

The main title 'Summary of work activities' in a bold, white, sans-serif font, set against a blue background.The author's name 'Céline Barnadas' in a white, sans-serif font, positioned below the main title.The subtitle 'European Public Health Microbiology Training Programme (EUPHEM), 2015 cohort' in a white, sans-serif font, positioned below the author's name.The section header 'Background' in a bold, blue, sans-serif font.

According to the European Centre for Disease Prevention and Control (ECDC) Advisory Group on Public Health Microbiology ('national microbiology focal points'), public health microbiology is a cross-cutting area that spans the fields of human, animal, food, water, and environmental microbiology, with a focus on human population health and disease. Its primary function is to improve health in collaboration with other public health disciplines, in particular epidemiology. Public health microbiology laboratories play a central role in detection, monitoring, outbreak response and the provision of scientific evidence to prevent and control infectious diseases.

European preparedness for responding to new infectious disease threats requires a sustainable infrastructure capable of detecting, diagnosing, and controlling infectious disease problems, including the design of control strategies for the prevention and treatment of infections. A broad range of expertise, particularly in the fields of epidemiology and public health microbiology, is necessary to fulfil these requirements. Public health microbiology is required to provide access to experts in all relevant communicable diseases at the regional, national and international level in order to mount rapid responses to emerging health threats, plan appropriate prevention strategies, assess existing prevention disciplines, develop microbiological guidelines, evaluate/produce new diagnostic tools, arbitrate on risks from microbes or their products and provide pertinent information to policy makers from a microbiological perspective.

According to Articles 5 and 9 of ECDC's founding regulation (EC No 851/2004) 'the Centre shall, encourage cooperation between expert and reference laboratories, foster the development of sufficient capacity within the community for the diagnosis, detection, identification and characterisation of infectious agents which may threaten public health' and 'as appropriate, support and coordinate training programmes in order to assist Member States and the Commission to have sufficient numbers of trained specialists, in particular in epidemiological surveillance and field investigations, and to have a capability to define health measures to control disease outbreaks'.

Moreover, Article 47 of the Lisbon Treaty states that 'Member States shall, within the framework of a joint programme, encourage the exchange of young workers. Therefore, ECDC initiated the two-year EUPHEM training programme in 2008. EUPHEM is closely linked to the European Programme for Intervention Epidemiology Training (EPIET). Both EUPHEM and EPIET are considered 'specialist pathways' of the two-year ECDC fellowship programme for applied disease prevention and control.

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This report summarises the work activities undertaken by Céline Barnadas, cohort 2015 of the European Public Health Microbiology Training Programme (EUPHEM) at the Statens Serum Institut, Copenhagen, Denmark.

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

Céline graduated from the university C. Bernard in Lyon (France) as a pharmacist in 2008. During her residency in Pharmacy, she undertook a Masters in microbiology and a PhD studying the molecular epidemiology and resistance to antimalarial drugs of *Plasmodium vivax* in Madagascar. She then undertook two postdoctoral positions, first at the Papua New Guinea Institute of Medical Research (3 years), then at the Walter & Eliza Hall Institute in Melbourne, Australia (4 years). During these years, Céline worked, mainly in the laboratory, on population based malaria surveys aiming to monitor drug resistance, assess intervention strategies and provide support for the evaluation of national malaria control programmes. She undertook the EUPHEM programme in order to broaden her expertise in public health microbiology and develop competencies in field epidemiology.

Methods

This report accompanies a portfolio that demonstrates the competencies acquired during the EUPHEM fellowship by working on various projects, activities and theoretical training modules.

Projects included epidemiological investigations (outbreaks and surveillance); applied public health research; applied public health microbiology and laboratory investigation; biorisk management; quality management; teaching and public health microbiology management; summarising and communicating scientific evidence and activities with a specific microbiological focus.

The outcomes include publications, presentations, posters, reports and teaching materials prepared by the fellow. The portfolio presents a summary of all work activities conducted by the fellow, unless prohibited due to confidentiality regulations.

Results

The objectives of these core competency domains were achieved partly through projects or activities (on-job services) and partly through participation in the training modules. Results are presented in accordance with the EUPHEM core competencies, as set out in the EUPHEM scientific guide¹.

1. Epidemiological investigations

1.1. Outbreak investigations

Foodborne outbreak following a high school gala dinner, Denmark, April 2016

Supervisors: Charlotte Kjelsø, Luise Müller, Steen Ethelberg, Lasse Rasmussen

At the beginning of April 2016, the department of Infectious Diseases Epidemiology of the Statens Serum Institut was requested to provide assistance for the investigation of an outbreak of gastroenteritis that had occurred at a high school in Zealand region, Denmark. The vice rector of the high school had reported around 15 cases of gastroenteritis for the period March 29th – April 8th. The high school had a canteen. A gala dinner had taken place at the school on Friday April 1st. At the same time, the Food and Veterinary authorities as well as the department of Infectious Diseases Epidemiology of the Statens Serum Institut were concerned by the possible occurrence of outbreaks of gastroenteritis linked to the consumption of Lollo bionda lettuce imported from France and contaminated by Norovirus genotype GI. An outbreak investigation was initiated to identify the source of the outbreak, in particular to determine if it was linked to contaminated Lollo bionda lettuce, and to prevent further cases.

The fellow became involved in this outbreak investigation by analysing the results from the cohort study. The fellow was responsible for writing the outbreak investigation report and she contributed to the communication with other authorities and the school and canteen personnel. The investigation showed that consumption of the gala diner starter (which included Lollo bionda lettuce) was strongly associated with developing gastroenteritis. This

¹ European Centre for Disease Prevention and Control. European public health Microbiology training. Stockholm: ECDC; 2017. Available from: <http://ecdc.europa.eu/en/publications/Publications/microbiology-public-health-training-programme.pdf>

outbreak was part of a series of 21 point source outbreaks identified in Denmark due to the consumption of lettuce from batches contaminated by Norovirus genotype GI in March-April 2016. Batches of lettuce suspected or confirmed as contaminated were called back in order to prevent further cases. The fellow co-authored a manuscript reporting on this series of outbreaks across Denmark and presented this outbreak investigation at ESCAIDE (oral presentation, 2016).

Foodborne outbreak in a language school, Copenhagen, April 2016

Supervisors: Luise Müller, Steen Ethelberg, Lasse Rasmussen

On April 19th 2016, the department of Infectious Diseases Epidemiology of the Statens Serum Institut was requested to provide assistance for the investigation of an outbreak of gastroenteritis that had occurred at a language school in Copenhagen, Denmark. Around 15 cases of gastroenteritis were reported among school employees for the period April 13th – April 16th. There was no information on the occurrence of cases among students. An outbreak investigation was initiated to identify the source of the outbreak and to prevent further cases. For practical reasons (ability to contact students, language barrier), it was decided to restrict the investigation to the school employees. It was hypothesised that the outbreak was caused by a meal eaten at the school canteen or shared among school employees. The fellow was involved in all steps of the outbreak investigation, from the first meeting with the school personnel, the kitchen inspection with the Danish Food and Veterinary authorities, the design of a cohort study and a study questionnaire, and the data analysis and reporting. The fellow also communicated with both the Infectious Disease Epidemiology department and the Department of Microbiological Diagnostics and Virology at the Statens Serum Institut which confirmed Norovirus positivity (GII) of six samples. An increased risk (6 times) of developing symptoms such as vomiting and or diarrhea was observed for individuals who ate at the school canteen on April 13th. It was not possible to determine if a dish or food item might have been contaminated by customers of the canteen, employees or if a specific ingredient was already contaminated prior to food preparation at the canteen kitchen. It was recommended to reinforce procedures of hygiene and good practices for food preparation and handling at the canteen kitchen.

Training modules

The methodology used to investigate outbreaks was taught to fellows during three modules. Each module included lectures and practical sessions in the form of case studies.

Introductory course, Spetses, Sept-Oct 2015 (3 weeks): Fellows learnt the importance of conducting outbreak investigation in a structured manner, using the 10 steps of an investigation.

Outbreak investigation module, Berlin, Dec 2015 (1 week): This module focused on practical aspects of an outbreak investigation with the writing of a questionnaire, data cleaning, description of the data (descriptive analysis) and design of an analytical survey (cohort study for example). Statistical analysis were realised with the software Stata.

Multivariable analysis, Zagreb, March 2017 (1 week): This module focused on the different methods used in multivariable analysis (linear regression, conditional logistic regression, Poisson and cox regression in STATA).

Educational outcome: To apply microbiological and epidemiological knowledge in outbreak situations; participation in a multidisciplinary outbreak team and involvement in outbreak investigations (case definition, case-finding, data collection, data analysis, laboratory typing methods, communication); dataset management; writing of report presentations and formulation of recommendations; scientific publication. Opportunity to learn about food items trace back and trace forward procedures.

1.2. Surveillance

Investigation of the burden of adenovirus infections in Denmark, 2010-2016, and molecular characterisation of adenoviruses

Supervisors: Jannik Fonager and Thea Kølsen Fischer

Human adenoviruses (HAdVs) are responsible for febrile respiratory tract infections, conjunctivitis, diarrhoea and more rarely haemorrhagic cystitis and meningitis. HAdV infections can be fatal in immunocompromised individuals. They are also a concern in neonates. In Denmark, hAdV infections have traditionally been diagnosed by immunological assays and more recently also by molecular assays. Positive HAdV samples are recorded in the Danish microbiological database, Miba. No typing of HAdV has so far been performed and therefore no microbiological surveillance of HAdV at a specie level currently exists in Denmark. The objectives of this study were to describe HAdV infection or carriage in Denmark, and to characterise the HAdV genotypes found in Denmark in

order to support the establishment of a laboratory based surveillance system for HAdV in Denmark and increase awareness about HAdV. In this project, the fellow performed typing of HAdV positive samples, sequence and phylogenetic analyses. The fellow also described the occurrence of HAdV infections among all stool, respiratory and eye swab samples received at the Statens Serum Institut during the study period. The study showed the diversity of HAdV circulating in Denmark and the existence of clusters of transmission. In the absence of a surveillance system aimed at detection and reporting HAdV infections it was not possible to determine if outbreaks of HAdV had occurred in Denmark over the study period. Further, we demonstrated that despite a relatively low proportion of HAdV positive samples, notably in stool and respiratory specimen, young children (<5 years old) were commonly affected by HAdV infections. HAdV infections often occurred concurrently with other viral pathogens suggesting a possible underestimation of the real HAdV burden or that HAdV acts via a 'co-infection threshold' for other viral pathogens or vice versa. We recommended that prospective cohort studies with regular sampling schemes irrespective of symptoms and of respiratory material as well as faeces from children be applied to investigate the contribution of HAdV to the burden of respiratory and diarrheal disease in young children.

The fellow drafted a manuscript reporting results for this study and presented this work at ECCMID (poster presentation, 2017).

Respiratory Syncytial Virus surveillance and detection survey to EU/EEA Member States

Supervisors: Thea Kølsten Fischer, Anne Teirlinck (RIVM, Netherland), Eeva Broberg and Pasi Penttinen (ECDC)

Respiratory Syncytial Virus (RSV) is a known cause of substantial morbidity and mortality in children worldwide. We designed a questionnaire based survey with the aim of providing an overview of current practices regarding RSV surveillance and virus characterization in EU/EEA member states. In particular, the survey aimed to collect information on practices around three main axes: RSV disease surveillance, RSV detection and RSV typing in EU/EEA states, in order to provide baseline data prior to the deployment of RSV vaccination in coming years and identify challenges for the generation of reliable data.

The fellow worked together with an EPIET fellow from RIVM and colleagues from ECDC to develop an electronic questionnaire. This questionnaire was sent by the ECDC to Influenza surveillance focal points and reference laboratories in each EU/EEA Member State.

The survey provided information on current RSV surveillance in EU/EEA countries (sentinel/vs non sentinel, included in Influenza surveillance system or not), information on RSV laboratory identification (central vs regional level, methods used, number of samples processed, number of laboratory confirmed RSV cases, etc.) and information on subtyping and genotyping of RSV (existing capacity (or not), methods used, subtypes identified). This overview of current practices in RSV surveillance (both from the epidemiological and laboratory sides) will provide a basis for future discussions on the establishment of a standardized RSV surveillance at the European level.

mecC methicillin resistant Staphylococcus aureus surveillance in Denmark

Supervisors: Anders Rhod Larsen, Anders Koch

As part of a research project in public health microbiology (see this section for more details on the project), the fellow was introduced to the methicillin resistant *Staphylococcus aureus* (MRSA) surveillance system in Denmark. The fellow analysed the epidemiological data extracted from the notification forms received on all *mecC* MRSA cases over the period 2007-2016. The fellow received support from co-EUPHEM fellow Andreas Petersen for the analysis of the *mecC*-MRSA epidemiological and laboratory data. The fellow was also introduced to the different analyses taking place in the laboratory to characterise these isolates: antimicrobial testing, genetic characterisation (*mecA* versus *mecC* gene, *spa* typing, etc.).

Training modules

The methodology used in surveillance was taught to fellows during three modules. Each module included lectures and practical sessions in the form of case studies.

Introductory course, Spetses, Sept-Oct 2015 (3 weeks): Fellows were thought about the different types of surveillance systems and the attributes of a surveillance system. They also worked on the development of questionnaires in surveys.

Outbreak investigation module, Berlin, Dec 2015 (1 week): This module focused on practical aspects of an outbreak investigation with the writing of a questionnaire, data cleaning, description of the data and the design of an analytical survey (cohort study for example). Statistical analysis were conducted with the software Stata. These skills were equally useful and relevant for the analysis of surveillance data.

Bioinformatics and Phylogeny module, Stockholm, Nov 2015 (3 days): This module focused on sequence analysis and phylogenetic analyses. Fellows were introduced to different software such as CLC, Bionumerics, etc. These tools were used by the fellow to analyse sequence data (Adenovirus project).

Educational outcome: Design and implement a survey to assess surveillance practices (questionnaire design, pilot survey, data analysis), public health management and communication, analysis of surveillance data (epidemiological and microbiological information), phylogenetic analyses to inform on pathogens circulating, writing of reports, scientific publications and presentations at conferences.

2. Applied public health microbiology research

Characterisation of five enteroviruses C105, C104 and C109 identified in Denmark in 2015 and 2016.

Supervisors: Sofie Midgley and Thea Kølsten Fischer

The potential for outbreaks due to Enteroviruses (EV) with respiratory tropism, such as EV-D68, and the detection of new and rare EV species C is a concern. These EVs are typically not detected in stool specimens and may therefore be missed by standard EV surveillance systems. Following the North American outbreak of EV-D68 in 2014, Denmark piloted an enhanced EV surveillance system that included the screening of respiratory samples. The objective of this study was to characterise rare EV detected. Positive EV samples received through the enhanced non-polio EV pilot surveillance system were characterized by sequencing fragments of VP1, VP2 and VP4 capsid proteins and clinical observations were compiled. Between January 2015 and October 2016, six cases of rare genotypes EV-C104, C105 and C109 as well as nine cases of EV-D68 were identified. Patients presented with mild to moderately severe respiratory illness; no paralysis occurred. Distinct EV-C104, EV-C109 and EV-D68 sequences argue against a common source of introduction of these genotypes in the Danish population. The enhanced EV surveillance system enabled detection and characterization of rare EVs in Denmark. In order to improve our knowledge of and our preparedness against emerging EVs, public health laboratories should consider expanding their EV surveillance system to include respiratory specimens.

The fellow was responsible for analysing the sequences available and writing the manuscript (published in the Journal of Clinical Virology, 2017). The fellow also presented this work at ESCAIDE (poster presentation, 2016).

Using whole genome sequencing to characterise all *mecC* MRSA cases in Denmark (1975-2016)

Supervisors: Anders Rhod Larsen and Marc Stegger

In 2011, the first *mecC*-positive MRSA was identified in the UK from a case of bovine mastitis. *mecC* is a homologue of the *mecA* gene, carried by a mobile genetic element, the Staphylococcal cassette chromosome *mec* (SCC*mec*) and both genes are responsible for methicillin resistance in *S. aureus*. *mecC*-MRSA has now been reported in 12 European countries and in a large number of animal species including livestock species, wild animals and companion animals. Animal (cow, sheep and pig) to human transmission has been confirmed in Denmark but the relative importance of the environment, livestock and companion and wild animal contact as risk factors of *mecC*-MRSA carriage and infection in humans is still unclear. The aims of this project were: 1/To perform a descriptive analysis of all *mecC* cases identified in Denmark between 1975 and 2016 (defined as *mecC* carriers or infected), 2/To describe the genetic diversity of all *mecC* isolates identified in Denmark during this period and the geographic distribution of clonal complexes, 3/To identify clusters of infections (geographically, hospital acquired infections) and investigate epidemiological links between cases, notably in possible outbreaks, 4/To search for the presence of genetic markers in the Danish *mecC* whole genome sequences which might support the hypothesis of a zoonotic reservoir of infection (eg. bovine adaptation factor) and support hypothesis regarding transmission and spread. Epidemiological information on *mecC*-MRSA cases was retrieved from the Danish MRSA notification system and all *mecC*-MRSA isolates were whole genome sequenced. This study showed that the incidence of *mecC*-MRSA is increasing and is higher in rural areas of Denmark. Geographical clustering of isolates genetically related and household transmission demonstrated the importance of a local and environmental reservoir of *mecC*-MRSA and the importance of the community in the spread of *mecC*-MRSA. With the need for specific laboratory methods to identify the presence of this gene, it is likely that the identification of *mecC* positive MRSA strains is underestimated in Europe. The environmental reservoir of infections needs to be investigated further in order to prevent the spread of these MRSA strains.

In this study the fellow was responsible for analysing epidemiological data on *mecC*-MRSA cases, performing phylogenetic analysis, mapping, and screening whole genome sequences for the presence of virulence factors. The fellow was also responsible for drafting a manuscript. The fellow was also introduced to the laboratory techniques

used routinely in the MRSA reference laboratory. Partial results from this study were presented at the International Symposium on Staphylococci and Staphylococcal Infections (ISSI) in 2016.

Training modules

Introductory course, Spetses, Sept-Oct 2015 (3 weeks): Fellows were trained in writing a study protocol and discussing study designs. Fellows performed exercises on scientific writing.

Bioinformatics and Phylogeny module, Stockholm, Nov 2015 (3 days): This module focused on sequence analysis and phylogenetic analyses. Fellows were introduced to different software such as CLC, Bionumerics, etc. The fellow used concepts of phylogeny and softwares such as SSE, Mega, CLC in her Enterovirus and MRSA projects.

Initial Management in Public Health Microbiology, Stockholm, Feb 2016: The module covered aspects such as time and person management, communication, which are important aspect in research activities.

Educational outcome: Preparation of a study protocol, adherence to ethical principles, gaining expertise in data analysis with STATA, data mapping, analysis of next generation sequencing data and phylogenetic analyses, communication with different parties, presentations at conferences and writing scientific publications.

3. Applied public health microbiology and laboratory investigations

Development and validation of a serodiagnostic test for Mycoplasma genitalium

Supervisors: Jørgen Skov Jensen

Mycoplasma genitalium is responsible of urogenital infections. A prevalence of 1 to 3.3% has been reported in the general population in Western Europe and the USA. Most studies have used molecular methods (PCR) to identify *M. genitalium* infections. While highly specific, PCR only provides a snapshot of the burden of *M. genitalium* restricting diagnosis to ongoing infections. This limits investigations to characterise the association between previous exposure to the pathogen and a current condition such as infertility. Such investigations would require a serological method to detect antibody response to *M. genitalium*. Development of an ELISA has been hampered by the cross-reactivity with antibodies generated against *Mycoplasma pneumoniae*, acquired by most adults. Previous work conducted at SSI led to the identification of a new candidate gene *mg075* coding for an immunogenic protein of *M. genitalium* generating limited cross-reactivity with *M. pneumoniae* antibodies. His-tag MG075 protein fragments were generated and purified. Preliminary ELISA results on known samples showed generation of a specific signal but a low signal/noise ratio indicating that further assay optimisation was required. The fellow was responsible for the optimisation of the ELISA testing different parameters such as plates, buffers, sera and antigen dilutions, etc. Post optimisation, the fellow attempted to validate the assay on 150 sera of known serological status. The fellow performed ROC analysis and estimated the sensitivity and specificity of the assay. The fellow also assessed the assay repeatability. New protein fragments were also prepared by performing transformation in *E. coli* cells using available plasmids.

While urogenital clinical presentations due to *M. genitalium* have been well characterised, the association between infection and complications such as infertility has been found in some studies but not others. It is therefore critical to develop a reliable serodiagnostic assay that can be used in research studies to better understand the burden of *M. genitalium* infections and propose adequate recommendations.

Whole genome sequencing to characterise Influenza B viruses isolated in Denmark during seasons 2011-2016

Supervisors: Ramona Trebbien and Thea Kølsten Fischer

The contribution of Influenza B to all influenza cases is highly variable each year. Perceived as a more benign infection, influenza B has been associated with severe reports and high case fatality rate, notably in children. Influenza B viruses belong to two lineages, Victoria and Yamagata, which have distinct antigenic properties. The difficulties to predict which lineage will be circulating in the next influenza season makes the formulation of the trivalent vaccine challenging as it only includes one Influenza B lineage (the quadrivalent vaccine contains both lineages). Denmark recommends influenza vaccination with a trivalent vaccine and during the 2015-16 influenza season, vaccine effectiveness against influenza B among individuals age 65 and over was estimated at 4.1% (95% CI: -22.0-24.7). This study aimed to characterize influenza B viruses in order to inform on circulating strains and provide molecular data supporting the observed lack of protection induced by the trivalent vaccination in Denmark in 2015-16. The study included over 1800 influenza B samples from the national influenza surveillance system for

seasons 2011-12 to 2015-16. Lineages Yamagata and Victoria were identified by qRT-PCR. Ten samples from each lineage and each season were subsequently randomly selected for whole genome sequencing. Lineage Yamagata represented 96% of influenza viruses characterized from 2011 to 2015. The Victoria lineage was dominant in the 2015-16 season. Phylogenetic analyses of the different segments of the Influenza genome suggested multiple strains introduction during each influenza season. Focusing on the 2015-16 season, amino acid substitutions (using the strain included in the vaccine as a reference) were identified in the 120-loop, the 150-loop and 190-helix of the hemagglutinin. The change to the dominance of the Victoria lineage during the 2015-16 season, not included in the trivalent vaccine that year, was unforeseen. This unpredictability as well as the occurrence of mutations in the antigenic sites targeted by the vaccine explains the poor vaccine effectiveness observed that season in Denmark and represents an important challenge for public health authorities. It highlights the need for molecular surveillance of circulating strains and for the evaluation of the public health benefits of the quadrivalent vaccine. The fellow was responsible for conducting some laboratory work, phylogenetic analyses and drafting a manuscript reporting the study findings. The fellow also wrote an abstract that was accepted for a poster presentation at ESCAIDE 2017.

Detection and identification of *Acanthamoeba* spp. in corneal scrapings by real-time PCR and microbiome analysis (16S/18S analysis)

Supervisors: Rune Stensvold

Acanthamoeba is a free-living amoeba that can cause keratitis and lead to blindness if left untreated. The genus exhibits large genetic diversity, and to date, 20 genotypes (T1–T20) have been identified. Differential diagnosis of keratitis, which can also be caused by bacteria, fungi, and viruses, requires in-depth clinical expertise and is supported by microbiological diagnostics. A microbiome platform was developed for exhaustive detection and identification of ribosomal genes in clinical samples (16S/18S analysis), thus allowing detection of parasites, fungi, and bacteria. As it is difficult to collect corneal scrapings and disease outcome is affected by a late diagnosis, high diagnostic sensitivity is essential to detecting *Acanthamoeba*-associated keratitis. Results obtained by state-of-the-art diagnostics (real-time PCR for *Acanthamoeba*) were compared with total ribosomal gene (16S/18S) analysis (microbiome) in order to evaluate i) the sensitivity of 16S/18S analysis compared with real-time PCR, ii) the robustness of genotyping based on microbiome analysis, and iii) the clinical applicability of the microbiome platform in terms of simultaneous detection of fungi, parasites, and bacteria as causative agents of keratitis. The microbiome platform showed adequate sensitivity and allowed identification of *Acanthamoeba* genotype T4 in most infections. Additionally, ten organisms known to cause eye infections were identified in *Acanthamoeba* negative and positive samples. The microbiome diagnostics platform showed adequate sensitivity for the detection of *Acanthamoeba* infections and could be used in differential diagnostics of keratitis as it allowed simultaneous characterisation of other bacteria and fungi known to be involved in eye infections. Quicker diagnostics would improve care of patients.

The fellow became familiar with the laboratory workflow of microbiome analyses and data analysis aspects, and evaluated the relevance of the microbiome approach as a diagnostic tool when differential clinical diagnosis of an infection (in this project, keratitis) is difficult and early diagnosis is important to ensure adequate treatment and a positive prognosis. The fellow drafted a manuscript reporting the study findings and presented this work at ECCMID (poster presentation, 2017).

Training modules

Bioinformatics and Phylogeny module, Stockholm, Nov 2015 (3 days): This module focused on sequence analysis and phylogenetic analyses. Fellows were introduced to different software such as CLC, Bionumerics, etc. The fellow used concepts of phylogeny and softwares such as SSE, Mega, CLC in her Influenza B and *Acanthamoeba* projects.

Biorisk and Quality Management module, Stockholm, Feb 2016: During this module, fellows were introduced to quality management practices which were applied when the fellow undertook laboratory investigations.

Educational outcome: Application of virology, bacteriology, and immunology concepts to the discipline of public health discipline; understanding the use and limitations of diagnostic and typing methods and their interpretation; developing expertise in analysis of next generation sequencing data and microbiome analysis; development and assessment of laboratory methods to improve surveillance and diagnostics procedures.

4. Biorisk management

Laboratory preparedness in Denmark

The fellow was responsible, together with EUPHEM fellow Andreas Petersen, of preparing a short presentation on laboratory preparedness in Denmark and emerging or re-emerging pathogens. Fellows conducted interviews with

different actors of infectious disease surveillance and public health microbiology in Denmark. They prepared a presentation focused on infectious disease surveillance and reporting systems in Denmark, and on the risk posed by antimicrobial resistance. They also provided information on the laboratory capacity in Denmark (containment levels available).

Characterisation of non-tuberculous mycobacteria

Supervisor: Michael Rasmussen

Together with EUPHEM fellow Andreas Petersen, the fellow undertook an activity in the BSL3 laboratory dedicated to Mycobacteria. The aim of this activity was to become acquainted with laboratory practices and protocols associated with work undertaken in a level 3 containment facility. The fellows were introduced to the laboratory workflow for the growth and identification of mycobacteria. They perform DNA extraction from bacterial cultures as well as library preparation in order to sequence the genome of non-tuberculous mycobacteria and identify organisms present. They were also introduced to antimicrobial susceptibility testing.

Training modules

Biorisk and Quality Management module, Stockholm, Feb 2016: During this module, fellows were introduced to quality management practices and trained in performing risk assessment and proposing mitigation measures. Fellows used an assessment tool to assess process management, quality control and documentation available. Fellows were also introduced to procedures for laboratory accreditation and certification. During this module, fellows also received training in international regulations and good practices for biological specimen shipment and obtained the WHO certificate for International Transport of Infectious Substances. The module included a visit to the Biosafety Level 4 Laboratory of the Folkhälsomyndigheten Institute (Stockholm, Sweden). During this visit, fellows were able to observe the rooms, equipments and discuss practices of the level 4 laboratory.

Educational outcome: Understanding and gaining experience with the processes associated with biosafety in the laboratory at different levels (BSL2/BSL3/BSL4), biorisk management, and international biosafety regulations. Understanding of the aspects to consider for improving laboratory preparedness against emerging and re-emerging pathogens.

5. Quality management

Seventh external quality assessment scheme for typing of verocytotoxin-producing *E.coli* (VTEC), self-funded participants

Supervisors: Susanne Schjørring, Mie Birgitte Frid and Flemming Scheutz

Verocytotoxin producing *Escherichia coli* (VTEC) are strains of *E. coli* that are characterised by the ability to produce verocytotoxins (VT), also called shiga-like toxins. Symptoms associated with VTEC infections vary from mild to bloody diarrhoea, with abdominal cramps, usually without fever. In 5%-10% of infections, a haemolytic uraemic syndrome (HUS) can occur. Human pathogenic VTEC may possess a number of virulence factors that are important in the development and progression of the disease. There are a large number of serotypes of *E. coli* identified as VT producers. Public health microbiology laboratories play a central role in the surveillance of VTEC infections, as well as in the preparedness for responding to outbreaks and in providing scientific evidence for the implementation of prevention and control measures. The WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella* at SSI has played a leading role in establishing a worldwide international network of quality evaluation and assurance for the typing of *E. coli* since 2002. This laboratory arranges annual external quality assurance (EQA) rounds for the national reference laboratories in the EU/EEA and non-EU countries on serotyping and virulence typing for VTEC. The overall aim of this EQA is the harmonization of the typing methods used for VTEC, in order to produce comparable typing data for VTEC strains between laboratories. Together with EUPHEM fellow Andreas Petersen, the fellow's role was to contribute to the management of the 2015-16 VTEC EQA for self-funded participating countries and laboratories, perform results evaluation and generate a report for each participant as well as an overall report describing performance and points for improvement.

Participation in an external audit – Measles and Rubella national reference laboratory for the WHO

Together with fellow Andreas Petersen, the fellow participated in an external audit of the Measles and rubella national WHO reference laboratory conducted by a team from the WHO. Fellows participated in the different

sessions of the audit: description of the laboratory activities, of the research and development undertaken, of quality management, and descriptions of the outcomes of the surveillance activities. Fellows also participated in the tours of the different section of the laboratory (eg. sample reception, serology, etc.) and in the feedback and conclusion session.

Internal audit of the Staphylococci reference laboratory

Together with fellow Andreas Petersen the fellow conducted an audit of the MRSA reference laboratory at SSI. The audit was focused on processes management and quality control in the laboratory, as well as the documentation available to describe procedures and show practices (eg. equipment management). A feedback meeting with staff from the laboratory and the laboratory head was organised to discuss the findings from this audit.

Training modules

Biorisk and Quality Management module, Stockholm, Feb 2016: During this module, fellows were introduced to quality management practices and trained in performing risk assessment and proposing mitigation measures. Fellows used an assessment tool to assess process management, quality control and documentation available. Fellows were also introduced to procedures for laboratory accreditation and certification. During this module, fellows also received training in international regulations and good practices for biological specimen shipment.

Educational outcome: Understanding and applying the principles and practices of quality assurance, administering, analysing and reporting the results of an external quality assurance scheme, understanding accreditation procedures, understand the role and missions of a WHO reference laboratory.

6. Teaching and pedagogy

Introduction to Infectious Diseases Epidemiology

Supervisor: Kåre Mølbak

This was a 3-day course organised for PhD students of the University of Copenhagen and also opened to employees of the Statens Serum Institut with an interest in infectious diseases epidemiology. The course was co-organised by the EPIET and EUPHEM fellows working at the Statens Serum Institut with the support of senior epidemiologists from the Infectious Disease Epidemiology department. Lectures and case studies were prepared and delivered by fellows and senior epidemiologists. In particular, the fellow facilitated two case studies on outbreak investigation and disease surveillance and delivered a lecture on molecular typing in outbreak investigations together with EUPHEM fellow Andreas Petersen. The fellows were also responsible for evaluating the course.

Role of the laboratory in complex emergency situations

Supervisor: Aftab Jasir

The fellow was responsible, together with another EUPHEM fellow, of updating and delivering a lecture during the RAS module (Rapid assessment in complex emergency situations) on the role of the laboratory in complex emergency situations.

Development of a case study for Epicentre, Paris

Supervisor: Marie-Helene Soulier (Epicentre)

The fellow was responsible for developing a case study based on a study conducted jointly by Epicentre and Médecins Sans Frontières. The case study aimed at training epidemiologists in designing a case control study and performing analytical steps. The case study was based on the reporting and investigations of cases of multi-organ failure in a paediatric population of Moronvia, Liberia, which after investigations were conducted were identified as paracetamol intoxications.

Lecture on viral infections of relevance in emergency medicine

Supervisors: Thea Kølsen Fischer, Sofie Midgley and Ramona Trebbien

The fellow was responsible for preparing and delivering a lecture on viral infections relevant to emergency medicine at the annual meeting of the Danish society of doctors working in emergency medicine (Middelfart, Denmark, April 2017). The fellow was assisted by senior virologist Sofie Midgley.

Educational outcome: Planning and organising a course for PhD students and/or public health professionals, structuring a case study, defining learning objectives, preparing lecture and case studies material and exercises, delivering lectures and facilitating group work around case studies, assessing a course.

7. Public health microbiology management

Fellowship's projects and activities

As part of the projects conducted during the fellowship the fellow performed public health microbiology management by having to communicate with different public health actors (SSI, Veterinary and food administration, Epicentre, ECDC, etc.). The fellow also participated in different management and communication exercises playing a leading role in a simulation exercise (management of a team in an Ebola response context). The fellow also had to communicate public health relevant findings from her work at different conferences. The fellows re-set her work priorities in order to manage the outbreaks she was involved in and was also part of collaborations between epidemiologists and microbiologists.

Training modules

Initial Management in Public Health Microbiology, Stockholm, Feb 2016: During this module fellows practiced communication (eg. giving and receiving feedback), discuss management styles, time management, leadership through multiple individual and group activities and exercises.

Educational outcome: Gaining experience in team and project management, planning and managing research and surveillance projects, respecting ethical principles regarding data protection and confidentiality, communication with different public health actors and the scientific community.

8. Communication

Publications

1. Müller L, Rasmussen LD, Jensen T, Schultz AC, Kjelsø C, **Barnadas C**, Sigsgaard K, Larsen AR, Widstrup Jensen C, Jeppesen S, Uhrbrand K, Hove N, Mølbak K, Ethelberg S. Series of Norovirus outbreaks caused by consumption of Green Coral lettuce, Denmark, April 2016. *PLoS Curr.* 2016.
2. **Barnadas C**, Midgley SE, Skov MN, Jensen L, Poulsen MW, Kølsen Fischer T. An enhanced Enterovirus surveillance system allows identification and characterization of rare and emerging respiratory enteroviruses in Denmark, 2015-16. *J Clin Virol.* 2017;93:40-44.
3. **Barnadas C**, Jelsbak Schmidt D, Fischer TK, Fonager J. Molecular epidemiology of human adenovirus-infections in Denmark, 2011-2016. *In preparation.*
4. **Barnadas C**, Stegger M, Petersen A, Holten Møller C, Koch A, Harrison E, Skov RL, Larsen AR. Molecular epidemiology of *mecC*-positive methicillin-resistant *Staphylococcus aureus* infections in Denmark, 1975-2016. *In preparation.*
5. **Barnadas C**, Poulsen MW, Rønn J, Vorborg K, Fischer TK, Trebbien R. Changes in Influenza B virus lineages circulating in Denmark, 2011-2016. *In preparation.*
6. **Barnadas C**, Mirbarati SH, O'Brien Andersen L, Vedel Nielsen H, Stensvold CH. Detection and identification of *Acanthamoeba* spp. in corneal scrapings by real-time PCR and microbiome analysis (16S/18S analysis). *In preparation.*

Reports

1. **Barnadas C**, Petersen A, Schjørring S, Scheutz F. External quality assessment scheme for the typing of verocytotoxin-producing *E. coli* (VTEC), self-funded participants 2015-16.
2. **Barnadas C**. Report on a foodborne outbreak at a gymnasium, Denmark, March 29th-April 8th 2016, Results from a cohort study. Outbreak investigation archives, Statens Serum Institut.
3. **Barnadas C**. Report on a foodborne outbreak at a language school, Copenhagen, April 11th – 14th 2016, Results from a cohort study. Outbreak investigation archives, Statens Serum Institut.
4. Petersen A, **Barnadas C**. WHO audit, Measles and Rubella reference laboratory, Department of Microbiological Diagnostics and Virology, Statens Serum Institut, February 2017.
5. **Barnadas C**, Petersen A. BSL3 laboratory activity: Characterisation of nontuberculous mycobacteria, Statens Serum Institut, August 2017.
6. **Barnadas C**, Petersen A. An insight into the WHO Euro office activities, September 2017.

Conference presentations

1. **Barnadas C**, Kjelsø C, Rasmussen LD, Jensen CW, Sigsgaard KV, Jensen T, Ethelberg S, Muller L. Norovirus outbreak following a high school gala dinner in Denmark, April 2016. ESCAIDE 2016, Stockholm, Sweden. Oral presentation.
2. **Barnadas C**, Midgley SE, Goldstein V, Skov MN, Jensen L, Poulsen MW, Fischer TK. Emerging Enterovirus C104, C105 and C109 in Denmark in 2015 and 2016. ESCAIDE 2016, Stockholm, Sweden. Poster presentation.
3. **Barnadas C**, Stegger M, Petersen A, Skov RL, Rhod Larsen A. Molecular epidemiology of *mecC* MRSA infections in Denmark, 2014-2015. International Symposium on Staphylococci and Staphylococcal Infections (ISSI) Seoul, Korea. Oral presentation (given by M. Stegger).
4. **Barnadas C**, Jelsbak Schmidt D, Fischer TK, Fonager J. Molecular epidemiology of adenoviruses circulating in Denmark, 2011-2016. ECCMID 2017, Vienna, Austria. Poster presentation.
5. **Barnadas C**, Mirbarati S, O'Brien Andersen L, Vedel Nielsen H, Stensvold CR. Detection and identification of *Acanthamoeba* spp. in corneal scrapings by real-time PCR and microbiome analysis (16S/18S analysis). ECCMID 2017, Vienna, Austria. e-Poster presentation.
6. Holten Møller C, Mølbak K, **Barnadas C**, Rhod Larsen A, Koch A. Emergence of *mecC Staphylococcus aureus* in Denmark. ECCMID 2017, Vienna, Austria. Poster presentation.
7. **Barnadas C**, Poulsen MW, Rønn J, Vorborg K, Fischer TK, Trebbien R. Changes in Influenza B virus lineages circulating in Denmark, 2011-2016. ESCAIDE 2017, Stockholm, Sweden. Accepted for poster presentation.

Other presentations

1. Short presentation on the work (research activities) conducted by the fellow prior to starting the fellowship, EPIET-EUPHEM forum (SSI, 23.09.2015)
2. Short presentation on Laboratory preparedness in Denmark for emerging and re-emerging infectious diseases. Panel: Head of ECDC, chief microbiologist and chief scientist. Followed by 10 min questions. Presentation delivered jointly with EUPHEM fellow Andreas Petersen. (ECDC, 12.02.2016)
3. 30 min presentation on „Five cases of emerging Enterovirus C104, C105 and C109 in Denmark in 2015 and 2016“, EPIET-EUPHEM forum (SSI, 06.04.2016)
4. 1,5h of presentation and discussions on the project: „Characterisation of five Enteroviruses C105, C104 and C109 identified in Denmark in 2015 and 2016“, Nordic mini project review (Stockholm, 18.04.2016)
5. 1,5h of presentation and discussions on the project: „Using WGS to characterise *mecC* MRSA cases in Denmark in 2014-2015“, Nordic mini project review (Stockholm, 19.04.2016)
6. 10 min presentation on „Norovirus outbreak following a high school gala dinner in Denmark, April 2016“, EPIET-EUPHEM forum (SSI, 17.08.2016)
7. 30 min presentation on „Molecular epidemiology of Adenovirus infections in Denmark, 2011-16“, EPIET-EUPHEM forum (SSI, 11.01.2017)
8. 1h seminar on „Feedback from ESCAIDE“ given by Steen Ethelberg, Lara Ricotta, Sophie Gubbels and the fellow, Epidemiology of Infectious Diseases department weekly meeting (SSI, 30.01.2017)
9. 30 min presentation on „Whole genome sequencing to characterise Influenza B viruses isolates in Denmark from 2011 to 2016“, EPIET-EUPHEM forum (SSI, 08.03.2017)
10. 1.5h presentation - discussions on „Detection and identification of *Acanthamoeba* spp. in corneal scrapings by real-time PCR and microbiome analysis (16S/18S analysis)“, Nordic mini project review (Helsinki, 04.04.2017)
11. 45 min presentation on „Detection and identification of *Acanthamoeba* spp. in corneal scrapings by real-time PCR and microbiome analysis (16S/18S analysis)“, EPIET-EUPHEM forum (SSI, 19.04.2017)
12. 1h presentation on „Approaches for sample purification prior to next generation sequencing and introduction to freely available tools for mapping (Microreact) and combining phylogenetic and epidemiological data (iTOL)“, Virology department weekly seminar (SSI, 11.05.2017)

Other

1. Team leader in situation simulation exercise conducted during Initial management in Public Health Microbiology EUPHEM module – preparation of a situation report and team management (scenario Ebola outbreak, team based in Guinea) (11.02.2016)
2. Simulation exercise during Initial management in Public Health Microbiology EUPHEM module – 5 min interview with journalist. (12.02.2016)
3. Feedback on internal laboratory audit (MRSA national reference laboratory), together with fellow Andreas Petersen (Sept. 2016)

Training modules – Teaching materials

1. 15 min presentation to EUPHEM fellows and facilitators on public health importance of carbapenem resistance (Spetses, 15.10.2015)

2. 1h presentation on „Hantavirus outbreaks in the 4 corners region (USA) in 1993 and 1998-99“, EPIET-EUPHEM forum (SSI, 17.02.2016)
3. 50min lecture on „Laboratory considerations in complex emergency situations“, RAS module (Athens, 25.06.2016). Attendees: EPIET and EUPHEM fellows.
4. Short presentation (10 min) prior to projection of movie 'Kuru: the science and the sorcery'. EPIET-EUPHEM forum, (SSI, June 2016)
5. Writing of a case study: Investigation of liver failure among children admitted to the MSF Bardnesville Junction Hospital, Monrovia, Liberia. Epicentre (Dec 2016)
6. Modification of an existing case study with inclusion of new materials: Paralytic illness in Ababo. (SSI, sept 2016)
7. 1h lecture on „Viral infections relevant to emergency medicine“. Annual day of the Danish society of doctors working in emergency medicine (Middelfart, 28.04.2017).

9. EPIET/EUPHEM modules attended

1. Introductory Course, Spetses, Greece, 28th September-16th October 2015
2. Bioinformatics and Phylogeny module, Stockholm, Sweden, 16th – 18th November 2015
3. Outbreak Investigation module, Berlin, Germany, 7th-11th December 2015
4. Biorisk and Quality Management module, Stockholm, Sweden, 1st-5th February 2016
5. Initial Management in Public Health Microbiology, Stockholm, Sweden, 8th - 12th February 2016
6. Rapid Assessment module, Athens, Greece, 20th - 26th June 2016
7. Project Review module, Lisbon, Portugal, 22nd - 26th August 2016
8. Multivariable Analyses module, Zagreb, Croatia, 13th - 17th March 2017
9. Project Review Module, Lisbon, Portugal, 28th August - 1st September 2017

10. Other training

1. Pre-deployment course with Médecins Sans Frontières (MSF), Bonn, Germany, 27th-30th May 2016.
2. Vaccinology Fun Massive Open Online Course (MOOC), Pasteur Institute, Dec 2016-Jan 2017.
3. Weekly EPIET-EUPHEM forums. Weekly meetings organised by fellows under the guidance of senior epidemiologists Kåre Mølbak and Steen Ethelberg, and public health microbiologists Thea Kølsten Fischer and Rune Stensvold. These meetings offered the opportunity to fellows to discuss current projects, outbreak investigations and receive feedback and advices in an unformal setting on both epidemiological and microbiological aspects of their projects.
4. Online course, Advanced security in the field. United Nations Department of Safety & Security. June 2016.
5. Visit to the WHO Euro office, Copenhagen, Sept 8th-9th 2017. Insight into the office's activities.

Rapid assessment survey of health needs and living conditions in a refugee camp, Greece

Supervisors: Kostas Danis (EPIET coordinator), Sandra Cohuet (Epicentre, Paris) – fellows leading the survey: Alex Spina, Aymeric Ung.

A group of fellows attending the Rapid Assessment in Emergency situations module in Athens in June 2016 were able to contribute to a rapid assessment survey organised by Epicentre to assess health needs and living conditions in a refugee camp. The fellow was involved by conducting 1 day of interviews together with another fellow and a translator (following a structured questionnaire) in a refugee camp. The survey indicated a limited access to basic hygiene materials in the camp and low access to appropriate care for chronic diseases.

Discussion

Coordinator's conclusions

One of the main goals of the EUPHEM programme is to expose fellows to diverse and multidisciplinary public health experiences and activities, thus enabling them to work across different disciplines. This report summarises all activities and projects conducted by Celine Barnadas during her two-year EUPHEM fellowship (cohort 2015) as an EU track fellow at the Statens Serum Institut in Copenhagen, Denmark. The projects described in this portfolio demonstrate the breadth of the public health microbiology work of Celine. Epidemiological studies included participation in local and national outbreak investigations with clear public health outcomes while surveillance activities extended from addressing the burden of Adenovirus infections in Denmark to work on RSV surveillance

with member states working towards standardised RSV surveillance at the European level. The laboratory and epidemiologically based projects covered a diverse range of disease programmes involving multidisciplinary team work with colleagues in other roles and specialities; clinicians, statisticians, epidemiologists, laboratory technicians, public health officers and government officials and colleagues in different member states, which demonstrates how the fellow has developed strengths in adapting to different environments and contexts.

Activities were in line with the 'learning by doing' and 'on the job training' ethos of the EUPHEM programme and fulfilled the core competency domains described for professionals in their mid-career and beyond. Activities were complemented by nine training modules providing theoretical knowledge. Projects had a clear outcome, with results communicated in scientific journals and at conferences. Céline has been active in contributing to training of others during her fellowship with the development of new case studies as well as direct training and facilitation activities which highlights the contribution fellows can make to capacity building beyond the programme.

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

Supervisor's conclusions

Céline Barnadas was already professionally very experienced with international work-settings and research within infectious diseases when she initiated her fellowship at SSI.

During the fellowship Céline acquired and demonstrated thorough understanding and conduct of all major core domains of the programs. Thus she has gained and grown strong competencies within Public Health Microbiology including field epidemiology, outbreak investigation and surveillance activities. She has been leading major projects in both bacteriology, parasitology and virology at SSI, worked very independently, and also co-led an ECDC-supported international survey on RSV surveillance activities in EU together with an EPI-ET fellow from RIVNM, Netherlands. At SSI, she has had close collaboration with both EUPHEM and EPI-ET fellows, and Epidemiology and Microbiology Departments and thereby bridging and strengthening the collaboration among public health microbiology and epidemiology departments and staff at SSI. She has demonstrated solid professional competencies as well as excellent social skills as a responsible, well-respected and well-liked manager of projects and people.

It is our conclusion that Céline adapted quickly to routines at SSI, encountered challenges with a positive and open mindset, and she has "infected" others with her openly great enthusiasm and interest in our projects and work. She is a great ambassador for the EUPHEM program and for Public Health Microbiology in general and completes her program from SSI with the highest possible scores.

Personal conclusions of fellow

Driven by public health, I joined the EUPHEM programme to gain insights into public health microbiology and field epidemiology, and get hands on experience in projects spanning the disciplines of virology, bacteriology, parasitology and epidemiology. Two years later this has been achieved and I have also been involved in outbreaks investigations which was an extremely valuable experience. I am now looking forward to applying these skills towards the prevention and control of infectious diseases to help improving people's health. Last but not least the programme offers the wonderful opportunity to develop a professional network among public health actors in the European region which will be very valuable in my future professional roles.

Acknowledgements of fellow

Many thanks to all at the Statens Serum Institut who have been supervising and providing me support over the past two years and have made these two years a fantastic experience and in particular, my acknowledgements to my site supervisors, for your enthusiasm, scientific inputs and leadership. My acknowledgements to the EUPHEM head of programme, front- and second line coordinators for your feedback and guidance all along the fellowship. A big thank you to all EUPHEM, EPIET and FETP-PAE coordinators, and the amazing C2015 fellows, to not only make the programme and modules a great learning experience but also fun times. Thanks to past and present EPIET and EUPHEM fellows of SSI for guiding me through my first steps (and quite many after...) in Denmark, and for your friendship. Last but not least a big thank you to my little family who moved across the world to embark on this adventure.