EUPHEM

EUPHEM REPORT

Summary of work activities

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European Public Health Microbiology Training Programme (EUPHEM), 2016 cohort

Background

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 Laura Bubba, cohort 2016 of the European Public Health Microbiology Training Programme (EUPHEM) at the Public health England, Colindale, 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.

Pre-fellowship short biography

The fellow studied and gained the Master degree in Biologia Applicata alla Ricerca Biomedica (Biology Applied to Biomedical Research) at the University of Milan in 2008 and continued to work in the same University where she also obtained her doctorate (PhD) in public health working within the virology unit. Her PhD thesis focused upon congenital cytomegalovirus (CMV) infection and development of an alternative tool for a rapid, sensitive and specific test for potential neonatal screening, in order to rapidly identify new cases and follow up for prompt therapy intervention.

During and after her PhD she was involved in the regional (Lombardy, northern Italy) Rotavirus, Poliovirus, Measles, Rubella and Influenza surveillance systems as a post-doc scientist, combining laboratory work with the analysis and interpretation of the data along with teaching responsibilities to University student during their one-year laboratory training.

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 guide[1].

1. Epidemiological investigations

1.1. Outbreak investigations

Supervisors: Sooria Balasegaram, Charlotte Anderson

A. Group A streptococcus outbreak among people who inject drugs (PWID) and homeless

A cluster of invasive group A streptococcus (iGAS) *emm*66 among people who injected drugs (PWID) and the homeless people was identified in October 2016 and spread within England and Wales. Upon further investigation, it was possible to identify more cases retrospectively (from January 2016) and prospectively for a total of 64 cases. The *emm*66 is an uncommon GAS type and from 2005 only 18 cases were reported within the iGAS surveillance system in England and Wales. All ten steps of an outbreak investigation were undertaken. Following the establishment of the outbreak investigation team (OIT), the number of cases was confirmed, defining the case definition and identifying new cases with the creation of an appropriate alert and questionnaire. From the information collected in the questionnaire, it was possible to describe and analyse the data, using EpiData and R, comparing the ongoing outbreak with the historical data available from the surveillance system, in order to help with the identification of appropriate control measures and identify public health recommendations. The circulation of GAS *emm*66 is still ongoing and it is being monitored despite the fact that the outbreak investigation was closed in May 2017. The route of transmission has not been identified, due to the hard-to reach population involved and

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

the limited number of environmental samples and cases among the general population. However, the hypothesis of a new, more successful clone of *emm*66 circulating among PWID and the homeless people which started to circulate in this population in 2016, was addressed by a specific research project (see research project section below). The EUPHEM fellow was involved in the outbreak from the beginning, participating within OIT meetings, generating the EpiData questionnaire, keeping the line list of cases updated, checking the microbiological output, undertaking the network analysis, presenting the results in both national and international conferences and contributing towards the publication and the outbreak report.

B. *Salmonella* Give outbreak in Malta

Supervisors: Amy Mikhail, Tim Dallman

An outbreak of *Salmonella* Give occurred in Malta in October-November 2016, involving 33 suspected cases. The outbreak investigation revealed the hypothetical source of infection could be linked to a local food manufacturer and/or local farms where infected animals were identified. In order to confirm/confute the epidemiological link, colleagues in Malta analysed 26 samples by whole genome sequencing (WGS). These samples were collected from eleven laboratory confirmed cases, seven veterinary, two from working staff and four from food specimens. Ready-to-eat antipasti from the same manufacturer were supplied to four restaurants that reported cases. For the WGS analysis, the EPIET fellow Alastair Donachie primary investigator of the outbreak investigation involved the EUPHEM fellow Laura Bubba based in UK. Using the Public Health England (PHE) pipeline established for the analysis of Salmonella sequences, it was possible to identify two clusters, distinguishable by more than 6,000 single nucleotide polymorphisms (SNPs). The first cluster, with a range of 0-2 SNPs differences, included 14 Maltese isolates, linking eight cases with the food collected at restaurants, the same manufacturer and the two staff samples. Interestingly, using the extensive PHE database it was possible to link with the first cluster, two British cases who travelled to Malta during the outbreak. The second cluster comprised two of the confirmed Maltese cases and all veterinary samples with a range of 0-7SNPs; with a British case reported a travel history in Spain in August 2016 fitting this second cluster. Deviations in following safety food procedures in the Maltese manufacturer were identified and the appropriate public health response was undertaken. WGS coupled with the international collaboration were very useful tools to identify the two clusters, strengthening the infection chain hypothesis and identifying the possible source of infection for two British cases who travelled within Malta during the outbreak; the third British case still remains with an unknown route of infection, as the travel history was incomplete. The EUPHEM fellow was the main link between the Maltese and English public health structures, analysed the sequences with the support of PHE's bioinformatics and pipeline, generated the network analysis and contributed towards to the final publication.

C. Training modules

During the EPIET/EUPHEM fellowship have been provide the basic of the outbreak investigation and the analytical tool that can be used to assess the ten step of an outbreak investigation. In particular, during the "introductory course" the 10 steps of an investigation were presented such as the logic and the statistical approach to each step, while the "Outbreak investigation" module provided the software packages such as EpiData, STATA and Microsoft excel. In the following module "Multivariable analysis" were also provide the statistical principles to build up the optimal model (linear, logistic, Poisson and Cox regression) to use in the setting under investigation. Moreover, in "Rapid assessment and survey" module it was possible to explore other tool for questionnaires and mapping, useful for the investigation on the field.

Educational outcome: Knowledge of the ten steps of an outbreak investigation and application of microbiological and epidemiological knowledge at each situation, participation to outbreak investigation as part of OIT, participation to all steps contributing both as epidemiologist and microbiologist, dataset management, presentation of the outbreak during the "Outbreak module", writing the outbreak report and scientific publications.

1.2. Surveillance

Supervisor: Theresa Lamagni

A. Influenza as risk factor of increasing activity on the incidence and severity of invasive group A streptococcal disease: a retrospective study (2008-2016) in England.

During the 2015/16 and 2016/17 seasons an increase in invasive Group A Streptococcal (iGAS) infection reports were observed during the end of an unusually prolonged influenza virus season. Despite previous reports of increased iGAS activity during influenza seasons, the role of influenza virus infection remains unclear and this study aimed to assess whether late influenza activity played a role in increasing iGAS incidence. A confirmation of influenza virus as a risk factor for increases in iGAS infections can inform the public health management of contacts of community-acquired iGAS infection and influenza vaccine policy. With this purpose, a retrospective surveillance data analysis from 2008/09 to 2016/17 seasons from cases in England was undertaken, merging laboratory-confirmed notifications of iGAS and influenza infection laboratory data by extraction of information from the national surveillance system (SGSS). During the study more than 45,000 cases were analysed with STATA, identifying a higher number of coinfections during 2010/11 and 2015/16 seasons, with an increased case fatality

rate (CFR) among children up to 5 years old. Both seasons (2010/11 and 2015/16) were characterized with a higher circulation of influenza virus H1N1, however, minimal information on the influenza strains were available in the second generation surveillance system (SGSS) database. Therefore, the study confirmed the increase of iGAS/influenza co-infections in 2015/16 season and, after further investigation also upon the influenza strains; these data can provide information and help to making decisions for vaccine policy and contact management. The EUPHEM fellow merged the two datasets, analysed the data with STATA and prepared and presented the results to conferences and a final publication in a scientific journal.

B. Malaria in England: a revision of the surveillance system between 2008-2017

Supervisor: Colin Southerland

Malaria is a tropical disease commonly imported into the UK, with 1300-1800 cases reported each year, and 2-11 deaths. The WHO recommendation for first line treatment of *P. falciparum* malaria is artemisinin-based combination therapy (ACT), however in sub-Saharan Africa artemether-lumefantrine (AL) therapy is most widely used. In Asia, a reduction in artemisinin susceptibility mediated by specific mutations in the parasite *pfk*13 locus has been observed, but the same variant has not been observed in Africa. No systematic studies are available, but the PHE Advisory Committee on Malaria Prevention in UK Travellers recommend that it is good practice to repeat a blood film and full blood count approximately 14 days after treatment, particularly if patients have had severe malaria or have received artemisinin therapy. In 2015/16, a recent study observed in four patients who were treated with AL for *P. falciparum* malaria in a UK hospital, recurrence of malaria symptoms caused by *P. falciparum* parasitemia within six weeks of treatment and with no intervening travel. No evidence of *pfk*13-mediated artemisinin resitence was identified, however other mutations carried in the *pfcrt*, *pfmdr*1, *pfap*2*mu* and *pfubp*1 genes were observed. Rapid identification of potential treatment failure is essential for the correct management of the patient, to inform clinicians on the most appropriate therapy and to build the basis of a more sensitive surveillance system. To date, the identification of potential treatment failure cases has not been systematic due to the surveillance system being based upon the collection of malaria cases on a single database that does not record all events as patient-based but as episode-based. Therefore, a review of the data collected from 2008 to 2017 has been addressed in order to evaluate the surveillance system in terms of completeness, validity, sensitivity and specificity, timeliness, usefulness, representativeness, flexibility and acceptability. In particular the aim was to assess the ability of the current surveillance system to capture potential treatment failures, in order to inform the phicisians for a better patient managment and set up recommendation for a better capture of this cases. The EUPHEM fellow collected and analysed the data using STATA in order to prepare and present the results to a scientific journal and report the results to the stakeholders.

C. Circulation of non-polio enterovirus (EV) and human parechovirus (HPeV) strains in EU/EEA Member States, 2015-2017

Supervisors: Heli Harvala, Eeva Broberg

Enterovirus (EV) and parechovirus (HPeV) are viruses mainly transmitted by faecal-oral and respiratory routes and result in a wide range of clinical outcomes, spanning from asymptomatic, gastroenteritis or cold symptoms to life threatening encephalitis, meningitis, paralysis and hepatitis, affecting manly children up to 5 years. Recently several outbreaks and severe outcomes associated with these viruses have also been reported in Europe. Despite the existence of poliovirus surveillance including standardized laboratory protocols coordinated by WHO in Europe, a laboratory based non-polio EV and HPeV surveillance system does not currently exist. A recent European study by Harvala *et al*. collected information about the microbiological methods used for non-polio EV detection and the existence of dedicated surveillance systems. They observed that the methods used for EV typing are variable across European countries but the majority (65%) are able to type EV by sequencing. Typing data are necessary to establish an appropriate surveillance system and to correctly inform physicians. In fact, the severity of the outcome is type-related and a surveillance including type information will be able to inform public health actions. Therefore, a study was assessed in order to describe, analyse and characterize the circulating EV and HPeV strains across EU/EEA Member States. An aggregate file was therefore, sent to the National Focal Points (NFPs) of each EU/EEA Member State via the Coordinating Competent Bodies (CCBs) of the European Centre for Disease Prevention and Control (ECDC). It was required to complete an aggregate file reporting the number of EV detected and typed by month and year of study (2015-2017), the clinical symptoms, the age and the sample type by EV/HpeV type. More than 16,500 EV and 900 HPeV were reported by 24/31 EU/EEA member states of which approximately 70% and 19% were typed respectively; neurological symptoms were the most frequent and amongst young children who represented the majority of the study population. The dataset created within this study is the largest in Europe and confirmed the wide circulation of these viruses in all European member states, assessing their epidemiology and identifing priorities and standards for the future establishment of a European-wide surveillance network. The EUPHEM fellow prepared all the informative material to send to the National focal points (letter of invitation, aggregate file), managed the website database, collected and analysed the data, maintained contact with each country, drafted and presented the results to scientific journal and to an international conference.

D. Evaluation of an enhanced Methicillin-resistant *Staphylococcus aureus* surveillance system in England, April 2017- March 2018

Supervisors: Angela Kearns, Russel Hope

Methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated within one year of the introduction of methicillin in the UK in 1961 and its incidence among *S. aureus* bacteraemia cases gradually increased to about 40% in the early 2000's (surveillance data). The spread of MRSA has been characterized by the international emergence of new epidemic clones (EMRSA) associated with different antimicrobial susceptibility profiles and identified using various genotypic techniques. However, these techniques lacked discriminatory power when one or a few clones were predominant, implying a lack in understanding clonal transmission. Recently, the introduction of whole-genome sequencing (WGS) has afforded key public health advantages enabling better description of transmission events in both healthcare and community settings, outbreak investigations and surveillance activities. The introduction of WGS in the routine surveillance system and weekly "chase-for-data" approach was introduced in the new integrated system established in April 2017. The identification of high risk clones in the population increased concerns about MRSA clones circulating suggesting that an enhanced survey, chasing for the isolates and including the WGS analysis could lead to a better and more efficient surveillance, rapidly identifying potential high risk clones, outbreaks and monitoring further changes in MRSA epidemiology. A retrospective evaluation of the first year of implementation of an enhanced MRSA surveillance system in England from April 2017 to March 2018 was undertaken in order to investigate whether the new system improved the surveillance coverage (that was about 40% in the pilot study and about 12% before the introduction of "chase-for-data" strategy) and evaluated the epidemiology of the circulating clones during that period. From the analysis, 76%of bacteraemia isolates were submitted to the reference laboratory for further investigation and were succesfuly matched with the case reported to the notification system. The majority of infections were community acquired and new clones are emerging as the high-risk clonal complex 5 (CC5) representing about 11% of circulating clones in 2017/18 and being the second most frequent clone both among hospital and community acquired cases. These results highlighted that an enhanced and more comprehensive surveillance system would lead to a better understanding of circulating clones, rapid detection of clusters and improvments in patient management. The EUPHEM fellow merged and analysed the two datasets with STATA, and presented the results to conferences and writing the final paper.

E. Training modules

During the "introductory course" the basis of the different approach of surveillance systems were delivered, including all logical and analytical tools in use to develop, validate and evaluate the attributes of a surveillance system. During the "Rapid assessment and survey" module was also possible to explore the more appropriate tools in use for nutritional, morbidity and mortality survey on the field or in emergencies situation.

Educational outcome:

During all surveillance projects, the fellow had the opportunity to apply different approaches and several tool such as the use of surveillance data to answer specific public health questions, the evaluation of a surveillance system to improve and implement surveillance attributes, the use of surveillance approach to build up the base line for an European surveillance system and evaluation of a surveillance system after the implementation of a new microbiological method. In all project, different tool have been used, from STATA, to Microsoft excel and mapping, interacting at each stage with different authorities and formulating specific public health recommendation. The final outputs were reported in peer review journal.

2. Applied public health microbiology research

A. Invasive Group A Streptococcal (iGAS) *emm*66 outbreak investigation during 2016 in England and Wales

Supervisors: Juliana Coelho, Vicki Chalker

In response to an outbreak of a rare iGAS type emm66 among people who inject drugs, homeless and people reporting problematic alcohol abuse in England and Wales, 2016 -2017 (see outbreak investigation section above), a research project was established to better understand the genetic diversity of *emm*66 GAS/iGAS strains, identify any geographical sub-clusters and epidemiological links that may have occurred in the ongoing outbreak and evaluate whether the current outbreak was caused by a new strain or by the expansion of a historical strain. Moreover, the evolutionary rate of *emm*66 was assessed by SNP and BEAST analyses. Knowledge of the changing virulence and fitness of the pathogen was also addressed and this would inform strategies for prevention and treatment. With these objectives, WGS was performed on 55 *emm*66 GAS isolates (n=50 *emm*66.0, n=5 *emm*66.1);

including 34 cases, 3 contemporaneous (2016) patients not fitting the case definition and 18 sporadic historical isolates (2005-2015). All sequences collected from outbreak cases and contemporaneous patients merged into the same clade with the *emm*66.0 historical cases collected in 2015 and resulted with more than 8,000 SNPs difference with other historical isolates, highlighting that the strain that caused the outbreak was a new clone probably introduced in 2015. WGS analysis confirmed epidemiological links that were difficult to define previously, identified geographical sub-clusters, redefined the case definition and increased the knowledge on this rare clone. The fellow prepared the research protocol, set up the experiment, analysed the WGS aligment file using the PHE pipeline, analysed the data, prepared and presented the results to national and international conferences and for the scientific journal.

B. *Training modules*

During the "introductory course" module it was possible for EPIET/EUPHEM fellow to understand the stage of a study protocol such as aim, objectives, methods and expected outcomes. Moreover, guidance on how to present the research outcome have been explored in the "introductory course", "Outbreak investigation" and "Multivariable analysis".

Educational outcome: The fellow conducted a research project where all stages were undertaken, from the assessment of a public health topic, to the plan of a study protocol and the elaboration of scientific paper, identifying limitations, future challenges and public health impact for the project. The final output has been presented in peer review paper.

3. Applied public health microbiology and laboratory investigations

Supervisors: Norman Fry, David Litt, John Duncan

C. Characterisation of *Bordetella pertussis* acellular pertussis vaccine antigens using ELISA to screen circulating UK strains and correlation with genotype and epidemiological data

Despite high vaccine coverage, UK cases of *Bordetella Pertussis* increased in 2015 (4,190) and 2016 (5,945) in comparison with the number of cases reported in the early 2000s (<500 cases/year). Several hypotheses have been proposed for the recent rise in pertussis infection, including sequence variation or lack of expression of vaccine antigens by the bacteria and changes in vaccination policy in affected countries. In fact, the rise in pertactin (PRN) negative isolates that occurred a few years after the switch to acellular pertussis vaccines for primary vaccination in several countries has led to a hypothesis that these vaccines are driving the selection of PRN negative mutants. Therefore, a study was set up in order to assess for the first time in England the PRN and pertussis toxin (PT) antigen expression in isolates collected at the National reference laboratory from 2013 to 2016, along with host factors such as age and vaccination history. Moreover, MLVA analysis and Sanger sequencing were performed on the same samples in order to compare genetic and phenotypic data. Three-hundred- sixty-five isolates were collected in the study period, quantifying the PRN and PT expression by ELISA and *ptxP*, *ptxA* and *prn* genotype. Clinical data from each patient was collected as part of Public Health England's enhanced surveillance programme for pertussis. From the preliminary results, it was observed that 57% and 64% of isolates did not expressed PRN in 2015 and 2016 respectively, whilst PT was expressed in all isolates. Genetic variances were not identified on the amplified regions of studied genes. The presence of PRN expression deficit in clinical isolates could explain why in England pertussis infections continued to be detected, rising a public health concern as they could potentially lead to vaccine escape clones and consequently to new clusters and outbreaks. The EUPHEM fellow selected all the pertussis positive isolates from 2013-2016, performed all the ELISA tests, linked the results to the genetic analysis and the clinical data, analysed the final results and drafted the paper for submission to a peer review journal.

Educational outcome:

In all module/course attended during the two-year fellowship, there was always the integration between epidemiological and microbiological approach. In particular, during the case studies, different microbiological tests have been discussed. The fellow has used her knowledge to identify the better approach to apply to outbreak investigation, surveillance system, research project and quality management. Moreover, the fellow had the opportunity to learn and apply new laboratory competencies. Outputs from the laboratory investigation have been presented to conference and peer review journal.

4. Biorisk management

A. WHO workshop: Preparation and dispatch of *Corynebacterium* spp. cultures

Supervisor: Androulla Efstratiou, Ginder Mann

Corynebacterium diphtheriae, *C. ulcerans*, *C. xerosis*, and *C. striatum* cultures were tested, prepared and dispatched for the forthcoming WHO workshop on "Laboratory diagnosis of Diphtheria" in Beirut, July 2018. Cultures were sent as freeze-dried specimens in compliance with current regulations (i.e. as biological substance, category B, packed to full UN3373 specifications).

B. Biorisk management exercise

After the "Biorisk management" module has been asked to the EUPHEM fellow to provide an exercise: laboratory audit in one of the laboratory in the training site. For the exercise the fellow has chosen the Diphtheria laboratory that resulted in line with the norms on sample management and control.

C. Training modules

The EUPHEM module "Biorisk management" provided the basis to assess biorisk and biosafety and indicating the appropriate mitigation actions and the WHO recommendation. During the module was also possible to visit a containment level 4 laboratory at the Public Health Agency of Sweden, Stockholm; the fellow had also the opportunity to visit the CL4 laboratory in Public Health England, Colindale, London.

Educational outcome: The fellow has the opportunity to obtain the certificate for international regulations on the transport of dangerous goods according to the International Civil Aviation Organisation (ICAO) and to practice during the "Biorisk management" module the correct procedures and appropriate measures for the safe transport of hazardous substances and pathogenic specimens. The fellow applied this knowledge for the dispatch of diphtheria panel in Beirut as part as the laboratory-based WHO workshop.

5. Quality management

A. Whole-genome sequencing analysis to rapidly detect Hepatitis C genotypes and antiviral resistance markers: clinical validation of the assay before the routine introduction in the UK diagnostic in 2018

Supervisors: Tamyo Mbisa, Daniel Bradshaw

Hepatitis C virus (HCV) is a drug resistant virus and a public health concern. In England and Wales more than 11,000 cases were reported in 2016 and surveillance is one of the main tools to rapidly identify new cases and monitor the circulation of drug resistant strains. To date, drug resistance testing is recommended as the baseline for the detection of key polymorphisms in genotype 1a viruses that affect the response to the protease inhibitor Simeprevir or non-structural (NS) 5a protein inhibitors. Following the increasing use of direct-acting antivirals (DAAs) in patients failing therapy, the request of a routine test for HCV resistance by clinicians has also increased. Currently, the detection of polymorphisms within NS3, NS5a and NS5b viral genes is requested and routinely delivered by Sanger-based sequencing of partial regions on target genes. Whole Genome Sequencing (WGS) assays can provide both the HCV genotype and susceptibility to all current and future DAAs in one single test with clear advantages for patients and public health, informing clinicians on the more appropriate therapy, reducing treatment failures and generating a large amount of data useful to epidemiologist to inform public health policy and interventions on long term and improving HCV surveillance and outbreak detection.
The clinical validation was set up following the Clinical Laboratory Improvements Act (CLIA) guidelines for laboratory developed tests (LDTs), selecting randomly 377 samples tested with Sanger sequencing previously, representative of the samples collected during the surveillance by genotype and genetic resistances. The concordance between Sanger and WGS resulted was evaluated with high concordance between the two methods both for the genotype and resistance genes identification. The EUPHEM fellow collaborates to identify, select and prepare the blinded clinical validation panel, collected the results, analysed the data and contribute in writing the final report.

B. Training modules

During the module "Quality management" an overview of the quality management systems in use in diagnostic have been provided, following the current norms and regulation. The rational and the approach of internal and external quality, accreditation, assessment and audit have been discussed.

Educational outcome: the fellow was involved in a clinical validation of WGS as diagnostic tool for genotyping and identification of resistance genes. During this experience the fellow had the opportunity to apply the knowledge on quality control during the validation process.

6. Teaching and pedagogy

A. Lab4Epi

The Lab4Epi module is a two-day course held at Public Health England (PHE) that takes place every year and in 2018 it occurred on 16th and 17th January respectively. The priority audience comprises EPIET and EUPHEM fellows, epidemiologists, health protection (HP) practitioners, public health speciality registrars, and is a core, mandatory training module for fellows of the UK Field Epidemiology Training Programme (FETP). The principal aim of this module is to improve the collaboration between epidemiologists and laboratory specialists building an understanding of perspectives and expectations. The course develops further each year following the data reported in the evaluation of the previous year, therefore this year the EUPHEM fellow Laura Bubba and the FETP fellow Ashley Sharp developed a "cheat-sheet" as an answer to the request to have something schematic, easy to read and to understand the main microbiological techniques that would be applied in a public health setting. The learning objectives spanned from basic information on microbiology to microbiological methods applied in diagnostic, surveillance, outbreak and research settings. At the end of each day a wrap-up was organized with a quiz and a game at the end of the two-day module. The course was very well evaluated and the majority of participants scored the presentations and the proposed activities as good and excellent. The EUPHEM fellow collaborated with the preparation of the programme and organization, developed the "cheat-sheet", facilitated all sections and the case studies and presented the wrap-up, the quiz and the game to the participants.

B. Teaching international assignment: WHO Workshop on Laboratory Diagnosis of Diphtheria

The WHO Global Collaborating Centre for Diphtheria is based within PHE. PHE, WHO Eastern Mediterranean Regional Office (EMRO) and WHO Headquarters provided a five-day laboratory-based workshop at the American University of Beirut, in Beirut, Lebanon (23-27 July 2018) in order to strengthen participants' skills and to build capacity in the laboratory diagnosis of diphtheria within the Eastern Mediterranean Region where the disease is endemic and has re-emerged in many countries. At the workshop, 12 participants from 12 Eastern-Mediterranean countries were involved. The fellow was involved in the organization and had the role to prepare and test all reagents that will be used during the workshop, including the control panel and simulated specimens, contributed (the molecular methods for toxin detection) the laboratory manual for the participants, collaborated in the coordination of the shipment of materials delivered a presentation on the molecular methods in use in diphtheria diagnosis and facilitated in the laboratory workshop.

C. Accreditation presentation ECDC

The fellow delivered a lecture focused on the norms and accreditation, during the "Management, Leadership and Communication in Public Health" module settle in Stockholm in February 2018. The lecture was shared with the EUPHEM fellow Lotta Siira and aimed to assess all norms and standards that are in place for laboratory accreditation.

Educational outcome:

The fellow had applied the knowledge presented during the "introductory course" both for the preparation and the delivering of teaching presentation. In particular she assessed the need of target group, planning, organising, generating new material and delivering tailored lectures and practical section. At the end of each section an evaluation of the teaching activity was undertaken followed by debrief with the colleague involved and a final reflective note or report.

7. Public health microbiology management

A. Implementation and state of the art of whole genome sequencing (WGS) in England for surveillance and outbreak management

In early 2018, the fellow performed a research study on the "Implementation and state of the art of whole genome sequencing (WGS) in England for surveillance and outbreak management". The results were presented to the Director, the Chief Microbiologist, and the Chief Scientist of ECDC, during the Initial Management in Public Health Microbiology module at Stockholm, February 2018.

B. Management during outbreak investigations and projects

During the outbreak investigation, surveillance projects and WHO workshop the fellow was involved in several meetings and she needed to communicate with different authorities in order to address the goal of each project. In particular, during the outbreak investigations she took part at all meetings of the OIM or created a bridge between the colleagues in Malta and the bioinformatics team in PHE, while during the EV/HPeV surveillance projects she keep the contact with all EU/EEA national/regional laboratories involved in the projects, such as with the ECDC; being involved in multidisciplinary settings.

C. Training modules

The EUPHEM module "Public health management and communication", was a module that aimed to provide tools for understanding of role and responsibilities in public health management. Different management styles were taken under consideration, such as the different approaches to a team in order to improve their performance, the delivering of feedbacks, time and stress managing. The communication part was developed with practical exercises that aimed to train the fellows for communication with higher authorities and the management of complex setting during emergency situation.

Educational outcome:

The fellow had the opportunity to apply all public health management and communication to all projects and setting where she was involved during the two-year fellowship, understanding the bases of the team, time, stress and feedbacks management that she can continue to use in her professional life.

8. Communication

Publications

A. Publications relating to EUPHEM fellowship

1. Bundle N, **Bubba L**, Cohelo J, Kwiatkowska R, Cloke R, King S, Rajan-Iyer J, Courtney-Pillinger M, Beck CR, Hope V, Lamagni T, Brown CS, Jermacane D, Glass R, Desai M, Gobin M, Balasegaram S, Anderson C.: Ongoing outbreak of invasive and non-invasive disease due to group a streptococcus (gas) type emm66 among homeless and people who inject drugs in England and wales, January to December 2016. Eurosurveillance, 22(3), 2017

2. Donachie A, Melillo T, **Bubba L**, Hartman H, Borg ML: National outbreak of *Salmonella* Give linked to a local food manufacturer in Malta, October 2016, Epidemiology and Infection 1–8. https://doi.org/10.1017/S0950268818001656

3. **Bubba L**, Bundle N, Kapatai G, Daniel R, Balasegaram S, Anderson C, Chalker V, Lamagni T, Brown C, Ready D, Efstratiou A and Coelho J: Genomic sequencing of a national emm66 group A streptococci (GAS) outbreak among people who inject drugs and the homeless community in England and Wales, January 2016-May 2017 – submitted

4. **Bubba L**, Broberg E, Jasir A, Harvala H, and the European study group: Circulation of non-polio enteroviruses in 24 EU/EEA countries between 2015 and 2017 – is it time to consider systematic surveillance?- in preparation

5. **Bubba L**, Nsonwu O, Pichon B, Davies J, Doumith M, Johnson A, Woodford N, Hope R and Kearns A. Exploiting Whole-Genome Sequencing for enhanced surveillance of methicillin-resistant *Staphylococcus aureus* bacteraemia in England- drafted

6. **Bubba L**, Guy R, Pebody R, Brown C, Ellis J, Chand M, Lamagni T: Effect of influenza activity on the incidence and severity of invasive group A streptococcal disease: a retrospective study (2008-2016) in England- drafted

7. **Bubba L**, Broberg E, Jasir A, Harvala H, and the European study group: Circulation of human parechovirus (HPeV) in 24 EU/EEA countries, 2015-2017- drafted

8. **Bubba L**, Afshar B, Southrland C: A retrospective surveillance study to determine the number of recurrent malaria cases and to genotype *Plasmodium falciparum* from these UK cases, 2008 to 2015- in preparation

9. **Bubba L**, Sandu P, Duncan J, Litt D, Amirthalingam G, Ribeiro S, Campbell H, Andrews N and Fry N: Molecular typing and the expression of pertactin and pertussis toxin vaccine antigens in *B. pertussis* clinical strains from the UK in 2013 and 2016- drafted

B. Other publications

1. Turner C, **Bubba L**, Efstratiou A: Gram positive book chapter: Pathogenicity factors in group C and G Streptococci- in press 2018

C. Reports

1. Outbreak of invasive and non-invasive disease due to group A Streptococcus (GAS) type emm66 among homeless and people who inject drugs in England and Wales, 1 January 2016 to 23 May 2017.
2. Whole-genome sequencing analysis to rapidly detect Hepatitis C genotypes and antiviral resistance markers: clinical validation of the assay before the routine introduction in the UK diagnostic pathway in early 2018.
3. Workshop on Laboratory Diagnosis of Diphtheria report.

D. Teaching materials

1. Lab4Epi reflective note
2. Lab4Epi cheat-sheet
3. Lab4Epi final evaluation
4. Quality management module, accreditation- presentation
5. Workshop on Laboratory Diagnosis of Diphtheria- presentation 1
6. Workshop on Laboratory Diagnosis of Diphtheria- presentation 2

E. Conference presentations

Oral presentation:

1. **Bubba L**, Guy R, Pebody R, Brown C, Ellis J, Chand M, Lamagni T: Effect of influenza activity on the incidence and severity of invasive group A streptococcal disease: a retrospective study (2008-2016) in England - selected as oral presentation at ESCAIDE 2017
2. **Bubba L**, Sandu P, Duncan J, Litt D, Amirthalingam G, Ribeiro S, Campbell H, Andrews N and Fry N: Molecular typing and the expression of pertactin and pertussis toxin vaccine antigens in *B. pertussis* clinical strains from the UK in 2015 and 2016- selected as oral presentation at EUpert 2017
3. **Bubba L**, Broberg E, Jasir A, Harvala H, and the study group: Circulation of non-polio enterovirus (EV) and human parechovirus (HPeV) strains in EU/EEA Member States, 2015-2017- selected as oral presentation at ESCAIDE 2018
4. **Bubba L**, Nsonwu O, Pichon B, Davies J, Doumith M, Johnson A, Woodford N, Hope R and Kearns A. National surveillance of methicillin-resistant Staphylococcus aureus bacteraemia following the implementation of Whole-Genome Sequencing in England, April-December 2017 -selected as oral presentation at ESCAIDE 2018

Poster presentation:

1. **Bubba L**, Bundle N, Kapatai G, Daniel R, Balasegaram S, Anderson C, Chalker V, Lamagni T, Brown C, Ready D, Efstratiou A and Coelho J: Genomic sequencing of a national emm66 group A streptococci (GAS) outbreak among people who inject drugs and the homeless community in England and Wales, January 2016-May 2017 – selected as poster presentation in Lancefield 2017
2. **Bubba L**, Nsonwu O, Pichon B, Davies J, Doumith M, Johnson A, Woodford N, Hope R and Kearns A. Exploiting Whole-Genome Sequencing for enhanced surveillance of methicillin-resistant Staphylococcus aureus bacteraemia in England-selected as poster presentation at ECCMID 2018

F. Other conference presentations

1. Bundle N, **Bubba L**, Coelho J, Kwiatkowska R, Cloke R, King S, Rajan-Iyer, Couthney-Pillinger M, Beck CR, Hope V, Lamagni T, Jermacane D, Glass R, Desai M, Gobin M, Balasegaram S, Anderson C: Ongoing outbreak of invasive and non-invasive disease due to group A Streptococcus type emm66, since January 2016, among

homeless and people who inject drugs in England and Wales- selected as oral presentation at Public Health England Conference 2017

2. Kearns A, **Bubba L**, Nsonwu O, Davies J, Doumith M, Thelwall S, Johnson A, Woodford N, Pichon B, Hope R Genomic insights into national surveillance of methicillin-resistant staphylococcus aureus bacteraemia in England – selected as poster presentation at 18th International symposium on staphylococci and staphylococcal infections (ISSSI 2018)
3. Bradshaw D, **Bubba L**, Mbisa T: Surveillance of resistance to NS5A inhibitors in HCV genotype 1a in the UK selected as poster presentation to Clinical Virology conference 2018

G. Other presentations

1. Invasive group A streptococcal *emm* 66 outbreak investigation in England and Wales, 2016: a piece of my experience –Outbreak investigation Berlin 2016
2. Effect of influenza activity on the incidence and severity of invasive group A streptococcal disease: a retrospective study (2008-2016) in England.- section presentation PHE 2017
3. Exploiting Whole-Genome Sequencing for enhanced surveillance of methicillin-resistant *Staphylococcus aureus* bacteraemia in England, April-September 2017 -WGS meeting 2018
4. Characterization of *Bordetella pertussis* acellular pertussis vaccine antigens using ELISA to screen circulating UK strains and correlation with genotype and epidemiological data.-Minimodule review 2017
5. Exploiting Whole-Genome Sequencing for enhanced surveillance of methicillin-resistant *Staphylococcus aureus* bacteraemia, April- December 2017 in England - Minimodule review 2018
6. Effect of influenza activity on the incidence and severity of invasive group A streptococcal disease: a retrospective study (2008-2016) in England.- Project review module 2017
7. Assessment: Norms and Accreditation- Biosafety and quality module, 2018

9. EPIET/EUPHEM modules attended

1. Introductory course (IC), Spetses, Greece, 2016 (three weeks).
2. Outbreak module, Berlin, Germany, 2016 (one week).
3. Multivariable analysis module (MVA), Zagreb, Croatia, 2017 (one week).
4. UK Mini Module, Copenhagen Bristol, England, 2017 (2 days).
5. Rapid Assessment and Survey methods (RAS), Athens, Greece, 2017 (one week).
6. Project review module (PRM), Lisbon, Portugal, 2017 (one week).
7. Initial management PHM and leadership/teamwork (IMPHM), Stockholm, Sweden, 2018 (one week).
8. Biorisk and quality control/quality management (BQM), Stockholm, Sweden, 2018 (one week).
9. UK Mini Module, Nottingham, England, 2018 (2 days).
10. Project review module (PRM), Lisbon, Portugal, 2018 (one week).

Discussion

Coordinator's conclusions

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. This report summarises all activities and projects conducted by Laura Bubba during her two-year EUPHEM fellowship (cohort 2016) as an EU track fellow at the Public Health England (PHE), London, United Kingdom. Laura is the fifth appointed EU track EUPHEM fellow in London. The portfolio includes laboratory and epidemiological projects covering viral, bacterial and parasitic pathogens across a variety of disease programmes, such as vector-borne diseases, sexually-transmitted diseases, food and waterborne diseases, respiratory tract infections, vaccine-preventable disease and antimicrobial resistance. The projects here described are in line with the 'learning by doing' approach of the EUPHEM programme and fulfilled the core competency domains described for professionals in their mid-career and beyond.

During the two-year fellowship, the fellow, supervisors and training site have demonstrated the capability of addressing communicable disease threats in a structured joint approach between public health microbiology and epidemiology such as the use of whole genome to detect HCV genotypes and antiviral resistant markers, outbreak investigations at national and international level (group A *Streptococcus* or *Salmonella* Give) and surveillance investigations (methicillin resistant *Staphylococcus aureus*). The projects have been nicely selected to cover not only important international and national public health topics such as malaria, diphtheria, pertussis, influenza, non-polio enterovirus infections or drug resistant bacteria among others but also a very broad panel of microorganisms and involved different professional groups, such as physicians, laboratory technicians, epidemiologists, statisticians, government officials, public health officers and logisticians, strengthening the fellow's ability to work in a multidisciplinary team and to adapt to different environments and contexts. Laura has been active in contributing to training of others during her fellowship with the development of new training materials as well as direct training and facilitation activities which highlights the contribution that fellows can make to capacity building beyond the programme. All projects had a clear outcome, with results communicated in scientific journals and at conferences and the activities were complimented by nine training modules providing theoretical knowledge. The contributions made by Laura 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.

Supervisor's conclusions

The EUPHEM programme was a tremendous opportunity for Laura and has provided her with the unique tools to find her niche in the field of public health microbiology and epidemiology whilst working with colleagues from very diverse disciplines, both nationally and internationally. For the host institution, it has provided the opportunity to build even more new bridges and strengthen collaboration between the different sectors within public health on both a national and international level. It has been a pleasure to mentor Laura for the past two years and it has been a sheer delight to see her develop within the programme and acquire many new skills, particularly within the field of public health and molecular epidemiology and microbiology. Her projects covered all the core domains within the programme and showed that she was able to work on these projects independently, only occasionally consulting colleagues and peers for advice. One of her greatest strengths is her desire and enthusiasm to do everything! In particular, her passion for international work in areas of public health and she was given the opportunity to do this via her teaching mission to Beirut, Lebanon. Laura excelled herself in Beirut and was a 'natural teacher' that impressed everybody, in particular WHO. Laura also had many opportunities to present her findings and outputs from her projects at various national and international meetings ranging from local PHE Meetings to International Congresses, for example the Lancefield International Symposium on Streptococci and Streptococcal Diseases in Fiji. Her scientific knowledge, technical and organisational skills, team spirit, enthusiasm and sheer dedication has been very much appreciated by all supervisors along with her kindness, open-mindedness, positivity, diligence and goal-oriented personality. It was a very great pleasure to have Laura as a EUPHEM fellow within PHE and we highly appreciate her contribution and achievements within the fellowship programme. I wish Laura every success for the future and shall follow her career with interest.

Personal conclusions of fellow

The EUPHEM fellowship gave the fellow the opportunity to be involved in numerous diverse projects within the fields of public health microbiology and epidemiology at both national and international levels, thus developing new competencies and skills within microbiology and epidemiology. The fellowship significantly improved the personal capacities giving at the EUPHEM fellows the opportunity to become expert not only among microbiology, but also to open their view in a multidisciplinary, dynamic and international setting. As fellow I was really fortunate to be part of PHE and be involved in projects far from my previous experiences and background, teaching me several new tools. The teaching approach by EUPHEM facilitators and project supervisors during the modules, case studies and projects allowed me to learn by experience, gain new knowledge and expertise, opening my views on public health microbiology and epidemiology. Moreover, the feedback approach was fundamental to identify any weakness and strength. During this two year fellowship, the enormous amount of inputs received will build a better microbiologist, able to be involved in microbiological and epidemiological projects, in both national and international settings and I am sure the learning objectives achieved during the modules and projects will be used in the future projects and working place I will be involved. From my point of view the networking created during the fellowship has been one of the main contributors thus building the future international environmental where to share multidisciplinary expertise.

In the future I hope to have the opportunity to be involved in international settings and projects and continue to improve my skills and competencies.

Acknowledgements of fellow

I'd like to think about the fellowship as an adventure that for two years drove me towards new knowledges, new skills, new people, new cities and new fantastic memories. No adventure will be possible without sharing new experiences with someone who supports, spurs and shares your goals both in the personal and working life.
Therefore, there are a huge number of people that I have had the honour to meet during the EUPHEM adventure that I really would like to thank.
Thank you to all coordinators, supervisors, facilitators and administrators that welcomed us in Greece during the introductory course and continued to guide us during the two-year fellowship. In particular, I would like to thank my front-line coordinators Aftab Jasir and Silvia Herrera, who closely followed my progress, supporting, discussing and encouraging me.
I would like to thank all my project supervisors at the training site that give me the opportunity to be involved in all these novel projects in different fields of the microbiology, guiding and teaching me, sharing their knowledge and helping me to achieve new skills and competences. In particular I would like to say a BIG thank you to Androulla Efstratiou, my main site supervisor who was always present for me and gave me the opportunity to improve my experience and advised me in a nice and professional way, sharing her expertise and in particular, involving me within the WHO diphtheria workshop that has left beautiful moments in my memory. The move to a new environment is always a challenge but everything is easier if you find a person like Bee (Baharak Afshar)! She has been my best supporter at work and there was always a smile for me when I was stressed out. As a former EUPHEM fellow herself, we had the opportunity to share several "fellowship adventures", some projects and a lot of funny lunches. I would wish to thank all the other fellows that shared this great two years with me. In conclusion, I would also like to thank my partner Karim who was the one that actually convinced me to participate within EUPHEM and pressed "Submit" during the online call. With courage and love he supported my adventure, moving in the UK with me and preparing delicious dinners when I was late! And if my partner is my family here in London, my parents, sister and Italian close friend never allowed me to miss their support from Italy, sending via phone energy, via mail fantastic home-made food and coming to visit us.
Without all their trust and their support I would not have been able to participate and enjoy this amazing adventure!