches treated with commercially available antimicrobial agents. XXIV International Congress of International Federation of Associations of Textile Chemists and Colourists, June 2016, Pardubice, Czech Republic.

5. Jánvári L, Damjanova I, Strupka V, Kurcz A, Máthé M, Farkas M, Urbán E, Osztie H, Tóth Á. The role of cross-border transmission in the emergence of NDM-producing *Enterobacteriaceae* in Hungary. 26th European Congress of Clinical Microbiology and Infectious Diseases, April 2016, Amsterdam, the Netherlands.
6. Tóth Á, Berta B, Tirczka T, Jekkel Cs, Ábrahám A, Prohászka Z, Bognár Zs, Erdősi T. First description of a rifampicin resistant *Neisseria meninigitidis* Y serogroup strain causing recurrent invasive meningococcal disease in Hungary. 26th European Congress of Clinical Microbiology and Infectious Diseases, April 2016, Amsterdam, the Netherlands.
7. Tóth Á, Dudás M, Erdősi T, Balla E. Molecular epidemiology of *Neisseria gonorrhoeae* in Budapest, Hungary, 2012-2014. European Scientific Conference on Applied Infectious Disease Epidemiology 2016 (accepted for poster presentation)

D. Other presentations

1. Tóth Á. Antibiotic resistance trends in Hungary. Scientific meeting, European Antibiotic Awareness Day 2014. November 2014, Budapest, Hungary.
2. Tóth Á. Evolution of the *in vitro* antibiotic susceptibility breakpoint systems. 20th Antibiotic Training Course. November 2014, Siófok, Hungary.
3. Tóth Á, Pászti J, Berta B, Lesinszki V. Microbiology of multidrug resistant Gram-positive pathogens: vancomycin resistant *Enterococcus faecium.* Roadshow 'Infections caused by multidrug resistant pathogens and *Clostridium difficile* in Hungarian healthcare system: new challenges and pratical solutions'. January 2015, Budapest, Hungary.
4. Tóth Á, Ungvári E. Microbiology of multidrug resistant Gram-positive pathogens: Methicillin resistant *Staphylococcus aureus.* Roadshow 'Infections caused by multidrug resistant pathogens and *Clostridium difficile* in Hungarian healthcare system: new challenges and pratical solutions'. January 2015, Budapest, Hungary.
5. Tóth Á, Kis Z, Bognár Zs. Ebola preparedness in Hungary. EUPHEM, Initial Management in Public Health Microbiology module, ECDC, February 2015, Stockholm, Sweden.
6. Tóth Á, Pászti J, Berta B, Lesinszki V. Microbiology of multidrug resistant Gram-positive pathogens: vancomycin resistant *Enterococcus faecium.* Roadshow 'Infections caused by multidrug resistant pathogens and *Clostridium difficile* in Hungarian healthcare system: new challenges and pratical solutions'. February 2015, Kecskemét, Hungary.
7. Tóth Á, Damjanova I, Pászti J, Jánvári L, Lesinszki V. Microbiology of multidrug resistant Gram-negative pathogens: *Acinetobacter baumannii.* Roadshow 'Infections caused by multidrug resistant pathogens and *Clostridium difficile* in Hungarian healthcare system: new challenges and pratical solutions'. February 2015, Kecskemét, Hungary.
8. Tóth Á, Naseer U. Vaccine against malaria. EUPHEM, Vaccinology module, April 2015, Krakow, Poland.
9. Tóth Á, Damjanova I, Pászti J. Typing of bacterial pathogens in outbreak situations. Outbreak investigations training course for field epidemiologists, National Center for Epidemiology, June 2015, Budapest, Hungary.
10. Tóth Á, Berta B, Tirczka T, Jekkel Cs, Ábrahám A, Prohászka Z, Bognár Zs, Erdősi T. Purulent meningitis caused by rifampicin resistant *Neisseria meninigitidis* Y serogroup strain, 2013, Hungary. EUPHEM, Project Review module, August 2015, Lisbon, Portugal.
11. Tóth Á. Antibiotic resistance trends in Hungary. 43rd Congress of Hungarian Society of Clinical Microbiology and Infectious Diseases, September 2015, Nyíregyháza, Hungary.
12. Tóth Á. How would be our world without antibiotics? "Researcher's Night", National Center for Epidemiology, September 2015, Budapest, Hungary.
13. Tóth Á. Deployment of stable MLST+ schema for *Klebsiella pneumoniae* with SeqSphere+ Ridom software" EUPHEM, Bioinformatics and Phylogenetics Module, November 2015, Stockholm, Sweden.
14. Tóth Á. Antimicrobial resistance in ophthalmology, Hungary, 2014. Congress of Societas Hungarica Ad Implantandam Oculi Lenticulam, April 2016, Siófok, Hungary
15. Tóth Á, Damjanova I, Pászti J. Detection and typing of bacterial pathogens in outbreak situations. Outbreak investigations training course for field epidemiologists, National Center for Epidemiology, June 2016, Budapest, Hungary.
16. Tóth Á. Antimicrobial resistance in ophthalmology, Hungary, 2014. Congress of Societas Ophtphalmoligica Hungarica, July 2016, Pécs, Hungary

E. Other

1. Tóth Á, Kristóf K, Tirczka T (2015) Szakmai tájékoztató az EUCAST antibiotikum érzékenység vizsgálati rendszerére való áttéréshez – Útmutató aerob és mikroaerofil baktériumok antibiotikum érzékenységének korongdiffúziós meghatározásához – EUCAST v5.0 verzió alapján. Mikrobiológiai Körlevél, 1: 1-30.
2. Albiger B, Glasner C, Struelens M, Grundmann H, Monnet D, the European Survey of Carbapenemase-Producing *Enterobacteriaceae* (EuSCAPE) working group (2015) Carbapenemase-producing *Enterobacteriaceae* in Europe: assessment by national experts from 38 countries, May 2015. Euro Surveill, 20:pii=30062.

3. Jánvári L, Tóth Á, Damjanova I (2014) Phenotypic and molecular investigation of carbapenemase-producing *Enterobacteriaceae* isolates, National Center for Epidemiology, Hungary, 2012- 2014. NCE Microbiological bulletin, 3-4: 22-34.
4. Tóth Á, Damjanova I. (2016) Situation of antimicrobial resistance in Europe and Hungary according to the annual report of EARS-Net 2014", Epinfo (Epidemiological Information Weekly) 23:14, released by the NCE

9. EPIET/EUPHEM modules attended

1. EPIET/EUPHEM introductory course, Spetses, Greece (three weeks)
2. Outbreak module, Berlin, Germany (one week)
3. Initial Management in Public Health Microbiology module, Stockholm, Sweden (one week)
4. Training module on Biorisk and Quality Management, Stockholm, Sweden (one week)
5. Multivariable Analysis module, Vienna, Austria (one week)
6. Vaccinology module, Krakow, Poland (one week)
7. Rapid Assessment and Survey Methods module, Athens, Greece (one week)
8. Project Review Module, Lisbon, Portugal (one week)
9. Bioinformatics and Phylogenetics Module, Stockholm, Sweden (three days)
10. Project Review Module, Lisbon, Portugal (one week)

10. Other training

Three-day ECDC training "Molecular typing and whole genome sequencing for sexually transmitted infections", Public Health England, London, England

Discussion

Coordinator's conclusions

Ákos Tóth was the second appointed MS track EUPHEM fellow in Hungary. He is a senior microbiologist focussing on multidrug resistant Gram-negative and Gram-positive bacteria, specified in antibiotic resistance mechanisms of multidrug resistant nosocomial bacterial pathogens. During the two years he has shown a great interest in other disease groups and acquired new competencies, especially in the domains of field epidemiology, public health management, quality management and surveillance. The projects described in this portfolio demonstrate the breadth of public health microbiology.
The outbreak activities comprised regional and national aspects and played a key role in these investigations. Akos showed that hungarian influenza sentinel surveillance system provides high quality data for national and international requirements and worked out suggestions for further improvements. With design of an applied public health microbiology research project Ákos facilitated the implementation of NG-MAST typing method at the NCE supported the collaboration with experts of Euro-GASP. Ákos was involved in proposed implementation of centralized rifampicin susceptibility testing of N. meningitidis strains in order to improve the effectiveness of the meningococcal public health management in Hungary. With his knowledge gained from an international study trip Ákos contributed to the implementation of molecular methods in Malaria detection in the NCE. With external quality assessment schemes the evaluation of results and recommendations for future improvements were proposed for typing of verocytotoxin-producing Escherichia coli (VTEC) and as well for EISN 2015 Influenza virus antiviral resistance detection EQA Programme and EISN 2015 Influenza virus rapid detection and culture EQA Programme.
The laboratory and epidemiologically based projects covered all diverse 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 environments. All activities undertaken by the fellow were in line with the 'learning by doing' and 'on-the-job' training approach of the EUPHEM programme and followed the core competency domains described for professionals in mid-career and above.
Ákos projects had a clear educational and public health outcome, with results communicated in scientific journals and at conferences. The EUPHEM coordinator team concludes that the fellow has succeeded in performing all his tasks to a high standard and with a very professional attitude. During the two years of his fellowship, Ákos has rapidly developed his managerial, professional and scientific communication skills. We would like to acknowledge Ákos excellent work, we would like to express our appreciation for his professional and cooperative attitude during the last two years and we wish him all the best for his future plans.

Supervisor's conclusions

This was the second time the National Center for Epidemiology (NCE) participated in the EUPHEM programme and **Dr. Ákos Tóth** was our second fellow. He is working at the NCE for years and leading the Hungarian National Reference Laboratory for multidrug resistant Gram-negative and Gram-positive bacteria. Ákos has an outstanding level of fundamental knowledge in several theoretical and applied sciences and he is very interested in possible application of the network theory to disciplines as microbiology and epidemiology. During the fellowship Ákos acquired competencies and high skills in virology, parasitology and epidemiology. He was always well prepared on the topics of the projects and started his work with greatest devotion. He also tried to perform immediately the new insights and knowledge learned from all the training modules. As the result of his outstanding work, skills and competencies gained during the two-year fellowships, he was appointed as the National Focal Point for Microbiology in Hungary. Ákos is modest, calm, well-minded and very empathic person with excellent organizational skills but also he is very good team player. On my own behalf and on behalf of our supervisors, I would like to thank Ákos for his excellent work and we are grateful to EUPHEM programme and coordinators for the very well-organized high quality training in the field of public health microbiology.

Personal conclusions of fellow

The EUPHEM programme allowed me to better understand the other fields of public health (e.g. epidemiology, infection disease control, management). During the two-year fellowship I worked in many disciplines, improving my knowledge of virology, bacteriology and parasitology. I found it especially useful to be in contact with people with a multidisciplinary background (epidemiologists, microbiologists, physicians, policy makers), who showed me different aspects of my field of work. I could improve my management and communication skills also which is important for good cooperation not only with the stakeholders of public health experts and desicion makers but the non-professional people also.
The EUPHEM training is a very well organized educational programme and an excellent concept. Through diverse projects, modules, the 'learning-by-doing' approach, and the close connection to the European Programme for Intervention Epidemiology Training (EPIET) network, the EUPHEM programme offers the excellent opportunity of strenghtening interdisciplinary collaborations and promoting networking between epidemiologists, public health microbiologists and other healthcare specialists in Europe.

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