

The main title 'Summary of work activities' in a bold, white, sans-serif font, set against a blue background.The author's name 'Regina Selb' in a white, sans-serif font, positioned below the main title.The subtitle 'European Public Health Microbiology Training Programme (EUPHEM), 2017 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.

The views expressed in this publication do not necessarily reflect the views of the European Centre for Disease Prevention and Control (ECDC).

Stockholm, September 2019

© European Centre for Disease Prevention and Control, 2015. Reproduction is authorised, provided the source is acknowledged.

This report summarises the work activities undertaken by Regina Selb, cohort 2017 of the European Public Health Microbiology Training Programme (EUPHEM) at the Robert Koch-Institute (RKI), Berlin, Germany.

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

Regina Selb has achieved her diploma in Biology with specialisation in Microbiology from the University of Vienna, Austria in 2010. She then continued her education at the Medical University of Vienna, Austria, where she completed her PhD in Immunology in 2015 with a specialisation in molecular Allergology. In parallel, she still pursued her career in Microbiology by working as a guest scientist at the Veterinary University of Vienna, focussing on bacterial systematics. In order to learn about Public Health, Regina then worked as Junior Scientific Officer at the European Food Safety Authority (EFSA) in Parma, Italy and at the German Federal Institute for Risk Assessment (BfR) in Berlin, Germany, focussing on Food Safety. Because of her keen interest in Microbiology, Immunology and Public Health, Regina took the opportunity of the EUPHEM fellowship to further develop and sharpen her skills in Public Health Microbiology and gain knowledge in Epidemiology. It is her aim to combine her previous knowledge in Microbiology and Immunology with her newly acquired skills for the benefit of population health in Europe.

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

Supervisors: Angelika Fruth, Bettina Rosner, Julia Enkelmann, Jennifer Bender, Yvonne Pfeifer

An ongoing outbreak of *Shigella sonnei* amongst men who have sex with men (MSM) in Germany and three additionally identified, unrelated *S. sonnei* clusters in the same time period

On 21. June 2018, an urgent inquiry from Public Health England (PHE) via the epidemic intelligence information system (EPIS) reported an increase in multi-drug resistant *Shigella sonnei* cases associated with transmission amongst men who have sex with men (MSM) in the United Kingdom. In Berlin/Germany, notified *S. sonnei* infections until week 26/2018 had exceeded those observed in the same time period of previous years (2018: n=48, 2017: n=10). A team consisting of the fellow, other epidemiologists and microbiologists of the Robert Koch Institute (RKI) and the Berlin state health authority initiated an outbreak investigation in order to identify the mode and vehicle of transmission, a possible connection to European-wide *S. sonnei* cases and to implement control measures.

Molecular characterisation by cgMLST, PFGE and phenotypic characterisation of *S. sonnei* isolates received at the national reference centre (NRC) for salmonellosis and other enteric pathogens at the RKI was intensified. In

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>

parallel, primary laboratories were contacted and encouraged to send *S. sonnei* isolates to the NRC, whenever occurring. The epidemiological department of the RKI initiated an investigation of available data and intensified further monitoring of *S. sonnei* notifications.

In total, 87 isolates of *S. sonnei* received between January and November 2018 were characterised at the NRC. Out of these, 36 cases were unobtrusive by phenotypic characterisation and further investigation by molecular typing was not performed.

By molecular characterisation, 27 isolates could be connected to the European-wide outbreak strain reported by PHE associated with transmission amongst MSM. All cases were male, on average 38 years old and mostly living in Berlin. Typical symptoms described in the notification database were abdominal pain, fever, vomiting and (bloody) diarrhoea. Hospitalisation was reported for two cases. One case reported that he had visited London previously to his illness. Possible MSM status was submitted via the reporting system as risk of infection for two cases. One of two MSM cases notified in Berlin and one additional case reported regular visits to Berlin clubs and events known to be frequented by the MSM community in Berlin. A questionnaire particularly targeting cases which were members of the MSM community was developed together with the unit for sexually transmitted infections at the RKI and the Berlin state health authority. Because of difficulties connecting cases identified by molecular typing with notified cases in the reporting system of the RKI, the questionnaire could not be implemented. The responsible authorities of the federal states affected by the multi-drug resistant *S. sonnei* were informed in order to take control measures.

In the course of the investigation, three additional clusters of *S. sonnei* were identified. Molecular typing by cgMLST confirmed these to be unrelated to the outbreak amongst MSM.

At the NRC, 14 further isolates, originating from a kindergarten outbreak in Berlin in September 2018, were investigated. Children and pedagogic personnel were affected. The outbreak was declared to be over in October 2018.

A cluster of seven isolates was additionally investigated, which was connected with attendance to a music festival in Poland in August 2018. Of these cases, six originated from Saxony, one from Berlin. No further transmission could be observed after August 2018.

Furthermore, three cases closely related in cgMLST were investigated. For one of the cases, travel to India had been reported. The other two cases could not be connected to the notification database.

Molecular and epidemiological evidence strongly suggested transmission of multi-drug resistant *S. sonnei* amongst the MSM community in Germany, which were closely related with isolates reported from other European countries including the UK. In health centres and clubs known to be frequented by the MSM community, information campaigns regarding sexual transmitted infections, including those transmitted by the faecal-oral route like *S. sonnei*, are in place. Local health authorities of the federal states were encouraged to continue and further intensify these preventive measures. Three other, self-limiting outbreak clusters were detected in the course of the investigation, which were unrelated to the initially investigated *S. sonnei* cases.

The fellow was involved in all stages of the outbreak investigation, including search for cases, confirming the outbreak, data analysis, informing the local authorities and writing the final outbreak report. In this outbreak analysis, the fellow took the leading role in coordination between the different stakeholders (epidemiology, microbiology, local authorities).

A transmission of Burkholderia cepacia complex in intensive care units in Germany

In August 2018, a company recalled a batch of mouth wash solution (MWS) from the German market because the product had been found to be contaminated with pathogens of the *Burkholderia cepacia* complex (BCC). In September 2018, the unit for nosocomial pathogens and antibiotic resistance of the RKI received eight BCC isolates for molecular characterisation, seven of those originating from patients in intensive care units of two German hospitals. The eighth sample was obtained from a contaminated MWS used at one of the hospitals. At his time point, the intensive care unit and the RKI were unaware that the product had been recalled previously. The RKI requested two isolates from the MWS manufacturer, one environmental sample from the filling station and one from a closed bottle of MWS. In October 2018, while the outbreak investigation by the RKI was still ongoing, a local group of investigators published a rapid communication in *Eurosurveillance*, reporting a BCC outbreak connected to MWS in the intensive care unit of a third German hospital. RKI had not been involved in solving this outbreak.

Molecular typing by pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (WGS)-based core genome multi locus sequence typing (cgMLST) was performed for all seven patient isolates, the isolate obtained from the MWS at the hospital and for the two BCC isolates received from the company. Additionally, the sequences published in the *Eurosurveillance* report were downloaded from the European Nucleotide Archive and added to the analysis.

The molecular analysis revealed two clusters. Six of the seven patient isolates received at the RKI, all originating from the same hospital, were closely related and clustered with the MWS isolate obtained at the same hospital. The last patient isolate, which was originating from another hospital, was not related to any of the other isolates and there was no evidence connecting this case to MWS. The two samples sent by the manufacturer isolated from MWS and from the filling plant were clustering with the BCC sequences described in the *Eurosurveillance* report.

MWS was confirmed as the vehicle of BCC cases in an intensive care unit in Germany. Specimen sent by the MWS manufacturer and comparison to another, published outbreak indicated multiple contaminations at the company's filling plant. Even though the contaminated product had already been recalled, the intensive care units continued to use the contaminated product due to insufficient forwarding of this information within the hospitals. The fellow was involved in molecular characterisation, supported the data analysis and wrote the outbreak report.

Training modules

During the EPIET/EUPHEM introductory course in September/October 2017, the fellow received insight into the basic concepts of outbreak investigations. Logistical and analytical approaches were provided in lectures, interactive sessions, case studies and group exercises. During the course, the fellow was introduced to the ten steps of outbreak investigations.

The "Outbreak Investigation" module in December 2017 enabled the fellow to perform analytical-epidemiological studies important for outbreak investigations. The interactive course methodologically led the fellow through the ten steps of outbreak investigations, from data entry, management and questionnaire design to data analysis including stratified analysis with the help of different software tools. The fellow was additionally taught when and how to perform descriptive or analytical studies including cohort studies and case-control studies.

In the "Management, Leadership and Communication in Public Health" the fellow gained experience in communication with higher authorities and communication in emergency situations. Furthermore, the course covered topics on how to lead and communicate effectively within a multidisciplinary team.

Educational outcome: The fellow gained substantial experience in Europe-wide, sexually transmitted outbreaks and in hospital-acquired outbreaks. The fellow sharpened her microbiological skills and applied them together with epidemiological knowledge in outbreak situations, covering all ten important steps of outbreak investigations. The fellow took the leading role in a multidisciplinary outbreak investigation team, coordinating actions between the microbiology departments, two epidemiological departments and local health authorities.

1.2. Surveillance

Supervisors: Andrea Hauser, Uwe Koppe, Norbert Bannert

Annual molecular HIV surveillance in Germany 2017

In Germany, an intensified molecular surveillance of newly diagnosed cases is in place and annual data analysis is performed in order to identify and understand current evolutionary developments of the HI virus and molecular infection dynamics. The surveillance of transmitted drug resistance in Germany is an official task of the Robert Koch Institute and part of a strategy paper of the German ministry of health with the overall aim to reduce HIV, hepatitis B and C, as well as other sexually transmitted infections and informs treatment guidelines.

Samples of HIV cases newly diagnosed in 2017 were received from a network of 82 sentinel laboratories in Germany together with official notification forms. NGS analysis of viral *pol*-sequences was performed. Circulating HIV variants and transmitted drug resistance mutations were analysed with the help of online tools.

In 2017, 1472 viral sequences were analysed, representing approximately 36% of all newly diagnosed HIV cases in Germany. HIV B was the predominant subtype circulating in Germany in 2017, causing 60.3% of all infections. Among non-B subtypes, the most common variants were the recombinant form CRF02_AG (8.6%), subtype A (8.0%) and subtype C (6.3%). The prevalence of transmitted drug resistance amongst newly diagnosed HIV cases accounted to 8.8% (CI95%: 7.5-10.4) in the year 2017. The majority of detected mutations affected the efficacy of non-nucleoside reverse transcriptase inhibitors (NNRTI, 3.4%), followed by nucleoside reverse transcriptase inhibitors (NRTI, 2.7%) and protease inhibitors (PI, 1.9%). In 0.2% of the investigated samples, mutations conferring resistance to integrase inhibitors (INI) were found. For 0.8% of the samples, resistance to two drug classes were observed, but no sample was multi-drug resistant. Resistance associated mutations resulted in predicted loss or reduction of drug efficacy, affecting mostly the drug class of NNRTI and NRTI, including the first-line agents tenofovir-disoproxilfumarate and emtricitabine. The efficacy of the drug classes PI and INI was considerably less affected, but mutations leading to reduced efficacy of the important antiretroviral agent elvitegravir were observed.

The integrated, molecular HIV surveillance in 2017 revealed a further shift towards non-B subtypes in Germany, with recombinant forms gaining increasing importance. Prevalence of transmitted drug resistance dropped below 10% in the year 2017. Transmitted drug resistance reducing the efficacy of first-line antiretroviral agents or agents used for HIV pre-exposure prophylaxis was observed and has to be monitored in particular. The current data indicate no necessary changes in treatment guidelines.

The fellow was involved in all stages of the project from sample preparation and data analysis to writing of the surveillance report. A new proofreading-based PCR method was implemented by the fellow, increasing the reliability of laboratory data.

Training modules

The EPIET/EUPHEM introductory course in September/October 2017 familiarized the fellow with basic concepts of surveillance systems and evaluation of surveillance data.

During the "Outbreak Investigation" and the "Multivariable Analysis" modules, the fellow gained the ability to use STATA for the comprehensive statistical analysis of large datasets.

In the "Rapid Assessment" module in May 2019, the fellow got an insight into sampling methodology and multidisciplinary research efforts in emergency situations.

Educational outcome: The fellow gained experience in the analysis and evaluation of surveillance data and extended her ability to work in multidisciplinary teams of microbiologists and epidemiologists. She gained experience in application of molecular surveillance data for policy development.

2. Applied public health microbiology research

Supervisor: Volker Rickerts

Molecular typing and resistance of *Cryptococcus neoformans* isolated in Germany between 2011 and 2017

Cryptococcosis is a fungal infection caused by *Cryptococcus neoformans* affecting the central nervous system. Immunocompromised patients including individuals with HIV are predominantly affected. Mortality from cryptococcosis infection is estimated to be 30% in patients in high-income countries like Germany despite antifungal combination treatment. Up to 50% of isolates with *in vitro* resistance to clinically used antifungals including fluconazole were reported from several countries worldwide, including Spain, South Africa and Taiwan. For Germany, no prospective data on susceptibility to antifungal drugs had been available. The aim of the study was the identification of subpopulations causing infections in Germany by molecular epidemiology and to estimate the percentage of drug resistance strains in Germany in order to inform treatment guidelines.

All *C. neoformans* isolates collected from individual patients' samples at the German reference laboratory for cryptococcosis between 2011 and 2017 (n=133) were characterised by multi-locus sequence typing (MLST) and phenotypic susceptibility testing to six antifungal drugs. Isolates with reduced susceptibility to fluconazole were additionally characterised by sequencing of the *ERG11* gene. Clinical data of two patients with resistant isolates were obtained from the respective medical centres with approval of the responsible ethical committee.

Genotype VNI isolates belonging to clonal complexes previously described from all over the world were the most prevalent agents causing cryptococcosis in Germany. Overall, isolates displaying minimal inhibitory concentrations (MIC) above the epidemiological cut-offs (ECV) were observed in 1.6% for fluconazole and 2.3% of 5-flucytosine. A novel mutation in the *ERG11* gene was identified, which might be associated with fluconazole resistance. A fluconazole/5-flucytosine double resistant isolate could be connected to a patient with a second relapse episode. The patient had been treated accordingly. A fluconazole resistant isolate was connected to a fatal case which had been treated with amphotericin B /fluconazole combination therapy.

The study provided evidence that drug resistance of *C. neoformans* isolates causing cryptococcosis in Germany is rare. Drug susceptibility testing may be important to reduce associated mortality and is especially recommended for relapse patients.

The fellow developed the study protocol, was the main investigator regarding sample processing in the laboratory and data analysis and wrote the manuscript.

Training modules

During the EPIET/EUPHEM introductory course in September/October 2017, the fellows learned how to plan, conduct and evaluate a scientific project. In a group exercise, the fellows developed a protocol in order to answer a research question. During special lectures and interactive sessions, the EUPHEM fellows were familiarized with the challenges of antimicrobial resistance.

The Management, Leadership and Communication in Public Health module was important for the fellows to gain personal skills for professional development, project management and communication in public health.

During the Project Review modules in August 2018 and 2019, the fellow had the chance to discuss important research outcomes with public health professionals, to sharpen recommendations accordingly and to advance public health communication skills.

Educational outcome: The fellow learned to conduct all stages of a Public Health Microbiology research project, starting from formulating a research question, plan the project accordingly, performing the necessary laboratory work and data analysis to writing the manuscript adapted to the interested scientific public health community in the field. The fellow got acquainted with the field of mycotic pathogens, current challenges and standard methodology in public health mycology.

3. Applied public health microbiology and laboratory investigations

Supervisors: Franziska Layer, Angelika Fruth, Sabine Santibanez, Christina Frank, Andrea Hauser

Characterisation of community-acquired methicillin-resistant *S. aureus* (CA-MRSA) isolated from children in the Rhine-Main area, Germany

Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is the most common cause of skin and soft tissue infections in otherwise healthy children and adults and can lead to severe skin abscesses. In contrast to hospital acquired MRSA strains, CA-MRSA are often associated with close communities like childcare facilities or sport teams, where bacteria can spread via close body contact. These strains differ genetically from classical nosocomial strains, having a fitness advantage and typical virulence factors. In this study, basic epidemiological information of affected children were collected and the respective MRSA strains were characterised, in order to get an overview on strains circulating in Germany and identify possible risk factors.

The RKI unit for nosocomial infections and antibiotic resistances received MRSA strains isolated from skin and soft tissue infections in children from a clinic in the Rhine-Main area in Germany. For all isolates, molecular characterisation and antimicrobial susceptibility testing was performed. For isolates with insufficient typing by classical methods, whole genome sequencing (WGS) was performed in addition.

Between 2015 and 2018, MRSA strains (n=114) were received and characterised. Patients were aged 0-18 years and the hospital reported a non-German background for 50% of the children. The major reason for hospital admission was abscess incision and drainage. All patient isolates were resistant to penicillin and oxacillin, but susceptible to ceftazidime, daptomycin, linezolid, mupirocin, fosfomycin, teicoplanin, tigecycline and vancomycin. Molecular characterisation demonstrated the presence of 48 different *spa*-types, *SCCmec* types typical for CA-MRSA, and 60% of the strains carried the characteristic virulence factor *lukPV*. Other virulence factors (*arcA*, *etd*, *seh*) typical for specific MRSA lineages were identified in addition. For few cases with non-German background and information available on country of origin, CA-MRSA lineages typical for these countries, as described in the literature, could be identified.

In conclusion, CA-MRSA strains able to cause severe disease are circulating in Germany. Currently, the origin and transmission of these strains is only insufficiently understood. We recommend the continuous surveillance of CA-MRSA in Germany and the collection of clinical and epidemiological data in order to take control measures.

The fellow performed molecular typing of the strains and was involved in antimicrobial susceptibility testing. Furthermore, she analysed the data and contributed to writing the final manuscript.

Rapid detection of *Campylobacter jejuni* capsule types associated with Guillain-Barré syndrome by WGS

Campylobacter sp. are responsible for approximately 70,000 cases of gastroenteritis each year in Germany. Guillain-Barré syndrome is a serious sequelae which was connected to *C. jejuni* infections caused by distinct capsule types. It was the aim of the project to generate a detection protocol of capsule genes based on whole genome sequencing (WGS) data, in order to extend the routine molecular surveillance in Germany and quickly and reliably evaluate occurring capsule types.

For raw WGS data, different assembly strategies were compared (SPAdes, Velvet, BWA). Available information from the literature regarding the composition of capsule genes and the different types were used to generate a task template in the Ridom SeqSphere software. The outcomes were compared to PCR results detecting capsule genes.

A new, reliable scheme to rapidly detect capsule types was developed on the basis of WGS data. SPAdes *de novo* assembly was found to be superior for raw read processing. In 55/97 isolates, the WGS results were in agreement with PCR typing. For the other samples, PCR had not been able to detect the capsule type or yielded PCR results were incorrect when evaluated in WGS. Because PCR was found to be unreliable to correctly detect capsule types, it is recommended to use the newly established scheme for extended molecular surveillance of *C. jejuni* in the future.

The fellow processed WGS reads generated by Illumina HiSeq sequencer, developed the scheme on the basis of published capsule type sequences, evaluated the yielded outcomes in comparison to classical PCR methodology and wrote the final project report.

Laboratory-based mumps virus (MuV) surveillance in Germany

Mumps is a notifiable disease in Germany since 2013. Despite childhood vaccinations, approximately 400-700 cases are reported each year in the country. The disease is currently not prioritized by WHO and no global sequence database is available as this is the case for measles virus. At the RKI in Germany, molecular surveillance of mumps virus (MuV) is systematically performed since 2008. Mumps data available at the National Reference Centre (NRC) for measles, mumps and rubella collected between 2008 and 2018 were evaluated in order to gain an overview on the population affected, possible immunity gaps and currently circulating MuV variants.

All samples from suspected mumps cases sent to the RKI between 2008 and 2018 were included in the study. The data was evaluated regarding basic epidemiological information (age, sex, vaccination status). "Confirmed mumps cases" were defined as those with PCR confirmed presence of the MuV genome. Serum samples positive for mumps-specific IgM were defined as "possible cases". These samples were retested with a different, more specific IgM capture ELISA and defined as "probable cases", whenever the testing result was positive. Available, MuV SH-sequences were named according to the WHO nomenclature for MuV and phylogenetic analysis was performed. Sequences from other European and world-wide locations were added to the analysis for each unique SH-sequence found in Germany.

In total, patient material from 1538 clinically suspected mumps cases was sent to the NRC between 2008 and 2018 and analysed. Mumps infection was confirmed by PCR in a minority of cases (n=186, 12.1%). Additionally, 28 probable mumps cases were identified by IgM capture ELISA. The majority of confirmed mumps cases were adolescents and young adults <30 years of age. Sequences for molecular typing were available for 116 cases; 112 belonged to genotype G. Several outbreaks were detected by phylogenetic analysis which had occurred in the investigated time period. Again, vaccinated adolescents and young adults were primarily affected.

In conclusion, only a minority of clinically suspected mumps cases could be laboratory confirmed. Adolescents and young adults were primarily affected by mumps genotype G in Germany between 2008 and 2018. For most cases, vaccination had been reported. As the currently used vaccination strain is genotype A and vaccination occurs in early childhood <2 years of age, insufficient and/or waning immunity might be responsible for infections in adults. It is recommended to implement PCR-based diagnostics in primary laboratories in order to provide early and reliable confirmation of mumps cases. Since vaccinated, young adults are primarily affected by mumps in Germany, data on occurring complications in relation to the infection have to be followed up in the affected population. The usefulness of a third vaccination dose has to be evaluated in further studies.

The fellow performed data analysis of routinely acquired laboratory data, was involved in performance of the capture ELISA and wrote the final report.

Evaluation of diagnostic tests for arbovirus infections available in Germany

In Germany, an increase of notified disease cases with arthropod-transmitted flaviviruses and alphaviruses infections has been noted in the last years. Cases are mostly imported by tourists/travellers but autochthonous transmission cannot be excluded for some of the viruses. Emerging viral infections have been observed in Southern European countries where vector populations are present, with the potential of spreading into Germany. While several nucleic acid-based methods are available for molecular diagnosis of these viruses in Germany, serology is currently the primarily used method in German diagnostic laboratories, which can only diagnose infections in later disease stages and may be problematic due to antibody cross-reactivity.

The fellow performed a literature review and internet search on commercial serology-based and rapid diagnostic tests available in Germany for dengue, West Nile, yellow fever, chikungunya and zika in order to summarize available tests and their reliability.

Non-nucleic acid based tests available are mainly antibody detecting ELISA and immunofluorescence tests. Cross-reactivity to other flaviviruses has to be expected for dengue, yellow fever and West Nile. For chikungunya, available test show no cross reactivity for dengue, but no further information is reported by the respective companies. Regarding zika, a specific ELISA test is available for which cross-reactivity with antibodies against other flaviviruses is low. A specific antigen-detection test is additionally available for dengue diagnosis, as well as several rapid detection tests for dengue and zika.

In summary, rapid tests and serology-based diagnosis of arthropod-transmitted viral infections in Germany may be unreliable due to cross-reactivity. We recommend implementation of nucleic-acid based or antigen-detection systems or to include cross-reactivity controls for molecular diagnosis of flavivirus and alphavirus infections.

The fellow performed the literature research and summarized the final data.

PCR protocol development used in molecular HIV surveillance

In the course of the surveillance project described in section 1 of this document, the fellow was involved in establishing a new PCR method for molecular HIV surveillance. A routinely used protocol had been in place since 2013, using *Taq* polymerase. In order to increase the data reliability, the laboratory aimed to switch to a protocol using a polymerase with proof-reading activity.

The performance of two polymerases, "Phusion" and "SuperFi", was compared to the established "one step" protocol using routine samples. Detection limit, ability to identify HIV subtypes, time exposure and costs were compared.

The polymerase "Phusion" was superior in detection of HIV sequences and subtypes compared to "SuperFi". When "Phusion" polymerase was used, detection limit and identification of HIV subtypes was comparable to the established methodology. Compared to the previous "one step" protocol, a price reduction of 25% was calculated and less RNA material was necessary. As a limitation, the newly established protocol is more time consuming.

The fellow was involved in laboratory processing of samples and data analysis and protocol development. The protocol generating higher data quality is now used routinely for molecular HIV surveillance.

Training modules

The introductory course in September and October 2017 supplied the fellows with a revision of important concepts of laboratory-based microbiological techniques and concepts of phylogenetic analysis. The course included case studies shaped especially for public health microbiologists interpreting laboratory results.

The "Outbreak Investigation" module in December 2017 provided more in-depth insights in sequence analysis and microbiological phylogeny important for laboratory studies.

The "Biorisk and Quality Management" module in February 2018 facilitated important concepts of quality control and biorisk management, which are crucial for all activities in microbiological laboratories.

During the "Project Review Module" in September 2019, EUPHEM fellows learned how to interpret laboratory results in the field of vaccine-preventable diseases.

Educational outcome: The fellow extended her skills in the application of standard laboratory methodology to identify and characterize viral and bacterial pathogens. The fellow got a good understanding of NGS and WGS techniques for viral and bacterial species and deepened her public health microbiology knowledge. Together with experts in different disciplines, she furthered her skills in interpreting laboratory results and how to use those for policy development.

4. Biorisk management

Supervisor: Astrid Lewin

Safe and secure handling of pathogens of the *Mycobacterium tuberculosis* complex in the BSL-3 laboratory

Bacteria of the *Mycobacterium tuberculosis* complex are highly pathogenic and have to be handled under S3 conditions. At the Robert Koch Institute, the BSL-3 laboratory for highly pathogenic bacteria had been moved to a new building; therefore, the viability of the strain collection had to be confirmed.

The fellow re-cultivated the collection of 41 wild type and genetically modified strains of the *Mycobacterium tuberculosis* complex, performed purity controls, prepared and documented the new strain collection. Additionally, for one *M. tuberculosis* complex wild type strain, DNA was isolated, controlled for sterility and safely discharged from the BSL-3 laboratory to BSL-2 conditions.

In the course of the project, the fellow received extensive theoretical and practical safety and security training suitable for BSL-3 conditions. The fellow participated in two emergency rescue trainings, practicing the safe and secure retrieval of unconscious, contaminated personnel out of the high-risk area. Additionally, the fellow received specialized first aid training, further preparing the participants for casualty situations.

The fellow acquired an official certificate issued by the unit for highly pathogenic bacteria at the Robert Koch Institute, allowing infectious work activities and assistance to infectious work activities under high-risk BSL-3 conditions.

Training modules

The "Biorisk and Quality management" module in February 2018 prepared the fellow for the work in the BSL-3 laboratory, providing the theoretical basis on biosecurity and on identification and mitigation of biorisks, including personal protective equipment, waste management and safe handling of infectious agents. In the course of the module the fellow achieved a WHO certificate for international transport of infectious substances.

Educational outcome: The fellow was enabled and trained to independently work under S3 conditions, including use of personal protective equipment, safe handling of highly pathogenic bacteria, waste management, controlling for sterility and safe discharge of material. The fellow was taught to assist in rescue and first aid of casualties happening under high safety and security conditions.

5. Quality management

Supervisors: Klaus Jansen, Dagmar Heuer

External Quality Assessment (EQA) of *Neisseria gonorrhoeae* antimicrobial resistance testing in primary laboratories in Germany

Antimicrobial resistance of *Neisseria gonorrhoeae* has become a challenge in Europe and worldwide. At the European level, external quality assessments (EQAs) of reference laboratories are in place provided by the Euro-GASP network. In Germany, no protocol has been available for assessment of primary laboratories until now. The German gonococcal resistance network (GORENET) was established by the Robert-Koch Institute (RKI) in collaboration with the German reference laboratory for Gonococci. In this frame, an EQA of primary laboratories was performed in order to assess the quality of antibiotic susceptibility testing, to collect information on laboratory procedures in place and to evaluate the impact of procedures on the results of antimicrobial susceptibility testing.

Laboratories were asked to test five strains of *N. gonorrhoeae* for drug susceptibility to azithromycin, cefixime, ceftriaxone, ciprofloxacin and penicillin and to submit minimal inhibitory concentration (MIC) results and interpreted susceptibility categorisations. Additionally, data on laboratory procedures and interpretation of results were collected via a standardized questionnaire. Statistical analysis was performed using generalized linear models and conditional inference trees.

21 laboratories participated and submitted data to the RKI. Depending on the antibiotic agent, 70%-96% of MIC results were reported within accepted deviations. Laboratory procedures, for example used agar base, incubation settings, the use of control strains and interpretation standards differed between laboratories. In statistical analysis, incubation time of cultures <24 hours was associated with correctly submitted MIC results. For azithromycin testing, 5% CO₂ concentration was associated with correct measurements compared to 3%. Conditional inference tree analysis confirmed the impact of incubation time and CO₂ concentration on testing results, and additionally identified humidity settings as an influencing parameter.

In conclusion, a novel protocol was developed to externally assess the quality of antimicrobial susceptibility testing of *N. gonorrhoeae* in primary laboratories in Germany. Laboratory methodology was heterogeneous and may significantly influence data quality. The development of a standard operating procedure is recommended, based on available protocols, the literature and on the findings of this study.

The fellow was involved in all stages of the project, including EQA protocol development, preparation of the trial, questionnaire development and data analysis. The fellow took the leading role in coordination between the epidemiologists and microbiologists in the reference laboratory for *N. gonorrhoeae* and was responsible for writing the final manuscript.

Quality audit of the reference laboratory for cryptococcosis

The fellow performed an internal audit of the reference laboratory for cryptococcosis at the Robert Koch Institute by interviewing the laboratory head and technical staff. Protocols, methods and workflows were revised. The audit yielded a general quality indicator percentage of 93%. Process management and quality control reached an indicator percentage of 97%. It was recommended to improve documentation procedures by further extending the electronic system.

Training modules

During the "Biorisk and Quality management" module in February 2018, the fellows learned about all aspects important for ensuring internal and external quality control in laboratories.

The "Multivariable Analysis" module in April 2018 provided the fellow with a more comprehensive understanding of the theoretical principles of statistical analysis and how to build optimal models using linear, logistic, Poisson and Cox regression using the STATA software.

During the Project Review modules in August 2018 and 2019, the fellow had the chance to discuss important study outcomes with public health professionals, to sharpen recommendations accordingly and to advance public health communication skills.

Educational outcome: The fellow developed a good understanding of quality control processes and learned how to perform internal and external quality assessments independently. The fellow learned about quality standards and guidelines provided by national, EU-wide and international committees and how these are applied on different levels nationally and locally.

6. Teaching and pedagogy

Organisation and implementation of the “Laboratory module” for fellows of the postgraduate training for applied epidemiology (PAE)

The “Laboratory Module” is organized annually at the RKI for fellows of the postgraduate training for applied epidemiology (PAE). Epidemiologists not familiar with the work in public health microbiology laboratories are participating with the aim to get an insight into the practical day-to-day work in laboratories at the Robert Koch Institute.

The fellow performed a pre-course assessment regarding learning needs and interests of the participants, recruited speakers and teachers for practical work and was responsible for time management. Additionally, the fellow presented an introduction to laboratory methods and taught her colleagues the principles of immunology in an interactive session. The course was evaluated with the help of a questionnaire and reflective notes.

Speakers were collaborative and motivated to teach the fellows, which allowed for a comprehensive coverage of microbiological topics. PAE fellows were actively participating to the lectures and especially interested in practical laboratory sessions. One major challenge was the heterogeneous background of the participants, including on the one hand already laboratory-experienced fellows and persons with no laboratory background. Therefore, a balance between facilitating basic knowledge and advanced topics was found.

The fellow was responsible for all stages of the organisation and implementation of the “Laboratory Module”. She actively participated in teaching activities and performed the final evaluation.

Organisation and implementation of vaccinology sessions for EUPHEM fellows

The “Vaccinology Module” is currently only available for ECDC fellows of the EPIET path. EUPHEM fellows of cohorts 2017 and 2018 submitted “expression of interest” documents with the aim of participating to the module. Moreover, annual programme evaluations performed by cohort representatives and the external evaluation of the fellowship programme clarified the need for vaccinology sessions for EUPHEM fellows. The head of EUPHEM, together with the cohort representatives, therefore decided to implement vaccinology sessions for EUPHEM fellows during the “Project Review” module in 2019, in order to provide an intermediate solution for fellows of cohorts 2017 and 2018.

The fellow took the leading role in organisation and implementation of the teaching activity. EUPHEM and EPIET fellows were invited to participate on a voluntary basis. Two sessions, 1.5 hours each, were planned. Day 1 included “Introduction to Vaccine Epidemiology” and an interactive discussion round with the topics “Vaccine Hesitancy” and “Mandatory Vaccination”. Day 2 included a lecture on “Advanced Vaccine Immunology” and two interactive case scenarios, focussing on population immunity and the evaluation of microbiological laboratory data on *B. pertussis* vaccination strategies and immunity to mumps viruses.

The fellow was involved and responsible for all stages of the teaching activity. She performed a pre-course assessment among EUPHEM fellows and organized the sessions together with the head scientific coordinator of EUPHEM, other cohort representatives and interested fellows. She was also involved in teaching in the sessions on population immunity and on mumps laboratory data interpretation.

Other teaching activities

During the introductory course in September/October 2017, the fellow researched and prepared material on the topic “carbapenemase mechanisms” in a joint group activity in “problem based learning”. The material was used to teach other EUPHEM fellows.

For the module “Biorisk and Quality Management” in February 2018, the fellow familiarized herself with the topic “Factors influencing laboratory results”, prepared a presentation on the topic and taught other EUPHEM fellows in the course of the module.

Training modules

The “Management, Leadership and Communication in Public Health” module in February 2018 supported the development of personal skills important for professional development and project management. The course covered topics on how to lead and communicate effectively within a multidisciplinary team.

Educational outcome: The fellow gained the ability to organize and coordinate teaching sessions, to conduct needs assessments and to integrate specific interests of participants. The fellow learned about lecturing a professional audience in topics important in public health microbiology according to the previous experience. Additionally, the fellow got acquainted how to perform teaching evaluations and how to summarize feedback of participants.

7. Public health microbiology management

Management of EUPHEM projects cross-cutting different areas

All projects of the fellow throughout the fellowship required comprehensive public health microbiology management. In particular for outbreak investigations described in section 1, coordination and communication between microbiologists, epidemiologists and local authorities was crucial in order to achieve the common goal of solving an outbreak and take respective control measures to contain the pathogen. For the quality management project described in section 5, coordination of the activities of reference laboratory and RKI staff, including epidemiologists, statisticians and microbiologists, required excellent management skills. Moreover, time and project management and coordination was crucial considering the 21 primary laboratories participating in the external quality assessment. Overall, the fellow further established her abilities in team building, coordination, negotiation and project management during the EUPHEM fellowship.

Further facilitation of the EUPHEM fellowship programme at the RKI

In the course of the EUPHEM fellowship at the RKI, the fellow together with her main supervisor Astrid Lewin observed that the aims and objectives of the EUPHEM fellowship among RKI staff and management were not fully evident. While the EPIET programme was well-known amongst epidemiologists, the role of Public Health Microbiology was less clear amongst microbiologists and epidemiologists at the institute. Therefore, the aim was to better introduce the EUPHEM programme at the RKI and point out differences in background of the fellows, curriculum and aims of the programme to management and staff.

In May 2019, the fellow introduced the EUPHEM fellowship in general and a selection of her projects in particular to the head scientific management and the scientific advisory board of the RKI. In September 2019, the fellow took the opportunity to explain the activities and achievements of her fellowship in detail to the RKI staff including management board, staff scientists, students and laboratory staff at the biweekly institute seminar.

In conclusion, the activities facilitated a good understanding of the achievements and skills of the fellow and objectives and potential of the fellowship programme. This can help future EUPHEM fellows and project supervisors at the institute to fully benefit from the potential the fellowship offers for the training site, the fellow and public health in Europe.

Training modules

The "Management, Leadership and Communication in Public Health" module in February 2018 supported the development of personal skills important for professional development and project management. The course covered topics on how to lead and communicate effectively within a multidisciplinary team.

Educational outcome: The fellow gained the ability to professionally and effectively manage Public Health Microbiology projects, including those in collaboration with multidisciplinary teams. The fellow learned how to effectively communicate with higher authorities, stakeholders and the public.

8. Communication

Publications

1. Selb R, Fuchs V, Graf B, Hamprecht A, Hogardt M, Sedlacek L, Schwarz R, Idelevich E, Becker SL, Held J, Kuepper-Tetzl CP, McCormick-Smith I, Heckmann D, Gerkrath J, Han C, Wilmes D, Rickerts V. Molecular typing and *in vitro* resistance of *Cryptococcus neoformans* clinical isolates obtained in Germany between 2011 and 2017. *Int J Med Microbiol.* 2019, <https://doi.org/10.1016/j.ijmm.2019.151336>.
2. Selb R, Jansen K, Eckardt M, Tamminga T, Dudareva S, Gassowski M, Graeber I, Guhl E, Heuer D, Buder S and the GORENET EQA study group. External quality assessment (EQA) of *Neisseria gonorrhoeae* antimicrobial susceptibility testing in primary laboratories in Germany. *Submitted manuscript*.
3. Selb R, Albert-Braun S, Weltzien A, Werner G, Layer F. Characterisation of community-acquired methicillin-resistant *S. aureus* (CA-MRSA) isolated from children: experiences of a hospital in the Rhine-Main area, Germany. *Manuscript in preparation*.

Reports

1. Surveillance report: HIV Surveillance – Annual report 2017
2. Outbreak report: *Shigella sonnei* Jan – Nov 2018 in Germany
3. Outbreak report: Spread of *Burkholderia cepacia* complex in intensive care units in Germany associated with mouth wash solution

4. Laboratory report: Development of an *in silico* method based on WGS data for rapid detection of specific *Campylobacter jejuni* capsule types known to be involved in manifestation of Guillain-Barré syndrome
5. Laboratory-based surveillance report: Mumps virus variants circulating in Germany 2008-2018

Conference presentations

1. Selb R, Visockaite V, McCormick-Smith I, Heckmann D, Han C, Rickerts V. Cryptococcosis: Molecular types and drug susceptibility of German clinical isolates (2011-2017). Poster Presentation: ESCAIDE 2018.
2. Selb R, Visockaite V, McCormick-Smith I, Heckmann D, Han C, Sedlacek L, Roll C, Wilmes D, Rickerts V. Cryptococcosis in Germany caused by *C. neoformans* var. *grubii*: *In vitro* resistance and molecular typing. Oral Presentation: Conference of the German Mycology Society 2018.
3. Selb R, Jansen K, Eckardt M, Tammimga T, Guhl E, Graeber I, Gassowski M, Heuer D, Buder S: External quality assessment (EQA) of *Neisseria gonorrhoeae* antimicrobial susceptibility testing in primary laboratories. Oral Presentation: ESCAIDE 2019.

Selected other presentations

1. Whole genome sequencing for surveillance and outbreak investigations in Germany. Oral presentation: ECDC, Management, Leadership and Communication in Public Health Module, February 2018
2. Introduction to EUPHEM – Fellowship at the RKI. Oral presentation: Robert Koch Institute, Meeting of the RKI Scientific Advisory Board, May 2019
3. EUPHEM Fellowship at the RKI – Regina Selb. Oral presentation: Robert Koch Institute, Robert Koch Institute Seminar, September 2019

Other

1. EUPHEM cohort 2017 representative

Training modules

During the introductory course in September/October 2017, the fellows were familiarized with ways of effective communication and presentation, writing of scientific articles and particularly targeting different audiences. The “Management, Leadership and Communication in Public Health” module in February 2018 thoroughly and in-depth prepared the fellows for successful and professional communication tailored to different audiences.

9. EPIET/EUPHEM modules attended

1. EPIET/EUPHEM Introductory Course; 25. September - 13. October 2017; Spetses, Greece
2. Outbreak Investigation Module; 04.-08. December 2017; Berlin, Germany
3. Biorisk and Quality Management Module; 05.-09. February 2018, Stockholm, Sweden
4. Management, Leadership and Communication in Public Health Module; 12.-16. February 2018, Stockholm, Sweden
5. Multivariable Analysis Module; 16.-20. April 2018; Nicosia, Cyprus
6. Rapid Assessment and Survey Methods Module, 14.-19. May 2018, Athens, Greece
7. Project Review Module, 27-31. August 2018, Lisbon, Portugal
8. Project Review Module, 26.-30. August 2019, Prague, Czech Republic

10. Other training

1. Geneious Bioinformatic Software Application Training; 26. January 2018, Berlin, Germany
2. Introduction to Linux and Raw Data QC for Next Generation Sequencing (NGS); 10.-11. January 2018, Wernigerode, Germany
3. 8th Next Generation Sequencing (NGS) Workshop: Shiga Toxin-Producing *E. coli* and *Mycobacterium tuberculosis*; 20.-22. March 2019; AGES Academy, Vienna, Austria

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 Regina Selb during her two-year EUPHEM fellowship (cohort 2017) as an EU-track fellow at the Robert Koch-Institute (RKI), Berlin, Germany. The projects described in this portfolio demonstrate the depth and breadth of the public health microbiology work of Regina. Regina's enthusiasm and ability to connect with people have given her the possibility to get involved in a number of projects that go from epidemiological studies including participation to 2 outbreak investigations with clear public health outcomes and molecular surveillance project on HIV. Her other projects and activities touched upon a wide variety of disease groups (from *Cryptococcus neoformans*, *S. aureus* (CA-MRSA), *Campylobacter jejuni*, mumps virus, arbovirus, *Mycobacterium tuberculosis*, to *Neisseria gonorrhoeae*) clearly demonstrating her capability to comfortably cover different disease groups. Regina has effectively contributed to the training site cascading her knowledge through teaching on various arguments and to a variety of audiences, including managing a 2-days sessions at Project Review Module 2019 on vaccinology. All projects here described were in line with the 'learning by doing' and 'on-the-job' training service approach of the EUPHEM programme and followed the core competency domains described for professionals in mid-career and above. Projects had a clear outcome, with results communicated in technical reports, scientific journals and at conferences. The EUPHEM Coordinator concludes that the fellow has succeeded in performing all her tasks to a very high standard and with a professional attitude, which indicates her development in leadership. We wish the fellow every success in her future career as a public health microbiologist.

Text

Supervisor's conclusions

Regina Selb's EUPHEM fellowship here at RKI has proved to be very fruitful for both Regina and the RKI. The supervisor team and the project supervisors all appreciated the opportunity to work with Regina. Her efficient work organization, independence and ability to cope with all sorts of people made her supervision very easy. Regina has pushed several projects forward and her reports and publications are of great value for the future work of the RKI. Her presentations in- and outside the institute made the importance of public health microbiology clear to the different audiences. Regina also contributed a lot to make EUPHEM better known in Germany and she made the German Scientific Advisory Board aware of EUPHEM's potential to strengthen public health. Several of Regina's projects covered both, aspects of microbiology and of epidemiology and required interaction of RKI's microbiologists and epidemiologist, thus strengthening their interaction. The fellowship has enabled Regina to reach a high level of expertise in public health microbiology and has prepared her well to take up a responsible position in the public health system. Thus we can highly recommend Regina Selb for employment in all kind of public health institution.

Personal conclusions of fellow

Through the EUPHEM fellowship I was offered the unique and valuable opportunity to sharpen my knowledge in a plethora of core competencies in public health microbiology. The learning approach applied throughout the fellowship, learning from public health experts while at the same time applying previous knowledge and skills is exceptional. Through courses offered by ECDC and learning opportunities at the Robert Koch Institute I was able to gain a solid basis in epidemiology and public health and to advance my skills in microbiology to the latest state of the art. At the same time, the theoretical and laboratory-based projects at the Robert Koch Institute allowed apply these newly acquired competencies and practical learning by doing. Last but not least, the fellowship provided me with a network of public health colleagues all over Europe, which is irreplaceable for coordinated future actions of public health experts for the benefit of population health in Europe.

Acknowledgements of fellow

First I would like to thank my EUPHEM supervisors at the Robert Koch Institute, Astrid Lewin, Andrea Hauser and Kathrin Keeren for their exceptional guidance throughout the fellowship. Especially Astrid's efforts to find suitable projects, to support me scientifically and personally during the last two years and her work for the EUPHEM fellowship at the Robert Koch Institute in general were immeasurable. I would like to thank all project supervisors for the offered opportunities, their guidance and support which made my fellowship unique and invaluable. I am very thankful to my front line coordinator, Loredana Ingrassio, who was guiding me throughout the fellowship and was supporting me in every way possible; make sure I was always on track with my fellowship and to gain the maximum possible learning experience. Moreover, I would like to thank Aftab Jasir for her commitment which made this fellowship experience possible for me and for her support throughout the last two years. All the people

involved in the training at ECDC and the course modules did excellent work to offer training and share their professional experience. Last but not least I would like to thank my EPIET/EUPHEM/FETP colleagues from cohort 2017 for all their scientific input and support throughout the fellowship, but especially for their friendship.