EUPHEM

Summary of work activities
Katherina Zakikhany, European Public Health Microbiology Training Programme (EUPHEM), third cohort

Background

According to the European Centre for Disease Prevention and Control's (ECDC) advisory group on public health microbiology ('national microbiology focal points'), public health microbiology (PHM) 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. The primary work function is to use microbiology to improve the health of populations in collaboration with other public health disciplines, in particular epidemiology. PHM laboratories play a central role in the detection, monitoring, outbreak response, and 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. PHM is required to provide access to experts with expertise and experience 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 or assist in the development of microbiological guidelines, evaluate/develop new diagnostic tools, arbitrate on risks from microbes or their products, and provide pertinent information to policy makers related to the above issues from a microbiology 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'. Therefore, ECDC has 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 Katherina Zakikhany, fellow of the third cohort of the European Public Health Microbiology Training Programme (EUPHEM) at the Health Protection Agency, London, UK.

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.

Material and methods

This report accompanies a portfolio of the outcome of different activities conducted during the EUPHEM fellowship. Activities were comprised of specific projects and theoretical training modules (not summarised in this report). Specific projects included laboratory surveillance, risk assessments, outbreak investigations, quality management, research, summarising and communicating scientific evidence, and activities with a specific microbiological focus, such as biosafety or different laboratory techniques. The outcome included 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

A cluster of Enterobacteriaceae-producing VIM metallo-β-lactamases (MBL) in England

The aim of this study was to characterise a cluster of carbapenem-resistant *Klebsiella pneumoniae* (VIM-1 metallo-β-lactamase (MBL)) in North West England (Cheshire County). *Enterobacteriaceae* with acquired MBLs are still very rare in most European countries, though VIM is common in *Klebsiella* in several southern European countries. The project was performed in liaison with both the Department of Healthcare-associated Infection and Antimicrobial Resistance and the Antibiotic Resistance Monitoring & Reference Laboratory with the objectives to (i) identify local, national or international (if applicable) links between VIM-type MBL-positive patient isolates from the region (descriptive epidemiology), (ii) to type VIM-type MBL-positive patient isolates, (iii) to type VIM-type MBL elements, and (iiii) to use the findings to make recommendations in order to prevent the spread/dissemination of VIM-type MBLs. In the timeframe investigated, 20 patient isolates were identified as carbapenem resistant; all of which were tested positive for VIM. Typing results suggested clonal transmission in the hospital and furthermore that VNTR types 1–4 were circulating in the community with no obvious connection to the clonal hospital cases; however, the retrospective analysis was hampered by incomplete contact histories.

Educational outcome: preparation of study protocol, design of questionnaires, participating in interpretative reading, interpretation of typing results, communication of results and outcomes to healthcare professionals, data analysis, and scientific presentation at a conference.

Molecular evolution of human norovirus

The aim of this research and development project was to analyse changes at a hotspot of mutation (Sites A and B) within the major capsid protein (VP1) of norovirus and investigate these changes as predictive markers for the emergence and/or switch of epidemic strains in the population. For this purpose, a high-throughput pyrosequencing assay was developed to screen clinical specimens collected between 2000 and 2011 (in England); protein modelling was applied to predict the effects of amino acid changes. Analysis of amino acids at Site A revealed that the majority (82.6%) belonged to one of three defined surface motif types (SMT-1, -2 and -3). Three years (2002, 2006 and 2009) were associated with higher-than-average NoV activity in the UK. The analysis showed that this correlated with the emergence of the different major surface area motif types (SMTs) for Site A. Based on the results, a model of antigenic evolution for norovirus was proposed, which could be used as a powerful tool to monitor and detect alterations of antigenic properties in order to foresee and control new epidemic waves.

Educational outcome: development of a technical protocol, understanding of requirements for technical and clinical validation of diagnostic methods, preparation of a scientific article.

Increase in invasive Streptococcus pyogenes and Streptococcus pneumoniae infections in England

The aim of this exercise was to work as part of an interdisciplinary team, consisting of epidemiologists, microbiologists and clinicians, in order to investigate a potentially increased risk for concurrent invasive bacterial and influenza infections in children and young adults in England (winter 2011). The analysis included preparation of a study protocol, data gathering, cleaning and analysis, and was performed together with an EPIET fellow. The

results suggested that the changes observed in invasive *S. pyogenes* may be due to other factors than influenza but other public health institutes are urged to be alerted to the possibility of similar observations.

Educational outcome: multidisciplinary teamwork, contribution to study protocol (epidemiology), contribution to data cleaning and data analysis, preparation of scientific article.

Supportive assays for case classification in low-incidence settings: measles immunoglobulin G (IgG) avidity testing of oral fluid samples

The aim of this study was to develop a platform for IgG avidity testing in oral fluid samples as a support tool for measles diagnostics in low-transmission settings. Currently, detection of immunoglobulin M (IgM) is the gold standard method to confirm recent infection, but as the incidence of measles infection is decreasing, the positive predictive value of single IgM tests is low. Evaluation of the modified EIA demonstrated that the avidity pattern in oral fluids paralleled that in serum. Oral fluid samples from 150 patients with suspected measles infections were subsequently investigated, and a statistically significant lower mean relative avidity index (%), indicating recent infection, was determined for IgM-positive samples (32.8%) compared to IgM-negative oral fluids (71.0%; $p<0.0001$). Further testing is in progress to determine sensitivity and specificity of the assay.

Educational outcome: broadened experience of serological diagnostics, development of a technical protocol, understood requirements for technical and clinical validation of diagnostic methods, understanding of principles of laboratory-based surveillance in low-transmission settings, preparation of a scientific article.

Comparison of external quality assessment (EQA) schemes performed in 2008 and 2010 for influenza virus rapid detection and culture for the Community Network of Reference Laboratories for Human Influenza in Europe (CNRL)

The aim of this project was to compare external quality assessment (EQA) schemes performed in 2008 and 2010 for influenza virus rapid detection and culture for the Community Network of Reference Laboratories for Human Influenza in Europe (CNRL) in order (i) to produce detailed assessments of the individual countries' capabilities, (ii) produce a technical report incorporating an analysis of all recent EQA results, (iii) contribute to the development of long-term EQA and future training plan for the network. The analysis showed that 29 (87.7%) laboratories returned results for both EQA panels (2008 and 2010). Improving trends in technical proficiency for rapid (molecular) detection and subtyping were observed from 2008 to 2010, but equally small trends in decreasing performance in virus culture proficiency were noted. Virus culture and strain characterisations were the most heterogeneous with only three laboratories achieving a maximum score in typing/subtyping/strain determination.

Educational outcome: familiarity with the EQA scheme for human influenza across Europe, authorship of a technical report, understanding of training needs and development of training plan for traditional and molecular detection methods, presentation at scientific conference.

Study of non-toxigenic **Corynebacterium diphtheriae**: the role of non-toxigenic toxin-gene-bearing strains (NTTBs) in the UK

The aim of this project was to determine the presence and characterise the prevalence of non-toxigenic toxin-gene-bearing (NTTB) strains among *C. diphtheriae* biovar mitis strains circulating in the UK. The circulation of NTTB strains was observed during and after the diphtheria epidemic in the Newly Independent States (NIS) and Russia and are considered an increased risk for disease, particularly amongst populations with low levels of immunity or amongst immunocompromised individuals. Research in this new field is still ongoing. Preliminary data have revealed the presence of NTTBs in Europe; these findings should not be ignored in terms of public health action.

Educational outcome: familiarity with identification and characterisation procedures, understanding of importance of laboratory-based surveillance and disease-specific networks, formulation of specific public health recommendations, preparation of scientific manuscript.

Facilitator and lecturer at laboratory training modules

- Organisation and facilitation of the laboratory modules for epidemiologists (EPIET fellows and external participants) at HPA, Colindale, in October 2011. Modules included: public health microbiology, sampling, safety transport, diagnostic and typing techniques, and case studies. This course was organised in liaison with an EPIET fellow and facilitated together with a EUPHEM fellow, former EPIET fellows and external facilitators.
- Teaching and facilitation at the 'European workshop on laboratory diagnosis of diphtheria', organised by the WHO Global Collaborating Centre for Reference and Research on Diphtheria, Athens, Greece (November 2011).
- Co-organisation and facilitation at the 'Use of laboratory results in epidemiological investigations' workshop for ECDC experts in epidemic intelligence, preparedness, response, and surveillance, and EPIET supervisors

(June 2012). The aim of the workshop was to teach and discuss laboratory methods used in outbreak investigations and for surveillance purposes.

Educational outcome: plan and organise a course, define learning objectives, teach laboratory and microbiology issues to epidemiologists and microbiologists.

International mission: A serosurvey for vaccine-preventable diseases, Tajikistan, 2010/2011

The objective of the mission was to evaluate and analyse the seroprevalence of tetanus and diphtheria antibodies amongst the surveyed population of Tajikistan and undertake a mission to Tajikistan in order to discuss and share the results of the serosurvey with the ministry of health of Tajikistan. The serosurvey in Tajikistan was conducted by the US Centers for Disease Control and Prevention (CDC) (Atlanta, USA), in collaboration with the ministry of health of Tajikistan, the World Health Organization (WHO) Regional Office for Europe and the Health Protection Agency. The analysis of the specimens (tetanus and diphtheria) was performed at the WHO Reference Laboratory for Diphtheria, HPA, London, UK. Briefing and debriefing visits to the WHO Regional Office for Europe (Copenhagen, Denmark) and the CDC were conducted before and after the mission to Tajikistan. The results of the serosurvey showed that population immunity for diphtheria is low and suboptimal for tetanus among children and young adults in Tajikistan. The findings highlighted the need to improve routine immunisation delivery in the country and support a proposal by the Tajik ministry of health for a nationwide one-time diphtheria-tetanus supplementary immunisation campaign to rapidly close immunity gaps and prevent diphtheria outbreaks.

Educational outcome: familiarity with laboratory procedures for tetanus and diphtheria serology; understanding of sampling strategies for serosurveys and the development of protocols for seroprevalence studies; understanding and discussion of political and scientific aspects of public health, familiarity with situations and conditions in Tajikistan (disease surveillance system, infrastructure, humanitarian aid, and political situation), scientific presentation to ministry of health (Tajikistan), scientific presentation at conference, preparation of scientific article.

Laboratory rotations: Identification and characterisation of pathogens in bacteriology and virology

The aim was to introduce the fellow to different identification and characterisation procedures performed in the different laboratories. The second aim was to gain insights into various identification strategies for organisms, national laboratory-based reporting systems, laboratory-based surveillance, the information flow within the agency, and the Laboratory Information Management Systems (LIMS). Several laboratories were visited over the course of two years; placement duration ranged from one day to two weeks.

Outbreak investigations

The aim was to be exposed to, and to participate in, the various steps of outbreak investigations as part of an outbreak control team (OCT). Investigations in which the fellow participated were a National Outbreak of *Salmonella* Enteritidis phage type 3 (2011) and a cluster of Group A streptococcal infections in a hospital in south-east London.

Educational outcome: participation in OCT meetings and teleconferences, participation in practical aspects of outbreak investigation (data gathering, trawling questionnaire, analysis, on-site hospital visits), participation in report writing, communication within an interdisciplinary team.

EPIET/EUPHEM modules attended

- EPIET introductory course (20 days)
- Computer tools in outbreak investigations (five days)
- Vaccinology (five days)
- Rapid assessment of emergency situations (five days)
- Introduction to ECDC (five days)
- Biorisk and quality management module (five days)
- Initial management in public health microbiology (five days)
- Project review module (two times five days)
- EUPHEM/EPIET project review module for fellows based in the United Kingdom (two time two days)

Other courses/modules

- ECDC stay (3 days)
- Containment level 3 training course* (3 days)
- Virus discovery in the clinical setting* (5 days)

(* HPA funded)

Publications

Peer-reviewed articles

1. Zakikhany K, Degail MA, Lamagni T, Waight P, Guy R, Zhao H, Efstratiou A, Pebody R, George R, Ramsay M. Increase in invasive *Streptococcus pyogenes* and *Streptococcus pneumoniae* infections in England, December 2010 to January 2011. Euro Surveill. 2011 Feb 3;16(5).

2. Zakikhany K, Efstratiou A. Diphtheria in Europe: current problems and new challenges, Future Microbiol. 2012 May;7:595-607.

3. Zakikhany K, Allen DJ, Brown D, Iturriza-Gómara M. Molecular evolution of GII-4 norovirus strains. PLoS One. 2012;7(7):e41625.

4. Khetsuriani N, Zakikhany K, Jabirov S, Saparova N, Sinavbarova N, Ursu P, Wannemuehler K, Wassilak S, Efstratiou A, Martin R. Seroepidemiology of diphtheria and tetanus among children and young adults in Tajikistan: nationwide population-based survey, 2010. (In preparation).

5. Zakikhany K, Brown DWG, Brown KE. IgG avidity testing as supportive tool for measles diagnostics (In preparation).

6. Zakikhany K, Neal S, Efstratiou A. Emergence and molecular characterisation of non-toxigenic tox gene bearing (NTTB) *Corynebacterium diphtheriae* in the UK (2003–2012). (In preparation).

Reports

1. Zakikhany K, Thompson C, Zambon M. Technical report: Comparison of external quality assessment (EQA) schemes performed in 2008 and 2010 for Influenza virus rapid detection and culture for the Community Network of Reference laboratories for Human Influenza in Europe (CNRL), 2012.

2. Group A *Streptococcus* cluster in a hospital (participation).

3. 2011 National Outbreak of *Salmonella* Enteritidis phage type 3 (participation).

Book chapter

Corynebacterium diphtheriae and related toxigenic species. Chapter: Surveillance
(contribution, in preparation).

Conference presentation

1. Khetsuriani N, Zakikhany K, Efstratiou A, Broughton K, Glumac J, Martin R. Seroepidemiology of diphtheria and tetanus in children and young adults in Tajikistan. (ESPID 2012).

2. Zakikhany K, Hill RLR, Blackburn R, Croall J, Woodford N, Atkinson J, Jarvis R, Livermore DM,Turton JF, Efstratiou A, Sheridan E. A cluster of *Enterobacteriaceae*-producing VIM metallo-β-lactamases in England (ECCMID 2011).

3. Zakikhany K, Allen DJ, Iturriza-Gómara M. A novel application for surveillance and outbreak investigations: Pyrosequencing of the hypervariable P2 domain in Norovirus. (ESCAIDE 2011).

Submitted abstracts (ESCAIDE 2012)

1. Zakikhany K, Brown DWG, Brown KE. Supportive assay for measles case classification in low incidence settings: Immunoglobulin G (IgG) avidity testing of oral fluid samples.

2. Zakikhany K, Valette M, Daniels R, McCauley J, Zambon M, Thompson C. On behalf of the members of the quality and training task group and the Community Network of Reference Laboratories (CNRL) for Human Influenza in Europe. External Quality Assessment for human influenza detection and characterisation in Europe, 2008 and 2010/11: evaluation of results.

3. Zakikhany K, Allen DJ, Brown DWG, Iturriza-Gómara M. Molecular evolution of human norovirus: predicting the emergence of epidemic strains.

4. Khetsuriani N, Zakikhany K, Efstratiou A, Jabirov S, Saparova N, Sinavbarova N, Broughton K, Glumac J, Wannemuehler K, Martin R. Diphtheria and tetanus seroepidemiology among children and young adults in Tajikistan 2010.

Other presentations

1. Zakikhany K. Seroepidemiology of diphtheria and tetanus in children and young adults in Tajikistan. Serosurvey for vaccine-preventable diseases in Tajikistan: Tetanus. To the ministry of health, Tajikistan, Dushanbe (2011).

2. Zakikhany K. Seroepidemiology of diphtheria and tetanus in children and young adults in Tajikistan. Serosurvey for vaccine-preventable diseases in Tajikistan. Initial management in public health microbiology module/ECDC. Stockholm, Sweden (2012).

3. Zakikhany K. European Network for Diagnostics of Imported Viral Diseases (ENIVD), The EUPHEM programme – activities 2010/2011. Antalya, Turkey (2011).

4. Zakikhany K. HPA Trainees Study Day. Health Protection Agency, Colindale, UK (2011).

5. Zakikhany K. The EUPHEM programme. HPA Microbiology Services, Division Colindale, International Day, The EUPHEM programme. London, UK (2010).

Discussion

One of the main goals of the EUPHEM programme is to expose the fellows to different public health experiences and activities, thus enabling them to work across various disciplines in the field of public health. This report summarises the different activities and projects conducted by Katherina Zakikhany, EUPHEM fellow (cohort 3), the second fellow placed at the Health Protection Agency, London, UK. The activities were in line with the 'learning by doing' approach of the EUPHEM programme; all projects had a clear educational outcome contributing into the development of a wide range of experiences and expertise. The activities provided the fellow with a variety of skills required in the field of public health microbiology and strengthened her ability to work in a multidisciplinary team.

Personal conclusions

Public health microbiology is the application of microbiological principles and practice to the prevention and control of infectious disease in a population. It requires microbiologists who are able to work effectively across different

disciplines, especially epidemiology and medical microbiology. The two-year EUPHEM programme offered me the possibility to be exposed to different aspects of public health microbiology, reaching across disciplines and being part of multidisciplinary teams. At present, however, public health microbiology is a discipline recognised only by few European countries, and the strengthening of the collaboration and networking within and between the different public health institutes at the national and international levels is vital to respond to future demands in public health. Through a variety of training activities, 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 unique opportunity of fostering interdisciplinary collaborations and promoting networking between epidemiologists, public health microbiologists and other healthcare specialists across Europe.

The EUPHEM programme aims to increase the expertise in this cross-cutting discipline and strengthen the capacity of the European public health microbiology system. Although the programme is still young and evolving, the importance of fruitful collaborations between microbiologists and other healthcare professionals has been well acknowledged, and the programme will continue to increase awareness and expertise in that area.

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