Summary of work activities
Jennifer Bender
European Public Health Microbiology Training
Programme (EUPHEM), 2019 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 in 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 provides experts in all relevant communicable diseases at the regional, national and international level with the tools they need to mount rapid responses to emerging health threats. This enables them to plan appropriate prevention strategies, assess existing prevention disciplines, develop microbiological guidelines, evaluate/produce new diagnostic tools, assess 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' which is why 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.

This report summarises the work activities undertaken by Jennifer K. Bender, cohort 2019 of the European Public Health Microbiology Training Programme (EUPHEM) at the Robert Koch Institute (RKI), Berlin, Germany. The work was conducted under the supervision of Dr Astrid Lewin & Dr Kathrin Keeren & Dr Jan Walter (site supervisors), Dr Loredana Ingrosso (Front Line Coordinator) and her project supervisors.

Jennifer Bender studied Biology at the Friedrich-Alexander-University Erlangen-Nuremberg, Germany (2000-2005). With a focus on bacterial human pathogens, she completed her diploma thesis at the University of Alabama, at Birmingham, United States. She continued her education in microbiology and finalised her PhD thesis in 2009 at the Robert Koch Institute (RKI), Berlin, Germany. Her expertise was further broadened by postdoctoral research at Melbourne University, Australia, and at Nanyang Technological University of Singapore (2010-2012). Jennifer returned to RKI in 2013 where she joined the Nosocomial Pathogens and Antibiotic Resistances Unit. Since then, she has been involved in the work of the National Reference Centre for Staphylococci and Enterococci and investigating the emergence and spread of antibiotic resistances. With the EUPHEM programme, she aimed to gain knowledge in Intervention Epidemiology and Public Health Management and Communication and to expand her skills in Public Health Microbiology in order to contribute to improving national and international health.

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.

Methods

This report accompanies a portfolio demonstrating 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; bio-risk management; quality management; teaching and public health microbiology management and the summary and communication of 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, with the exception of those prohibited for reasons of confidentiality.

Results

The objectives of these core competency domains were achieved partly through project or activity work and partly through participation in the training modules. Results are presented in accordance with the EUPHEM core competencies, as set out in the EUPHEM scientific guide[1].

1. Epidemiological investigations

1.1. Outbreak investigations

A. Factors preventing SARS-CoV-2 transmission during unintentional exposure in a GP practice: a cohort study of patient contacts; Germany, 2020
Supervisors: Dr Udo Buchholz, Dr Kai Michaelis

In early March 2020, two general practitioners (GPs) with a SARS-CoV-2 infection provided in-person patient care to patients of their joint medical practice before and after symptom onset, up until SARS-CoV-2 laboratory confirmation.

In a retrospective cohort study of all GP-patient contacts, we assessed the risk (frequency and determinants) of SARS-CoV-2 transmission from the GPs to their patients. Case definitions were based on self-reported symptoms/clinical criteria (onset of $\geq$2 COVID-19-symptoms = possible; when including pneumonia, anosmia, ageusia or dysgeusia = probable) and laboratory confirmation (SARS-CoV-2 PCR-positive = confirmed).

We interviewed 83/131 (69%) patients. The median age was 45 [IQR 7-87] years, and 56 (68%) were female. There were 25 (30%) current smokers (tobacco) and 54 (65%) reported underlying conditions. Due to multiple GP visits by the same contact-person, 89 contact-events were included. Patients spent a median of 10 minutes (IQR 10-20) with their GP. The GPs wore a medical facemask during 31 (35%) of 89 contact-events, more often during physical examination (25/56; 45%) compared to no physical examination (6/32; 19%; Fisher's exact *P*=.046).

We identified 22 (27%) cases: 17 (21%) possible, 3 (4%) probable, and 2 (2%) confirmed. All 22 identified cases had contact with a GP when the GP did not wear a mask, and/or when contact was $\geq$10 minutes. Importantly, contact <10 minutes with a GP wearing a facemask was significantly associated with a reduced risk (IRR 0.21; 95%CI 0.01-0.99) of COVID-19.

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[1] European Centre for Disease Prevention and Control. European public health training programme. Stockholm: ECDC; 2013. Available from: http://ecdc.europa.eu/en/publications/Publications/microbiology-public-health-training-programme.pdf

In conclusion, the use of facemasks for GPs and short consultation times helped limit the spread of SARS-CoV-2 from GPs to their patients. Our investigation lends support to current guidelines from WHO and the ECDC, both recommending that all healthcare workers continuously wear a medical facemask at work, especially in areas of community transmission.

Jennifer was part of the outbreak investigation team deployed to Nuremberg, Bavaria, to support the local public health authority with contact tracing. Together with cohort 2018 fellow Dr Sonia Boender, Jennifer was the principal investigator, developed the study protocol and questionnaire, interviewed contacts, and managed the overall study. They further developed a data entry mask in EpiData, entered, cleaned, and analysed the data with descriptive and inferential statistical analysis. As shared first authors, Sonia and Jennifer submitted an abstract to ESCAIDE 2020, which was accepted as a poster presentation. Together, they wrote a manuscript, which was published on the preprint server medRxiv and in Epidemiology & Infection, a peer-reviewed journal (#3).

B. Two doses of the mRNA vaccine Comirnaty effectively reduced transmission of SARS-CoV-2: evidence from a COVID-19 outbreak with SARS-CoV-2 Alpha in a nursing home, Osnabrück, Germany, January-March 2021
Supervisors: Dr Dorothea Matysiak-Klose, Dr Wiebke Hellenbrand

Shortly after completing vaccination with Comirnaty (BioNTech/Pfizer), an outbreak of SARS-CoV-2 infections occurred in a nursing home in Osnabrück, Germany. We assessed vaccine effectiveness (VE) and compared viral load and secondary attack rates (AR) in vaccinated and unvaccinated cases.

Cases were residents (living at the nursing home or receiving daycare) and staff testing PCR-positive between 3 January 2021 and 18 March 2021. Data on vaccination status, disease severity and SARS-CoV-2 testing were obtained for all cases, while the infection status of household members was assessed for SARS-CoV-2-positive staff only. We estimated VE as [1-RR], using Poisson regression models. For investigating vaccine effects, we analysed Ct-values as a proxy for viral load using linear regression models.

In this cohort, 95/124 (77%) residents and 72/128 (56%) staff were vaccinated twice with Comirnaty. AR among vaccinated and unvaccinated residents [24/95 (25%) vs. 10/29 (34%); P=.46, Chi-squared test] and staff [5/72 (7%) vs. 11/56 (20%); P=.06] were comparable. Of 50 cases, four were hospitalised [1/4 (25%) vaccinated] and five [2/5 (40%) vaccinated] died of COVID-19 (all residents). Variant Alpha was detected in 27/28 of typed cases. Age-adjusted VE was 88% [95%CI:37-98%; P=.04] against hospitalisation or death and 68% [95%CI:36-84%; P<.01] against symptomatic SARS-CoV-2 infection. With increasing duration between the second vaccine dose and diagnosis first PCR, the first Ct-value was significantly higher [0.31 25 units/day;95%CI:0.10-0.520.04-0.47; P<.01=.02]. The secondary AR was lower in households of vaccinated [2/9 (22.2%)] than of unvaccinated SARS-CoV-2-positive staff [12/18 (66.7%); P=.05, Fisher's exact test].

Two doses of BNT162b significantly reduced the risk of SARS-CoV-2 infection, disease, severe outcome, viral load, and secondary transmission, even within 14 days after the second dose. The incomplete protection emphasises the importance of maintaining non-pharmaceutical interventions after completed vaccination. When reconsidering non-pharmaceutical measures for fully vaccinated people, policy-makers must be made aware of age-impairing effects on VE and residual risks for infection, disease and transmission, especially among the elderly.

Jennifer and colleagues from the RKI were deployed to Osnabrück twice with an advisory function in order to support the outbreak investigation and for comprehensive data collection. Together with fellows Dr Emily D. Meyer and Mirco Sandfort (cohort 2020), Jennifer collected and subsequently analysed the data including data cleaning, descriptive epidemiology and univariate and multivariable regression analyses using R. Jennifer was involved in drafting an ESCAIDE 2021 abstract (accepted as poster presentation), writing the outbreak report and two manuscripts for submission to peer-reviewed journals (#5 & #6). The three fellows share first authorship in #5, while Jennifer and Emily are listed as first authors in #6.

C. Controlling an unprecedented nosocomial outbreak of vancomycin-resistant E. faecium in two central care hospitals in Southern Germany, October 2015 to December 2019
Supervisors: Dr Tim Eckmanns, Dr Sebastian Haller, Prof Dr Guido Werner

Over the course of four years, an outbreak with vancomycin-resistant *Enterococcus faecium* (VRE) has affected two hospitals in southern Germany. Between October 2015 and November 2019, more than 2 900 cases were notified. We aimed to comprehensively describe the outbreak dynamics by combining epidemiological and microbiological data and to assess implemented infection prevention and control (IPC) measures that eventually led to the end of the outbreak in November 2019.

In total, 1 337/2 903 (46%) of cases were female; the median age of all cases was 78 years (IQR 68-84). Of the 2 903 cases, 2 837 (98%) were colonised, whereas from 66 (2%) VRE could be isolated from otherwise sterile body fluids such as blood or surgical sites; case fatality rate was 0.6% (3/495).

We performed strain typing by means of whole genome sequencing (WGS) on a selected set of isolates (n>400) thereby revealing a predominant clone, sequence type (ST) 80/ complex type (CT) 1 013 at the beginning and at peak time of the outbreak. Nosocomial transmission was observed, as well as constant influx of VRE from outside, e.g. by patient transfer between hospitals. Additional WGS in early 2019 (n>90) demonstrated a genetic shift in genotypes over time.

Comprehensive IPC measures were implemented, including varying screening regimes, intensified training of staff, improvement of basic hand hygiene, and the implementation of an antibiotic stewardship core unit.

In conclusion, epidemiological and molecular analysis together with thorough IPC measures are indispensable to contain VRE in hospital settings.

Jennifer conducted data cleaning and descriptive epidemiological analysis. She further performed whole genome sequencing and phylogenetic analyses for the set of selected isolates. The results were discussed during the Project Review Module in August 2021. A peer-reviewed manuscript is currently in preparation.

Training modules related to the assignment/projects
The ECDC Introductory Course in September/October 2019 introduced Jennifer to the basic concepts of outbreak investigations, including the 10 steps of an outbreak investigation and matching epidemiological and microbiological analyses. This was achieved with interactive lectures, case studies, and group exercises. The Outbreak Investigation module in December 2019 provided in-depth insights into descriptive and analytical studies comprising everything from designing a questionnaire to data entry, data cleaning and management, descriptive and univariate and stratified analysis. The Multivariable Analysis module, held online in May 2020, provided a comprehensive understanding of different regression models. Using case studies and performing all analyses in R were helpful for the outbreak investigations Jennifer was involved in. The Management, Leadership and Communication in Public Health module taught her how to efficiently communicate with different stakeholders and higher authorities, even in emergency situations.

Educational outcome
Jennifer applied all steps of an outbreak investigation, which strengthened her epidemiological knowledge and data analysis skills. She had the opportunity to be deployed to local health authorities in Germany several times, which fostered her understanding of the challenges of the local health system and provided her with the unique opportunity to engage with an interdisciplinary team, including epidemiologists and public health officers. Jennifer further advanced her skills in drafting and publishing conference abstracts, posters, oral presentations, outbreak investigation reports, and peer-reviewed manuscripts.

1.2. Surveillance

A. Inferring measles virus endemicity from the variable MF-region as an improvement to measles surveillance in Germany

Supervisors: Prof Dr Annette Mankertz, Dr Sabine Santibanez, Dr Sébastien Calvignac-Spencer

Measles virus has been targeted for elimination by WHO. To reach this, a vaccine coverage of >95 %, an incidence of < 1 case/1 million inhabitants and the length of measles virus transmission chains (no transmission chain $\geq$ 12 months) must be certified. To genotype the virus, a 450 nucleotide sequence from the Nucleoprotein (N) gene of measles virus is used; however, the N-450 sequence alone lacks resolution; thus, WHO recommends to expand the sequence window to the highly variable MF-1000 intergenic region.

We aimed at assessing the suitability of the MF-region in Bayesian analyses to increase the discriminatory power of strain differentiation and to infer endemic transmission events within the German population in order to improve measles surveillance.

After we obtained statistical support for genetic divergence over time in the available MF-sequences (genotypes D8-Gir-Somnath n=136, B3-Dublin n=112, 2016-2019), we reconstructed phylogeny under the assumption of a molecular clock. Our results demonstrate that i) imported measles virus variants are very closely related to isolates obtained from German patients and for which no epidemiological information was available that might indicate a so far unknown linkage and/or transmission; ii) a possible introduction in early 2018 for D8-Gir-Somnath and a re-introduction in late 2018 for B3-Dublin. However, due to reduced statistical power, we realised that endemic transmission is difficult to infer from the MF-1000 region.

In conclusion, the WHO recommendation to complete the molecular surveillance of measles by including the MF-1000 region is insufficient when aiming at determining the transmission pattern and identifying endemic chains at country level. This is particularly true for countries with constant importations of Measles virus from multiple sources, such as Germany. We thus would like to propose to the WHO to encourage whole genome sequencing for increased resolution and data sharing in every perspective. Combining all those resources will allow reconstructing global phylogenies and will enable national surveillance systems to reliably assess endemicity of certain measles virus variant in the respective country.

Jennifer, as the principal investigator, gained knowledge about the surveillance mandate of a National Reference Centre and how this impacts the analyses and the interpretation of the results, e.g. in terms of representativeness and statistical support. She conducted all Bayesian analyses, reconstructed phylogeny, and discussed putative recommendation for public health action.

B. Routine national surveillance activities at the Robert Koch Institute

B.1. Monitoring of the Rapid Alert System for Food and Feed (RASFF)

Supervisors: Dr Bettina Rosner

The Rapid Alert System for Food and Feed (RASFF) provides a platform to rapidly share important food safety information between the food safety agencies of the member states. The RKI has committed itself to screen the RASFF alerts, as provided to the RKI by the German RASFF food safety contact point on a daily basis, for alerts regarding food items contaminated with notifiable infectious organisms that are related to human health. Potentially useful information is then forwarded to federal and local public health authorities in order to support their investigations of notified cases of the respective infectious disease. Jennifer was in charge of screening and summarising the available information for a total of two months, and of informing the respective authorities of potential sources of food-borne infectious diseases or disease outbreaks.

B.2. Event-based surveillance (EpiLag)

Supervisors: Nadine Zeitlmann

Since January 2009, the RKI has conducts a weekly telephone conference (EpiLag) with participants from federal state public health authorities, consultants for infection protection of the federal states, and representatives of the German armed forces. The conference provides a platform on which current infectious disease events can be discussed on a regular and structured basis as well as in a timely manner. Exchanges include information relevant to international events, subnational events, and events that affect the entire Germany, as well as other topics related to surveillance and response to infectious diseases. In addition to regular attendance of the conference, Jennifer supported EpiLag as an editor, which included collecting all the relevant topics and respective information for the teleconference from all stakeholders, preparing an update on the international and national COVID-19 situation and other infectious disease topics, and reporting about novel guidance documents (COVID-19) that were published or updated by the RKI in the preceding week. On the day of the conference, Jennifer took detailed notes of the discussions and reported them back to all participants and stakeholders in the public health sector.

Training modules related to the assignment/projects

During the EPIET/EUPHEM introductory course in September/October 2019, Jennifer was taught the basic concepts of surveillance, development and evaluation of a surveillance system. In the Outbreak Investigation module and the Management, Leadership and Communication in Public Health module, Jennifer received insights into event-based surveillance, which were further intensified in the Rapid Assessment and Survey Methods module in May 2021 with respect to complex emergency situations.

Educational outcome

Jennifer applied the concepts of event-based surveillance during her routine national surveillance activities at the RKI. By participating in the weekly epidemiological telephone conference (EpiLag), she developed an understanding of the advantages and pitfalls of a national notification system that was adjusted *ad hoc* to the ongoing COVID-19 pandemic. Working on the measles project, she further gained experience in evaluating surveillance indicators and their suitability to assess global virus elimination goals by conducting comprehensive bioinformatics analyses.

2. Applied public health research

A. Analysis of asymptomatic and pre-symptomatic transmission in a SARS-CoV-2 outbreak, Germany, 2020

Supervisors: Dr Udo Buchholz

In February/March 2020, a cluster of 59 SARS-CoV-2-positive, partially asymptomatic cases emerged around carnival events in a rural district in Germany. Due to its importance for public health management, we used this cluster to analyse the transmission of SARS-CoV-2 by pre-symptomatic, symptomatic, and asymptomatic cases to close contact persons.

We surveyed cases regarding possible exposures, symptoms, and information about household (HHC) and non-HHC/other contact persons (OC) in their infectious period (two days prior to 10 days after symptom onset) with a standardised questionnaire. In detail, cases recalled HHC/OC's test results and in which phase of infection the interaction occurred. We calculated secondary attack rates (SAR) for laboratory-confirmed SARS-CoV-2 infection

and relative risks (RR) with 95% confidence intervals (95%CI). Additionally, we reconstructed the most probable transmission chain.

Fifty-three cases (90%) participated in the survey. Among the 46 symptomatic cases, fatigue (80%), headache (54%), cough (52%), ageusia (43%), and fever (43%) were the most frequently reported symptoms. In total, cases reported to have 96 HHC and 398 OC. None of the 7 (13%) asymptomatic cases led to SARS-CoV-2-infection of their HHC or OC. Secondary attack rates for HHC for symptomatic cases was 15%. We observed the highest secondary attack rates for OC who met their source case in their pre-symptomatic phase (SAR=21%). The secondary attack rate for OC was 6.9% for meeting their source case in their symptomatic phase. The OCs of pre-symptomatic and symptomatic cases were 6.5-times (95%CI: $1.1-\infty$) and 1.8-times (95%CI: $0.14-\infty$) more likely to test positive for SARS-CoV-2 when compared with OCs of asymptomatic cases.

In conclusion, our study suggests that asymptomatic cases are unlikely to contribute substantially to the spread of SARS-CoV-2. COVID-19 cases should be detected and managed early to quarantine close contacts immediately and prevent pre-symptomatic transmissions.

Together with Michael Brandl (cohort 2019) and Nadine Zeitlmann (FG38, RKI), Jennifer developed the study protocol, designed the questionnaire, interviewed cases, designed the data entry mask, and imported and analysed the data using descriptive methods and statistical regression analysis. She presented the study at ESCAIDE 2020 (oral presentation) and drafted the manuscript that was accepted for publication in Emerging Infectious Diseases (#1). Jennifer and Michael share first authorship. She further presented the results at the bi-annual meeting of the RKI scientific advisory board in December 2020.

B. In-patient diversity and in-patient evolution of Mycobacterium abscessus in patients with Cystic Fibrosis
Supervisors: Dr Astrid Lewin, Dr Sébastien Calvignac-Spencer

Mycobacterium abscessus (MABS) is one species of non-tuberculous mycobacteria (NTM) that causes infections in mostly immunocompromised humans. In cystic fibrosis (CF) patients, MABS are increasingly detected and frequently cause deterioration of lung function. The species is intrinsically resistant to a plethora of antibiotics and known for its capability to develop further resistances upon antibiotic exposure.

In order to prevent infection and improve treatment strategies, it is important to know how the bacteria evolve during the course of infection, i.e. to assess whether there is a lung compartment-specific diversity, and secondly to ascertain when the resistance eventually emerged. We aimed to assess in-patient diversity and in-patient evolution by using MABS isolates that were collected from CF patients at a CF treatment centre in Berlin, Germany (2013 to 2018).

For in-patient diversity *M. abscessus subsp. masilliense* (n=22) isolates obtained from different lung compartments from one patient were analysed by determining single nucleotide polymorphisms (SNP) and by developing an *ad hoc* core genome MLST scheme. Our results demonstrate that within the 10 months' time difference between sampling, there is not much strain diversity present. In addition, there was no evidence for mixed infection and no strain specific colonisation of a certain lung compartment.

In-patient evolution was assessed using 30 *M. abscessus subsp. abscessus* isolates from another patient obtained between 2013 and 2017. SNPs were called by sequence read alignment to an isolate from this patient for which the genome sequence was determined previously. We aimed to estimate the mutation rate and therefore investigated the presence of a temporal signal by i) linear regression using TempEst (an exploratory, informal test), ii) comparison of molecular clock models assuming isochronous or heterochronous sampling using model marginal likelihoods derived from Bayesian Evolutionary Analyses Sampling Trees (BEAST) analyses, and iii) a date randomisation test carried out in R by using the TipDatingBeast package and BEAST analyses run on 20 simulated datasets. Unfortunately, we failed at detecting a temporal signal in our sequences, precluding any meaningful rate assessment. Repeating such analyses with a data set with a longer time span would allow estimating a mutation rate and ascertaining the emergence of e.g. resistant isolates during the course of infection and under antibiotic therapy.

Jennifer planned the project and carried out all bioinformatics analyses. These comprised a comprehensive quality control of whole genome sequences derived from Illumina® sequencing, generating an *ad hoc* cgMLST typing scheme, sequence read alignment (mapping), SNP filtering and Bayesian molecular clock analysis, including three different methods to assess genetic divergence over time (temporal signal). Due the request of a reviewer to determine the mutation rate of MABS in CF patients, Jennifer drafted a point-by-point answer on the basis of the results from assessing in-patient evolution, which were then included in the revised version of manuscript 'Genetic diversification of persistent *Mycobacterium abscessus* within Cystic Fibrosis patients' for which Jennifer was listed as co-author (#4).

Training modules related to the assignment/projects

During an interactive group exercise in the EPIET/EUPHEM introductory course, Jennifer was trained on how to frame a research question, plan, conduct, and evaluate a research project. In addition, an introduction to different study designs facilitated setting up a retrospective cohort study and downstream analyses of the data in STATA (also introduced during the introductory course). The Management, Leadership and Communication in Public Health module was useful for her to expand her personal skills in project management and communication in public health. During the Project Review module in August 2020, Jennifer discussed the research outcomes regarding 'Analysis of asymptomatic and pre-symptomatic transmission in a SARS-CoV-2 outbreak, Germany, 2020' with her fellow students and public health professionals. This fostered narrowing down the outcomes with respect to public health recommendations and to advance in public health communication skills.

Educational outcome

In the two projects, Jennifer experienced all stages of a public health microbiology research project, including framing the research question, planning the project, data collection (from patients through interviews or genome sequences from laboratories), performing the necessary data analyses, and drafting conference abstracts and a manuscript of interest to the public health scientific community. She advanced significantly in conducting Bayesian molecular clock analysis. Finally, Jennifer further improved her communication skills by presenting the study results to public health professionals.

3. Applied public health microbiology and laboratory investigations

Supervisors: Dr Regina Selb, Dr Dagmar Heuer

Neisseria (*N.*) *gonorrhoeae* is the causative agent of gonorrhoea, the second most frequent reported STI in the European Union. Within the German Gonococcal Resistance Network's (GORENET) *N. gonorrhoeae* sample collection, azithromycin-resistant *N. gonorrhoeae* isolates increased from 4.3% in 2016 to 9.2% in 2018. Resistance against azithromycin is mediated, amongst others, through chromosomal mutation within – and increased expression of – the multiple transferable resistance operon *mtrRCDE*. This mosaic operon has previously been described for azithromycin-resistant US isolates of NG MAST ST12302.

We aimed at describing the genetic relationship of azithromycin-resistant *N. gonorrhoeae* clinical isolates of an emerging sequence type (ST12302) obtained from German patients in order to investigate *N. gonorrhoeae* molecular epidemiology and to infer possible routes of transmission.

We applied whole genome sequencing on selected *N. gonorrhoeae* clinical isolates of NG-MAST genotype ST12302 (n=27). We developed an *ad hoc* core genome MLST (cgMLST) typing scheme and compared the *mtr* locus between German and US isolates.

When examining the *mtr* operon and the *mtrR* promoter of the strains we found that the *N. gonorrhoeae* German clinical isolates display 100% sequence identity to the US strains. Minimum spanning trees revealed two cluster of closely related strains (<15 alleles difference) that contained both German and US isolates suggesting a possible epidemiological linkage. As we only had limited or no whole genome sequences available for German *N. gonorrhoeae* clinical isolates of NG-MAST ST12302 before 2018, we cannot hypothesise on the direction of transmission from one country to the other.

Our results have proven the suitability of *ad hoc* cgMLST schemes to determine genetic relatedness. Our data suggest that, together with horizontal gene transfer of resistance determinants and well-established point mutations, international spread of resistant lineages plays a major role regarding azithromycin resistance in Germany. We were able to describe the emergence of these isolates for the first time in Europe.

Jennifer generated the *ad hoc* cgMLST scheme. Based on this, she analysed the sequences derived from whole genome sequencing. In addition, Jennifer contributed to the revision of the manuscript. The results were published in a peer-reviewed article with Jennifer as a co-author (#2).

Training modules related to the assignment/projects

In the EPIET/EUPHEM Introductory Course in September/October 2019, Jennifer attended a catch-up lecture on important concepts of laboratory-based microbiological typing techniques and concepts of phylogenetic analysis. This included case studies with a substantial amount of public health microbiology and interpretation of laboratory results. Phylogenetic analyses and the interpretation together with epidemiological data were trained in more detail during the Outbreak Investigation module in December 2019. The Management, Leadership and Communication in Public Health module in February 2020 provided concepts about how to communicate and work in a multi-disciplinary team.

Educational outcome

With this project Jennifer mastered her understanding in analysing WGS data with respect to rapid development of a high-resolution typing scheme for bacterial pathogens. By combining different approaches to genotypically characterise the isolates, she improved her ability to interpret laboratory results. By overlaying with epidemiological data, Jennifer improved her Public Health Microbiology knowledge in studying global threats imposed by microbial pathogens.

4. Biorisk management

Safe and secure handling of pathogens of the Mycobacterium tuberculosis complex in the BSL-3 laboratory
Supervisors: Dr Astrid Lewin, Elisabeth Kamal, Dr Daniela Jacob

Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis, which is one of the top 10 leading causes of death worldwide. Mtb is transmitted through air by inhaling Mtb-containing droplets by a susceptible host. Due to its very low infectious dose (1-200 inhaled bacilli), handling of Mtb must be conducted using biosafety level 3 (BSL-3) practices and equipment. In order to provide reference material to colleagues at RKI and third parties upon request, we extracted DNA from reference strain M. tuberculosis H37Ra.

Jennifer received extensive theoretical and practical safety training to securely handle pathogenic organisms under BSL-3 conditions. She practiced assistance to another colleague and performed work under high-risk BSL-3 conditions herself. She wrote the final report and received an official certificate issued by the unit for highly pathogenic microorganisms at the Robert Koch Institute, allowing her to independently perform infectious work activities under those conditions.

Training modules related to the assignment/projects

The Biosafety and Quality management module was cancelled.

Educational outcome

Jennifer was trained to assist and work independently under BSL-3 conditions. Theoretical training and practical work included accessing the BSL-3 facility and required documentation, adequate usage of personal protective equipment, safe handling of highly pathogenic bacteria, controlling for sterility and safe discharge of DNA material and last, waste management.

5. Quality management

Internal and external monitoring audit of the National Reference Centre (NRC) for Salmonella and other Bacterial Enterics and the NRC for Staphylococci and Enterococci
Supervisors: Dr Janine Kleymann-Hilmes, Dr Angelika Fruth, Dr Birgit Strommenger

The German Federal Ministry of Health appoints and funds National Reference Centres (NRCs) since 1995 in order to monitor important human pathogens. The NRC for *Salmonella* and other Bacterial Enterics and the NRC for Staphylococci and Enterococci, both at RKI, represent laboratories that provide secondary, often confirmatory analysis of bacterial pathogens. The two NRCs are accredited for DIN EN ISO/IEC 17025 - 2018 (testing and calibration laboratories) and DIN EN ISO 15189 – 2014 (medical laboratories) compliance for certain methods of their portfolio which requires re-assessment by the national accreditation body for the Federal Republic of Germany (DAkkS, Deutsche Akkreditierungsstelle) on a periodical basis. We aimed to achieve accreditation within the framework of the adjusted DIN EN ISO/IEC 17025:2018 and accreditation with a flexible scope for certain methods.

We conducted an internal audit to assess the process (laboratory inspection including documents revision) and the system (documents and control). As reviewed by the RKI QM representatives in the internal audit report, some requirements were formulated that needed revision in preparation for the external audit by the DAkkS. The external monitoring audit by the DAkkS representatives resulted in a report framing two non-critical deviations for both the NRCs, which were granted two months to react upon and revise accordingly, which resulted in approval of the requested accreditation, eventually.

Jennifer assisted the RKI QM representatives during the internal audit and the DAkkS during the external process and systems audit. She further supported both NRCs in preparation for the external monitoring audit including summarising and communicating the results of the internal audit report to the unit representatives.

Training modules related to the assignment/projects

The Biosafety and Quality management module was cancelled.

Educational outcome

By participating in both an internal and external monitoring audit, Jennifer majorly advanced her knowledge in quality control, especially in the context of accredited processes and systems of German National Reference Centres. She obtained an understanding of the complex concepts of the RKI's laboratory quality management system as well as the national accreditation prerequisites that apply for diagnostic laboratories.

6. Teaching and pedagogy

A. Training of Containment Scouts in the context of COVID-19 contact tracing

In order to support local public health authorities (LPHA) in Germany, the Ministry of Health employed 600 students in May 2020, so called containment scouts, that were deployed to LPHA to support COVID-19 contact tracing activities. Jennifer participated in the evaluation of the initial training material, adapted the presentation thereafter and recorded the lecture which was then delivered to the scouts and mobile teams/staff at LPHA who would benefit from the training.

B. Organisation and implementation of the Laboratory module for fellows of the Postgraduate Training for Applied Epidemiology (PAE)

The Laboratory Module is organised annually for PAE/EPIET fellows of current cohorts at RKI Berlin and at state/local health authorities in Germany. Participants not familiar with the work of microbiology laboratories get an insight into the practical everyday activities of laboratories at the institute. Jennifer was responsible for all stages including conceptualising, organising and implementing the module online. She presented an introduction to laboratory methods and evaluated the course with the help of an online questionnaire.

Training modules related to the assignment/projects

During the EPIET/EUPHEM Introductory Course, the fellows were familiarised with the concepts of teaching. The Management, Leadership and Communication in Public Health, February 2020, supported development of project management skills and how to communicate effectively in a multidisciplinary team. The Project Review module in August 2020 was very valuable to learn how to give and receive feedback.

Educational outcome:

Jennifer improved her ability to communicate effectively and deliver scientific content to non- but also to public health professionals. Jennifer gained experience in organising a full online course, in defining learning objectives and in evaluating the teaching performance by summarising the feedback of participants.

7. Public health microbiology management

A. Implications of changing diagnostic approaches for the detection of Giardia and Cryptosporidium for Public Health Management

Supervisor: Dr Anton Aebischer, Dr Christian Klotz, Dr Laetitia Kortbeek

Intestinal parasitic protozoa can cause severe infections and constitute a serious health problem. Giardiasis, caused by *Giardia (G.) duodenalis* mostly affects infants, young children, travellers and immunocompromised individuals and together with Cryptosporidiosis accounts for a substantial amount of diarrheal disease in children <5 years old. In Germany and the Netherlands, state laws allow imposing employment bans or restricted access, if a person is tested positive for one (Germany: for both) parasite. With changing diagnostics and more asymptomatic cases reported, a problem arises, as no harmonised country-specific and/or international guidance documents exists for interpretation of these results. However, this is of utmost importance as it remains unclear how to manage colonised individuals with respect to treatment and, most importantly, how to lift enforced employment bans or bans to participate in communal activities in institutional settings.

We developed a questionnaire to assess how *Giardia* and *Cryptosporidium* are currently diagnosed in both countries and to survey the measures implemented by doctors and local public health authorities upon positive test result. Unfortunately, and due to the COVID-19 pandemic the project was put on hold, as there was no capacity for diagnostic laboratories or health authorities to participate in the survey. Nevertheless, the output of this project can be used immediately, if capacity allows it.

Together with Dr Kamelia Stanoeva, Jennifer designed the bi-national questionnaire including questions specific to either country prerequisites and wrote the final report. Jennifer further formulated a Letter of Invitation to engage local health authorities during the weekly epidemiological telephone conference between the federal states and RKI and finalised the Data Protection Questionnaire from the data protection advisory board at RKI.

B. Participation in the German SARS-CoV-2 crisis management at local and national public health authorities

Supervisors: Dr Jan Walter, Dr Katharina Alpers

As of 26 June 2021, SARS-CoV-2 has caused 90 616 deaths among 3 724 806 people who became infected in Germany ever since the first case was notified on 27 January 2020. On 24 January 2020, the RKI launched its Emergency Operations Centre (EOC = situation room). Ever since, a plethora of public health measures were recommended by the RKI such as intensified contact tracing and quarantine guidelines, testing strategies, and in general comprehensive health education of the expert audience. In addition, employees of the RKI were sent to local public health authorities (LPHA) in case support was requested according to §4 of the German Protection against Infection Act.

Over the course of one and a half years, Jennifer carried out various tasks. First, she operated a citizen's hotline for SARS-CoV-2/Coronavirus disease COVID-19 at the local public health office 'Berliner Senatsverwaltung für Gesundheit, Pflege und Gleichstellung' in Berlin, Germany. Second, she supported the work of the RKI situation room at different positions such as 'tasks', 'situation protocol', 'international communication', 'outbreak screening' and 'data analysis'. Last, Jennifer was deployed to the LPHA of Freising, Nuremberg and Osnabrueck, Germany, in order to carry out contact tracing and support local SARS-CoV-2 outbreak investigations. She summarised her experiences and learning outcomes in a final report.

C. Assistance of RKI to the local public health office of Sonneberg district to consult in SARS-CoV-2 outbreaks within retirement homes

Supervisor: Dr Tim Eckmanns

On 3 July 2020, Sonneberg district was the only administrative district in Germany with a seven-day incidence that exceeded the threshold of 50 new COVID-19 cases/100 000 population. Thus, a request for assistance was issued to the RKI to assist the district health office in advisory capacity. Together with Tim Eckmanns, Jennifer visited the local health office. Following a briefing by the crisis management team, they visited one retirement home and one institution for mentally handicapped people. The retirement home has recently reported an outbreak with 19 cases of COVID-19 and already implemented infection prevention and control (IPC) measures; as has the institution for handicapped people that was inspected thereafter. In a debriefing, Tim and Jennifer recommended to the crisis management team of the public health office to further improve IPC measures such as i) regular inspection or personal discussions with all inpatient nursing homes of that region in order to improve communication between these institutions and the local public health authority; ii) monitoring of potential symptoms of residents/patients under care and of staff by daily interviews, by means of pulse oximeters and fever testing; iii) increasing compliance among healthcare staff by means of RKI recommendations and iv) sensitive testing in all healthcare settings as soon as symptomatic (even with less specific COVID-19 symptoms) cases occur and possibly also of asymptomatic suspected cases. Jennifer further wrote the final mission report.

Training modules related to the assignment/projects

The Management, Leadership and Communication in Public Health module in February 2020 was very useful for developing communication strategies addressing different audiences, such as healthcare professionals, managers, the media and the general public.

Educational outcome
Jennifer obtained a broad understanding of the responsibilities with respect to management and communication at different levels within the German public health system. She further sharpened her knowledge to formulate public health recommendations and to deliver information to a wider audience including non-public health professionals.

8. Communication

Publications related to the EUPHEM fellowship

1. **Bender J** & Brandl M, Hoehle M, Buchholz U, Zeitlmann N. Analysis of Asymptomatic and Presymptomatic transmission in SARS-CoV-2 Outbreak, Germany, 2020. Emerg Infect Dis. 2021 Apr;27(4):1159-1163. doi: 10.3201/eid2704.204576. Epub 2021 Feb 18.

2. Banhart S & Selb R, Oehlmann S, **Bender J**, Buder S, Jansen K, Heuer D. J Infec Dis. 2021; https://doi.org/10.1093/infdis/jiab091.

3. Boender S **& Bender J**, Michealis K, Krueger A, Buchholz U. Factors preventing SARS-CoV-2 transmission during unintentional exposure in a GP practice: a cohort study of patient contacts; Germany, 2020. Epidemiol Infect. 2021 Jul 2;149:e161.doi: 10.1017/S0950268821001503.

4. Lewin A, Kamal E, Semmler T, Winter K, Kaiser S, Schaefer H, Mao L, Eschenhagen P, Grehm C, **Bender J**, Schwarz C. Genetic diversification of persistent Mycobacterium abscessus within Cystic Fibrosis patients. Virulence 2021 Dec;12(1):2415-2429.doi: 10.1080/21505594.2021.1959808.

5. Meyer ED & Sandfort M **& Bender J**, Matysiak-Klose D, Doerre A, Bojara G, Beyrer K, Hellenbrand W. Two doses of the mRNA BNT162b2 vaccine effectively reduced severe outcomes, viral load and transmission: evidence from a SARS-CoV-2 Alpha outbreak in a nursing home, Osnabrück, Germany, January-March 2021. 2021. *Manuscript submitted*.

6. **Bender J** & Meyer ED, Sandfort M, Matysiak-Klose D, Bojara G, Hellenbrand W. Low sensitivity of rapid antigen tests to detect SARS-CoV-2 infections prior to and on the day of symptom onset in staff and residents of a nursing home, Germany, January-March 2021. *The Journal of Infectious Diseases, jiab528,* *https://doi.org/10.1093/infdis/jiab528*.

Reports

1. Surveillance report: Inferring measles virus endemicity from the variable MF-region as an improvement to measles surveillance in Germany.

2. Surveillance report: Routine surveillance activities at the Robert Koch Institute.

3. Outbreak report: 'Untersuchung eines SARS-CoV-2 Alpha-Ausbruchs unter Geimpften und Ungeimpften einer Pflegeeinrichtung im Landkreis Osnabrück, Niedersachsen, 2021'.

4. Public Health Management report: Implications of changing diagnostic approaches for the detection of Giardia and Cryptosporidium for Public Health Management.

5. Public Health Management report: Participation in the German SARS-CoV-2 crisis management at local and national public health authorities.

6. Public Health Management report: 'Bericht zum Einsatz des RKI im Landkreis Sonneberg am 03.06.2020'.

7. Biorisk management report: BSL3 activity – DNA extraction from *Mycobacterium tuberculosis*

Conference presentations

1. Bender J, Brandl M, Hoehle M, Buchholz U, Zeitlmann N. Comparison of secondary attack rates resulting from asymptomatic, presymptomatic and symptomatic cases in a COVID-19 outbreak, Germany, February-March 2020. Oral presentation: ESCAIDE 2020.

2. Boender S, Bender J, Michaelis K, Krueger A, Buchholz U. The risk of SARS-CoV-2 transmission by infected general practitioners (GPs): a cohort of contacts from a GP practice, Germany, February – March 2020. Poster presentation: ESCAIDE 2020.

Other presentations

1. What do genomes tell us about the epidemic success of nosocomial pathogens? Oral presentation: Robert Koch Institute, NGS User Meeting, December 2019

2. Fighting SARS-CoV-2 – Insights into the work of a EUPHEM fellow. Oral presentation: Robert Koch Institute, FG16 Seminar series, November 2020.

3. Asymptomatic, but not pre-symptomatic, transmissions are unlikely to contribute substantially to the spread of SARS-CoV-2 – results from a COVID-19 outbreak in Southern Germany, 2020. Oral presentation: Robert Koch Institute, Meeting of the RKI Scientific Advisory Board, December 2020.

Other activities

1. N/A

Other training modules

1. Healthcare-associated Infections module, 04/06 – 05/06/2020, virtual

9. EPIET/EUPHEM modules attended

1. Introductory Course, 23/09 – 11/10/2019, Spetses, Greece

2. Outbreak Investigation module 09/12 – 13/12/2019, Nicosia, Cyprus

3. Management, Leadership and Communication in Public Health module, 10/02 – 14/02/2020, Stockholm, Sweden

4. Multivariable Analyses module, 20/04 – 24/04 2020 & 18/03/2021, virtual

5. Project Review module 2020, 24/08 – 28/08/2020, virtual

6. Rapid Assessment and Survey Methods, 27/04/2021 & 05/05 – 06/05/2021, virtual

7. Vaccinology module, 14/06 – 18/06/2021, virtual

8. Project Review module 2021, 23/08 – 26/08/2021, virtual

10. Other training

1. All-day training on the revised DIN EN ISO 17025, 22/10/20169, Berlin, Germany

2. R course at RKI, 23/10 – 24/10/2019, Berlin, Germany

3. R course at ECDC, 25/11 – 26/11/2019, Stockholm, Sweden

4. Safe and Secure Approaches in Field Environments (SSAFE), 22/09 – 24/09/2020, Turin, Italy

5. Antibiotic Resistance Surveillance Workshop, 02/12/2020, virtual

6. GOARN 1.5 Virtual Workshop, 01/07 – 02/07/2021, virtual

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 Jennifer Bender during her two-year EUPHEM fellowship (cohort 2019) as an MS-track fellow at the Robert Koch Institute (RKI) in Berlin, Germany.

After two years of international experience, Jennifer was back at RKI and was based at Wernigerode, where she joined the Nosocomial Pathogens and Antibiotic Resistances Unit. She undertook the ECDC Fellowship with the aim of approaching and obtaining a better understanding of public health microbiology. Her genuine enthusiasm, capacity, intelligence, and collaborative attitude were highly appreciated by everyone. In particular, being her frontline coordinator proved to be an easy and highly rewarding task. As a fellow, she offered a friendly, direct interaction that made it a pleasure to interact with and provide her with feedback; as a scientist, she proved herself with a variety of successful projects with relevant outputs. For her cohort fellows at RKI she was a valuable colleague, as testified by several joint abstract and publications: indeed, the best advertisement for EUPHEM/EPIET collaboration. Jennifer, in addition to obtaining her objectives in the several core competencies, has actively contributed to the COVID-19 response both at regional and national level, and was deployed in the field to assist in outbreak management. Her scientific quality put at the service of public health are only paired by her good spirit and collaborative attitude. As her frontline coordinator, together with the EUPHEM team, we conclude that Jennifer has succeeded in performing all her tasks to a very high standard and with a professional attitude. I wish her every success in her future career and congratulate the training site for a successful training of the fellow.

Supervisor's conclusions

Jennifer Bender was the first MS track EUPHEM fellow at RKI, which is one of the reasons why the course of her fellowship attracted great interest from colleagues in the institute. Her fellowship was considered a great success by everyone. In particular, in the special situation of the COVID-19 pandemic, Jennifer's work was a big support for the RKI. She participated in several COVID-19 outbreak investigations and worked closely with epidemiologists at RKI as well as different regional public health institutions. In this way, she broadened her horizons to understand the organisation of and procedures in regional public health institutions and the approaches to epidemiological studies. Her microbiologic knowledge, on the other hand, enriched the work of the epidemiologists. Her skills in public health communication and management were effectively trained by the exchange with other public health institutions. In addition, Jennifer provided a lot of support to the COVID-19 Emergency centre and thus relieved her colleagues from too much workload during this difficult time. Apart from participating in COVID-19-related work, Jennifer has pushed forward various projects in different units of RKI. Both the supervisor team and the project supervisors appreciated working with Jennifer. Her efficient work organisation, independence, and initiative made supervision easy. In the course of the fellowship, Jennifer obtained a comprehensive knowledge of public health issues, including in addition to public health microbiology public health management, research, and epidemiology. The fellowship has prepared her perfectly for a responsible and leading position in the public health system.

Personal conclusions of fellow

The EUPHEM fellowship gave me the unique opportunity to receive two years of constant training of professional and personal skills. I have gained insights into and improved my knowledge of public health microbiology, epidemiology, and infectious disease surveillance, including a better understanding of the German local/national public health system and processes, and I have also further advanced my communication abilities. In addition, I have obtained comprehensive bioinformatics skills that will be of major importance for my future work as a laboratory scientist. The programme brings together public health professionals, and I am certain that the network our cohort has established during these years will represent a long-lasting entity of future fruitful exchange and collaborations. As an MS-track fellow, I have and will especially benefit from the collaborative work conducted with colleagues from many different units at RKI. This is valuable not only for my own personal development but will eventually facilitate in-house collaborations and mutual support. Finally, as part of the EPIET/EUPHEM alumni network, I will now have the opportunity to share my experiences and knowledge with future fellows and the broad public health community.

Acknowledgements of fellow

I am deeply grateful to my EUPHEM site supervisors at RKI, Astrid Lewin and Kathrin Keeren, who have provided excellent guidance, constant and valuable feedback, and a pleasant working environment throughout my fellowship. They were dedicated and supportive in every sense. I would further like to thank Jan Walter for his supportive epidemiological guidance and all my project supervisors who enabled me to personally develop and engage in so many interesting projects that extended my scientific knowledge in various aspects. I am grateful to my frontline coordinator Loredana Ingrosso for her respectful collaboration, valuable discussions and feedback that kept me on track with all the objectives that needed to be fulfilled.

Special thanks goes out to Sébastien Calvignac-Spencer who never got tired of answering questions about molecular clock analyses; to Regina Selb who introduced me to EUPHEM and with whom I was lucky enough to work with during my fellowship; to Guido Werner and Martin Mielke, who strongly supported my participation in the programme; to Aftab Jasir for her commitment to all the EUPHEM fellows; to Nadine Zeitlmann, Michael Brandl, Sonia Boender, Emily Meyer, and Mirco Sandfort, all fellows at RKI, with whom I had the opportunity to collaborate with. Last, but not least, I want to thank all trainers at ECDC for their effort in providing a multi-faceted learning experience, and my lovely cohort 2019: stay crude and unadjusted.