

The section header 'Summary of work activities' in a white, sans-serif font, set against a blue background.The author's name 'Claudia Lucarelli' in a white, sans-serif font, set against a blue background.The title of the report 'European Public Health Microbiology Training Programme (EUPHEM) 2013 cohort' in a white, sans-serif font, set against a blue background.The section header 'Background' in a bold, blue, sans-serif font.

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

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

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

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

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Stockholm, August 2015

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This report summarises the work activities undertaken by Claudia Lucarelli of the 2013 cohort of the European Public Health Microbiology Training Programme (EUPHEM) Member State -track at Istituto Superiore di Sanità (ISS), Rome, Italy. Claudia Lucarelli is a microbiologist from Italy. Before EUPHEM she was working as a post-doctoral fellow at the Istituto Superiore di Sanità in the Gastroenteric and Neurological Bacterial Diseases Unit, where she was involved in foodborne and waterborne disease surveillance.

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 through specific projects, activities and theoretical training modules.

Specific projects included epidemiological investigations (outbreaks and surveillance), applied public health research, applied public health microbiology and laboratory investigations, 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 represents a summary of all work conducted by the fellow, unless inclusion is prohibited for reasons of confidentiality.

## Results

The objectives within these core competency domains were achieved partly through project/activity work and partly by participating in training modules. Results are presented in accordance with the EUPHEM core competencies defined in the EUPHEM scientific guide<sup>1</sup>.

### 1. Epidemiological investigations

#### 1.1. Outbreak investigations

##### *A. Outbreak of *Campylobacter jejuni* in a kindergarten in the north of Italy, November 2013*

Project supervisors: Dr Ida Luzzi, Dr Fortunato D'Ancona

*Campylobacter* spp. is the most commonly reported gastrointestinal pathogen in humans worldwide, although in Italy reporting is very low.

An outbreak of *Campylobacter jejuni* occurring among children at a kindergarten in northern Italy was investigated in collaboration with Tommi Kärki (EPIET fellow, Cohort 2013). In addition, the fellow performed phenotypic and molecular typing of the strains.

We defined a case as a child with diarrhoea between 11–30 November 2013, attending the kindergarten and having a laboratory-confirmed diagnosis of *Campylobacter jejuni*.

Stool samples from the kitchen staff and environmental samples from the kindergarten kitchen were examined for enteric pathogens. Since food leftovers were not available, the menu logbook of the refectory was reviewed to identify a possible food vehicle. In addition, the food preparation process was revised.

*Campylobacter jejuni* strains were tested for antimicrobial susceptibility and subtyped using Pulsed Field Gel Electrophoresis (PFGE) and multilocus sequence typing (MLST).

We identified 20 cases among 247 children (attack rate = 8%): 70% of the cases were male, median age in years (range: 2–5). Since food leftovers were not available for microbiological investigation, due to a delayed outbreak warning, we reviewed the menu logbook of the refectory to identify a possible food vehicle. All the cases had lunch in the kindergarten. A turkey roast, one of the more common sources of *Campylobacter jejuni*, was served two days before the outbreak started.

Stool samples from the kitchen staff and environmental samples from the kindergarten kitchen were negative for enteric pathogens. There was no apparent lack of hygiene measures in food preparation.

<sup>1</sup> <http://ecdc.europa.eu/en/publications/Publications/microbiology-public-health-training-programme.pdf>

The *Campylobacter jejuni* strains (n=5) examined were sensitive to all the antimicrobials tested and with Pulsed Field Gel Electrophoresis (PFGE) four of them showed an identical PFGE profile, while the fifth strain showed a PFGE pattern similarity of 89%.

Using multilocus sequence typing (MLST) all the five strains were assigned to a single sequence type (ST), ST451 (clonal complex, CC21), an ST very frequently reported in poultry. The assignment of the outbreak strains to a clonal complex/ST mainly found in poultry supports the hypothesis of turkey roast, served two days before the occurrence of the first cases, as a possible source. The hypothesis is also supported by the usual incubation period of *Campylobacter* spp.: two to five days, with a range of 1–10 days. However due to delayed investigation no microbiological information was available on the turkey batch.

Our study confirms the importance of molecular typing and the need for a timely start in outbreak investigations. We recommended that pediatricians and general practitioners should ask local public health authorities to start investigations as soon as possible and implement control and preventive measures rapidly.

### **B. Outbreak of *Ralstonia mannitolilytica* in an oncological day ward, September 2013–June 2014, Rome, Italy**

Project supervisors: Dr Annalisa Pantosti, Dr Loredana Ingrosso

Several outbreaks have been described as caused by disinfectant and solutions contaminated by *Ralstonia* spp. this occurrence may be easily explained by their ability to survive in different hospital disinfectants and to pass through 0.2- $\mu$ m filters. Furthermore, cases have frequently been described of oncological patients bearing central venous catheters (CVC) being infected with these bacteria.

An outbreak caused by *Ralstonia mannitolilytica*, occurred in oncological patients in a hospital in Rome. An outbreak investigation including epidemiological, microbiological and environmental investigations was conducted in order to identify the vehicle of transmission and prevent further cases. In addition, further characterisation of the strains was performed. The fellow participated in the outbreak investigation, contributing to the relevant stages of outbreak investigation, and carried out phenotypic and molecular typing of the strains.

A case was defined as an oncology outpatient attending the day ward from September 2013 to June 2014 with positive blood and/or central venous catheter (CVC) culture for *Ralstonia* spp. We reviewed medical records of the cases and investigated the medical procedures to identify manoeuvres possibly involved in the dissemination of infectivity. We undertook environmental investigations, including surface swabs from therapy rooms, surface swabs from drug preparation rooms, samples from liquid soaps in use and tap aerators in the day ward. *Ralstonia* spp. was identified by 16S rRNA sequencing, typed by Pulsed Field Gel Electrophoresis (PFGE) with *SpeI*, biofilm production was evaluated by the BioFilm Ring Test; resistance to carbapenemes was investigated by phenotypic and molecular methods.

All patients had had CVC flushed with saline before receiving chemotherapy or to retain previous venous access. A review of the medical procedures revealed that between August 2013 and September 2013, multiple-dose bottles of saline solution were in use for CVC flushing but they were no longer available for testing. Environmental samples were negative for *Ralstonia mannitolilytica*.

All the isolates had an indistinguishable PFGE profile, while unrelated strains showed a different PFGE profile, demonstrating the discriminatory power of the technique for this species.

All the isolates were also weak biofilm producers and showed resistance to carbapenems but were negative for carbapenemase genes.

Although the culprit is still unidentified, CVC colonisation following flushing with contaminated saline solution is likely to have caused the clonal outbreak of *Ralstonia mannitolilytica* in oncology patients. This common environmental pathogen may represent a new threat to vulnerable patients, given the unexpected mode of transmission and unusual carbapenem resistance. We recommend paying attention to previously unforeseen sources of infection, use of single dose solutions whenever possible and the introduction of regular saline batch quality checks.

### **C. Training modules**

The EPIET/EUPHEM introductory course familiarised participants with the logistical and analytical approach to outbreak investigations. The module 'Computer tools in outbreak investigations' taught essential data management skills (data entry, validation and cleaning) and key epidemiological techniques using appropriate software packages. The fellow also attended a workshop on strategies for investigating outbreaks in healthcare settings in Warsaw, Poland.

Educational outcome: Participation in outbreak control meetings and teleconferences, risk assessments, infection control assessments, involvement in outbreak investigations (case definitions, active case finding, data collection, data analysis, on-site visits), implementation of prevention measures, use of molecular typing techniques in an outbreak scenario, writing of reports and scientific articles.

## 1.2. Surveillance

### *A. ECDC project to determine the sensitivity of the European Union 2012 case definition for hepatitis B and C*

Project supervisors: Dr Erika Duffell, Dr Aftab Jasir

Viral hepatitis B and C infections are recognised as a public health problem worldwide and it is estimated that up to 520 million people are chronically infected, while every year 1.5 million people die as a consequence of these diseases. In 2011, ECDC rolled out an enhanced surveillance programme for acute and chronic hepatitis B and C across the EU/EEA and this programme included new case definitions that proposed to differentiate between acute and chronic cases.

The implementation of this programme has highlighted considerable difficulties in sorting cases as acute or chronic. In fact, many cases are reported as 'unknown', particularly with regard to hepatitis C. Acute hepatitis B and C are difficult to diagnose clinically and serologically as they are largely asymptomatic. In addition, due to the use of different case definitions among EU countries, it is difficult to compare the surveillance data collected on these diseases. The aim of this project was to validate the EU 2012 case definitions for hepatitis B (hepatitis B virus-HBV) and hepatitis C (hepatitis C virus-HCV) to determine their sensitivity in identifying acute and chronic hepatitis B and C. The EU 2012 case definitions were applied to clinical records and analysed to estimate their sensitivity in identifying acute and chronic hepatitis B and C. Several hospitals and clinical centres in Germany and Italy were selected in order to review clinical and laboratory records available for patients with a diagnosis of acute/chronic hepatitis B and C. Germany is a country with low HBV prevalence. A recent population-based survey revealed an anti-HBc antibody prevalence of 5.1% in the general adult population due to former infection, and 0.3% (95% CI [0.2; 0.6]) show signs of current infection or carrier status. In 2013, a total of 1 947 hepatitis B cases were reported in Germany. Of these, 691 (35%) cases corresponded to the reference case definition. The incidence of hepatitis B in Germany in 2013 was 0.8 cases per 100 000 population. A local clinical centre – University Hospital of Leipzig – was selected to sample acute hepatitis B cases. According to the German case definition, cases were defined as acute when registered under code B16.9 (ICD10) in local clinical databases. The fellow was given full access to all clinical and laboratory records in order to collect data. UKL has approximately 15 cases of acute hepatitis B per year. An approximate sample size of 100–150 cases was collected by analysing medical records between January 2004 and December 2014. Clinical, serological and biochemical data from these records were analysed.

In total, 294 patient disease histories were validated for the period 2004–2014. These patients had been diagnosed with acute hepatitis B and treated at the University Hospital of Leipzig. In total, 118 followed German case definition and could be used for further analysis, 105 were detected elsewhere without comprehensive data and 71 had either chronic hepatitis B or other liver disease.

In Italy, two clinics in Lazio region were enrolled. The fellow collected clinical data for chronic cases of hepatitis C diagnosed from 2014 onwards until she had a total of 130 cases for each clinic. Data were also collected for all the acute cases of hepatitis C available.

To estimate the sensitivity of EU 2012 case definitions, the diagnoses made at the selected clinical centres were used as a gold standard. All data with further analysis were used to create a comprehensive report for ECDC, combining results collected by two EUPHEM fellows, in Germany and Italy. The data will enable ECDC to decide whether to keep or modify the current case definition for acute and chronic hepatitis B and C.

### *B. Surveillance of malaria imported case in Italy, 2011–2013*

Project supervisors: Dr Carlo Severini, Dr Daniela Boccolini

In Italy malaria is a mandatory reportable disease and its surveillance is essential due to the presence and abundance of malaria vectors. The cases are notified to the Ministry of Health and laboratory confirmed by the Istituto Superiore di Sanità. The aim of this study was to identify the main affected categories in order to plan and/or implement information campaigns specifically directed at risk categories by analysing national data on malaria cases reported during the years 2011–2013. The fellow contributed to data digitalisation and analysis.

Malaria case reports, comprehensive demographic information, country visited, reason for travelling, prophylaxis information, clinical and microscopic diagnosis were analysed.

A total of 1 958 malaria cases were reported, about 652 cases per year; 71.4% of cases occurred in males and 44.1% were in the 25–44 year age group. No deaths were reported during the study period. All the cases were imported except for a probable autochthonous case (*P. vivax*, 2011) and a transmitted blood transfusion case (*P. malariae*, 2013).

In total, 18.7% of cases occurred in Italian travellers and the most common reason for travel was work (40.3%), followed by tourism (23.1%) and voluntary work (22.5%). Overall, 81.3% of cases were foreigners (born outside Italy), of which 82.1% were visiting friends and relatives (VFRs) and 11.2% were refugees. In all, 21.6% of cases claimed to have taken chemoprophylaxis: 44.7% of whom had not completed chemoprophylaxis.

The predominant species was *P. falciparum* (84.6%), followed by *P. vivax* (10.4%), *P. ovale* (2.9%), *P. malariae* (1.7 %) and mixed infections (0.4%). Most of infections were acquired in Africa (92%), followed by Asia (7.2%), South-Central America (0.6%) and Papua New Guinea (0.1%).

Our study showed a stable trend in malaria cases during the period 2011–2013, confirming the trend of the last five years, and a still scarce compliance of travellers with requirement to take chemoprophylaxis. We recommend specific information campaigns to educate travellers, in particular VFRs, about the need for prophylaxis and other protective measures when visiting malaria endemic countries.

### C. Training modules

The EPIET/EUPHEM introductory course familiarised participants with the basic approach to developing and evaluating surveillance systems; analysis of surveillance data.

**Educational outcome:** Writing a study protocol/scientific article. Collaboration with clinicians and analysis of medical records; analysis of national epidemiological and laboratory-based surveillance systems; formulation of specific public health recommendations and contribution to national/EU surveillance strategies and policy.

## 2. Applied public health microbiology research

### A. High prevalence of anti-hepatitis E virus antibodies among blood donors in Central Italy, February–March 2014

Project supervisors: Dr Enea Spada, Dr Patrizio Pezzotti

Hepatitis E virus (HEV) is emerging as an under-diagnosed disease in industrialised countries, with many sub-clinical infections. The clinical disease mainly affects middle-aged and elderly men. In Italy, an HEV diagnostic test is not routinely performed and previous seroprevalence studies suggested a prevalence from 1–6%. In order to estimate the prevalence of HEV antibodies and evaluate potential exposures, a cross-sectional study was carried out among blood donors from L'Aquila province, in central Italy. The fellow participated in the study design, elaborated the questionnaire and performed the analysis.

In February–March 2014, we carried out a cross-sectional study among blood donors attending the blood transfusion unit in L'Aquila. We analysed sera for anti HEV IgG and anti HEV IgM using ELISA. All the sera were also tested for HEV RNA using NAT: where samples were positive, RNA was sequenced. All the blood donors were interviewed. Participants were asked to fill in a questionnaire collecting information on demographics and putative risk factors for HEV infection (professional and recreational activities, contact with domestic or wild animals, eating habits and travel history).

The data collected were analysed by means of univariate and multivariable analysis, estimating prevalence rate ratios (PRR) by binomial regression.

Between February and March 2014, 327 blood donors attended the blood transfusion unit, of whom 313 (80.5% male; age 18–68 years, median 48 years) were suitable for donation and completed the questionnaire. Almost all were Italian (98.7%) and resided in Abruzzo (98.4%), where 83.7% of donors were also born.

The overall anti-HEV IgG prevalence was 48.9% (153/313; 95% CI, 0.43-0.54). The prevalence was higher, though not significantly, in males ( $p=0.24$ ). A quite high anti-HEV IgG prevalence ( $> 35\%$ ) was observed in all age groups, without significant differences among them ( $p=0.19$ ). However, the prevalence increased with age and people over 55 years showed the highest rate.

Univariate analysis resulted in several potentially-at-risk exposures having an association with anti-HEV IgG positivity, with a  $p$ -value of less than 0.2: birth in a rural area; contact with dogs; home vegetable gardening and eating raw dried pig-liver sausage. Multivariate analysis, also adjusting for age and sex and only eating raw dried pig-liver sausage resulted in independent association with anti-HEV IgG positivity (Adjusted Prevalence Rate Ratio=2.35, 95% CI: 1.36-4.08,  $p=0.007$ ).

Anti-HEV IgM and HEV RNA were detected in two donors, each of which were asymptomatic, without alanine aminotransferase (ALT) abnormalities. Of two HEV RNA positive donors, one was anti-HEV IgG and IgM positive, the other was anti-HEV IgG and IgM negative. Both donors were carrying HEV genotype 3, a ubiquitous genotype also found in domestic and wild mammals, such as pig, wild boar, deer and rodents.

The high prevalence detected in healthy donors indicates that further studies are required at national level of the incidence of the disease, HEV infectivity and transmissibility by blood products, before considering implementing HEV NAT in blood donors. We recommend an information campaign, targeting those categories at risk, the elderly and immunocompromised patients, to avoid consumption of raw dried pork liver sausage in order to minimise the risk of infection and disease. In addition we recommended HEV RNA detection for IgM and IgG positive blood donors in order to exclude risk of transmission to recipients of blood.

## B. Training modules

While the EPIET/EUPHEM introductory course focused on the development and presentation of study protocols, the module 'Computer tools in outbreak investigation' taught essential data management skills (entering, validating and cleansing data) and dataset management. The module 'Multivariable analysis' focussed on understanding the principles of multivariable analysis and the identification of the type of multivariable analysis that is adapted to a specific study design.

**Educational outcome:** Preparation of study protocols; design of data collection forms; understanding of sensitivity and specificity of molecular diagnostic methods relative to HEV; sero-epidemiological analysis; using STATA software for data analysis; presentations and article writing.

## 3. Applied public health microbiology and laboratory investigations

### A. Capsular sequence typing for non-viable *Streptococcus pneumoniae*: prevalent serotypes circulating in Italy in the 1980s

Project supervisors: Dr Romina Camilli, Dr Annalisa Pantosti

More than 90 serotypes of *Streptococcus pneumoniae* have been recognised to date and the gold standard for streptococcal serotyping is the Quellung reaction, a technique that is expensive, time-consuming, requires a certain technical expertise and is applicable only to viable strains. In recent years a number of molecular approaches have been investigated as alternative serotyping methods. As part of a European project aimed at the standardisation and harmonisation of *S. pneumoniae* typing methods, a new method known as Capsular Sequence Typing (CST) has been developed at European laboratories participating in the surveillance of invasive bacterial diseases (EU-IBD).

The aim of this study was to assess the feasibility and usefulness of CST by testing a collection of lyophilized but non-viable invasive isolates, obtained in Italy during the 1980s from children and adults. The fellow carried out the study design and performed DNA extraction, PCRs and sequences analysis.

The method was applied to DNAs extracted from 88 non-viable strains from 1980s. If the method indicated multiple serotypes for a strain, a serotype specific PCR was carried out.

Among the 88 non-viable invasive isolates, a single serotype was identified for 62.5% of isolates while for 5.7% of isolates only the serogroup was obtained. For the remaining isolates multiple serotypes were indicated and single serotype/serogroup determination was obtained by type-specific PCR except for 1 isolates. In total, 25 serotypes were identified, the most prevalent being: 3 (17.9%), 14 (16.7%), 19A (6.4%), 4 (5.1%) 8 (5.1%) and 22A/22F (5.1%). 41.4% of the strains belonged to serotypes not currently included in the PCV13 vaccine.

The study demonstrated that the method was successful in serotype determination of non-viable strains, revealing a proportion of non-PCV13 serotypes that were circulating in the pre-vaccine era in Italy.

CST seems a promising technique for pneumococcal serotyping on non-viable specimens, even if in some cases an additional PCR is required to obtain the single serotype/serogroup. Compared with multiplex PCR, it has the advantage of being cheaper and faster. In addition it could be used by labs with the modest capability for conventional PCR to perform quite comprehensive capsular serotype surveillance of pneumococci isolates and to identify pneumococci and serotypes within certain clinical specimens such as cerebrospinal fluid, and nasopharyngeal aspirate.

## B. Comparison of the performance of an in-house WNV IgG ELISA with a standard commercial ELISA kit.

Project supervisors: Dr Giulietta Venturi, Dr Giovanni Faggioni

West Nile virus (WNV) is continuously spreading across Europe, with the number of cases in Europe increasing from 24 in 2008 to 232 in 2012 and different outbreaks has been reported in Italy.

Most infected humans remain symptoms-free, but in a minority of cases the infection can develop into a neuroinvasive form, causing life-threatening encephalitis and threatening meningitis. An early, specific, and accurate diagnosis of WNV infection is important, particularly in regions where multiple flaviviruses co-circulate.

The current diagnostic methods for WNV infection include serological tests, viral isolation, virus nucleic acids and infected cells detection. Serological tests, including detection of specific antibodies and virus proteins, are considered specific and convenient. Commercial ELISA kits based on IgM and IgG antibodies detection are commonly used for diagnosis, as well as for the performance of seroprevalence studies due to the ease of use and because they enable a large number of samples to be analysed. Seroprevalence studies are essential in order to monitor the diffusion of the WNV.

The aim of the project was to compare the performance of an in-house WNV IgG ELISA with a standard commercial ELISA kit. If the in-house kit will produce results comparable to that of the commercial ELISA kit, it could be used to provide a cheaper, timely, and accurate diagnosis of WNV. In addition it could be used, with a substantial cost decrease, for seroprevalence studies. The fellow participated in the study design, performed the in-house WNV IgG ELISA and analysed the results.

A panel of 60 sera collected among healthy subjects, randomly selected, residing in a Bolivian rural area, reflecting the total adult population with respect to age and sex, were tested with the in-house WNV IgG ELISA. The sera included were West Nile virus positive, negative and 'false positive' samples (Dengue positive), formerly tested for anti Dengue and anti WNV IgG antibodies using commercially available ELISA kits. The positive samples, to further confirm diagnosis, were subsequently tested using plaques reduction neutralisation tests (PRNTs), both for WNV and Dengue virus.

The in-house ELISA assay showed 63.6% sensitivity and 53.1% specificity versus a commercial ELISA kit and 64.3% sensitivity and 63.2% specificity versus PNRT.

The low specificity of this ELISA test is probably due to cross reaction with other flavivirus. Further studies are needed to improve the test in order to use it for diagnosis and seroprevalence studies. This studies are essential in order to monitor the diffusion of the WNV and subsequently decide on public health action, such as screening of blood donors for WNV and vector control campaign.

**Educational outcome:** Application of molecular identification methods and comparison with phenotypic methods; understanding limitations of molecular methods; application of laboratory methods to detect immunological evidence of past infection; writing of a scientific article.

## C. Training activity in the NRL for malaria diagnosis, Istituto Superiore di Sanita (ISS), Italy

Supervisors: Dr Daniela Boccolini, Dr Michela Menegon

To gain some background knowledge for the project on surveillance of malaria imported cases. This training course provided practical insights into the identification of plasmodium species using microscopy, rapid diagnostic test and molecular methods.

## 4. Biorisk management

### A. Theoretical and practical biosafety level 3 (BSL3) training, Istituto Superiore di Sanità, Italy

Project supervisors: Dr Lanfranco Fattorini, Dr Alessandro Mustazzolu

The training consisted of a five-day theoretical part and a practical part and took place at the microbiology BSL3 laboratory of Istituto Superiore di Sanità. The course included a comprehensive overview of local and international health and safety regulations as they apply to laboratory work with infectious organisms, theoretical and practical training in biosafety engineering, safety checks, audits and techniques for working with hazard group 3 organisms.

## B. Training modules

The EUPHEM module 'Biorisk management' provided training on techniques for biorisk/biosafety assessment and mitigation, including WHO recommendations on biosafety management in laboratories. Formal assessment and certification was also provided for international regulations on the transport of dangerous goods, as prescribed by the International Civil Aviation Organization (ICAO).

**Educational outcome:** Understand processes associated with BSL3/BSL4 laboratories; experience different personal protective equipment; understand and experience the principles and practices of biorisk management; biorisk assessment and biorisk mitigation. Practice appropriate methods for the safe transportation of diagnostic, hazardous substances and pathogenic specimens.

## 5. Quality management

### A. Participation in the external audit of the European Union Reference Laboratory for Parasites

Supervisors: Dr Patrizia Rossi, Dr Alessia Possente

The European Union Reference Laboratory for Parasites at ISS, is currently accredited under EN ISO 17025:2005 standard. The fellow participated, as an observer, in a two-day external accreditation audit. This will enable the fellow to implement similar processes in other laboratories.

### B. Evaluation of the EQA performance of the Italian laboratories participating in the EARS-NET during the period 2009–2013

Project supervisor: Dr Annalisa Pantosti

External quality assessments (EQA) are of major importance to evaluate and assure the diagnostic quality of microbiological laboratories. Italy participated in the European Antimicrobial Resistance Surveillance Network (EARS-Net) through a national surveillance system, AR-ISS (Antibiotico Resistenza-Istituto Superiore di Sanità), coordinated by the Istituto Superiore di Sanità, and based on the participation of approximately 30–40 sentinel hospital laboratories, from different areas of the country. Most of the sentinel laboratories participate in the EQA proposed by EARS-Net.

The aim of this project was to evaluate the EQA performance of the Italian laboratories participating, during the period 2009–2013, in the EARS-NET for bacterial identification and antimicrobial susceptibility test of the seven bacterial species included in the surveillance and compare it with the European results. The fellow drew up the study design and performed the data analysis.

During this five-year period, we observed an important change in the type of guidelines used. In 2009, 93% of laboratories used CLSI guidelines but over the years a high percentage of laboratories started to use EUCAST guidelines, with the percentage reaching 90% in 2013. With regard to bacterial identification, a match of 98–100% was registered among the participating laboratories, except for *Enterococcus faecium/faecalis*, in which 11% and 4% of laboratories respectively determined only the genus in 2010 and 2011.

With regard to the antimicrobial susceptibility test, there were no systematic errors but most of the errors were due to particular phenotypes that borderline MIC values. In addition, some categorisation mistakes were observed, possibly caused by outdated automatic systems.

In general, the results obtained by Italian laboratories matched the results reported by other European laboratories.

A continuous update of automatic diagnostic systems was recommended in order to avoid the incorrect categorisation of results.

## C. Training modules

The EUPHEM two-day 'Quality Management' module provided an overview of quality management concepts in diagnostic laboratories, according to the ISO 15189 standard. Topics covered included factors influencing quality in laboratories, internal and external quality control, norms and accreditation, assessments and audits, documentation and record keeping, sample management, stock purchase and inventory management, management of equipment and temperature-controlled devices, process improvement, customer service and international health regulations.

**Educational outcome:** Understand the principles and practice of quality assurance; analyse the results from an external quality assessment exercise; contribute to an external accreditation audit; understand local and European accreditation procedures.

## 6. Teaching and pedagogy

### Infectious diseases module on the course 'Health Systems through Conflict and Recovery (HSCR)', Scuola Superiore di Sant'anna, Pisa-April 2015

Together with two EPIET fellows, Flavia Riccardo and Tommi Kärki, the fellow organised a course with lectures and an exercise. Topics of the lectures included: how protracted crises and insecurity act as to put pressure on health systems, impact infectious disease transmission adding to and/or creating public health emergencies and how infectious disease outbreaks can themselves put pressure on health systems, mimicking the type of disruption observed in conflict and post-conflict situations. Examples from the recent Ebola outbreak in West Africa were presented and discussed. The topic of the exercise was how to select indicators for assessing the future impact of the 2014–2015 Ebola outbreak on the Liberian health sector.

**Educational outcome:** Plan and organise lectures; define learning objectives; teach infectious diseases and public health emergencies; develop learning materials.

## 7. Public health microbiology management

### A. Public health microbiology management during outbreak investigation and other projects

Public health microbiology management was an integral component of all projects and activities during the fellowship. This included laboratory management, work flow management, consideration of ethical issues, team building and coordination, time management, working with external collaborators, as well as working in a multidisciplinary team comprising microbiologists, physicians, laboratory technicians, clinical scientists, epidemiologists, statisticians and public health officers.

In all projects the fellow needed to communicate with experts from various disciplines to effectively progress and finish the projects. She learned to plan, coordinate and lead projects. Specifically, during the outbreak investigation she facilitated the contact between the laboratory and the epidemiological investigators of the outbreak control team. During the ECDC hepatitis project she communicated with ECDC experts and EUPHEM colleagues to elaborate the study protocol and with clinicians in order to enrol their clinics in the project and she planned and organized data collection. In the pneumococcus project she planned and organized laboratory work and analyses.

### B. Training modules

A one-week module entitled 'Initial management in public health microbiology' focussed on the understanding of roles and responsibilities in public health management. Topics included the identification of different management styles, team roles and team evolution, the delegation of tasks, time management, communication to special audiences and stress management.

**Educational outcome:** Experience of working in a multidisciplinary public health team; understanding team management; understanding roles and formal responsibilities in public health microbiology; planning, scheduling and organising research projects.

## 8. Communication

### A. Publications

1. Lombardi D, Malaspina S, Strippoli A, Lucarelli C, Luzzi I, Ripabelli G. *Salmonella enterica* serovar Virchow meningitis in a young man in Italy: a case report. J Med Case Rep. 2014 May 6;8:139.
2. García P, Malorny B, Rodicio MR, Stephan R, Hächler H, Guerra B, Lucarelli C. A chromosomal region encoding multidrug-resistance to ASSuT is responsible for the lack of the second-phase flagellar antigen in a widespread clone of *Salmonella enterica* subsp. *enterica* serovar 4,[5],12:i (in final preparation for submission to Front. Microbiol).
3. Lucarelli C†, Spada E†, Taliani G, Chionne P, Madonna E, Marcantonio C, et al. High prevalence of anti-hepatitis E virus antibodies among blood donors in central Italy (submitted to Eurosurveillance).
4. Lucarelli C, Dionisi AM, Trezzi L, Farina C, Passera M, Kärki T, et al. Molecular and epidemiological analysis of an outbreak of *Campylobacter jejuni* in a kindergarten in the north of Italy, November 2013 (submitted to Foodborne Pathogens and Diseases)

5. Lucarelli C, Bracco D, Toma L, Prignano G, Fortunati M, Pelagalli L, et al. An outbreak of *Ralstonia mannitolilytica* in an oncological day ward, September 2013-June 2014, Rome, Italy (in final preparation for submission to hospital of infection)
6. Boccolini D, Lucarelli C, Menegon M, Gradoni L, Pezzotti P, Pompa MG, et al. Surveillance of malaria imported case in Italy, 2011-2013 (in preparation)
7. Errico G, Lucarelli C, D'Ambrosio F, Del Grosso M, Ingrosso L, Camilli R, et al. Capsular sequence typing for non-viable *Streptococcus pneumoniae*: prevalent serotypes circulating in Italy in the 1980s (in preparation).
8. Lucarelli C†, Nikisins S†, Tosti ME, Zimmermann R, Jasir A, Duffell E. Evaluation of the sensitivity of the European Union 2012 case definition for hepatitis B and C (in preparation).

† Contributed equally

## B. Reports

1. Lucarelli C, D'Ambrosio F, Monaco M, Ingrosso L, D'Ancona F, Annalisa Pantosti A and AR-ISS laboratories. 'Evaluation of the EQA performance of the Italian laboratories participating in the AR-ISS during the years 2009–2013' (in final preparation for Rapporti Istituzionali).
2. Lucarelli C. Comparison of the performance of an in-house WNV IgG ELISA with a standard commercial ELISA kit, June 2015.
3. Lucarelli C, Tommi Karki. Outbreak of *Campylobacter jejuni* in a kindergarten in the north of Italy, November 2013. April 2014.

## C. Teaching materials

1. Riccardo F, Kärki T, Lucarelli C. 'Epidemics and healthcare systems' presented at Health Systems through Conflict and Recovery (HSCR)', Scuola Superiore di Sant'anna, Pisa, April 2015.
2. Riccardo F, Kärki T, Lucarelli C. Exercise: Choosing indicators to assess the future impact of the 2014–2015 Ebola outbreak on the Liberian health sector (developed for and delivered at the 'Health Systems through Conflict and Recovery (HSCR)', Scuola Superiore di Sant'anna, Pisa, April 2015).

## D. Conference presentations

1. Lucarelli C, Bracco D, Toma L, Prignano G, Fortunati M, Pelagalli L, et al. An outbreak of *Ralstonia mannitolilytica* in an oncological day ward, September 2013–February 2014, Rome, Italy. Accepted as poster at the 25<sup>th</sup> European Congress of Clinical Microbiology and Infectious Diseases (ECCMID 2015). Copenhagen, Denmark, 25–28 April 2015.\*
2. Lucarelli C, Spada E, Chionne P, Madonna E, Marcantonio C, Pezzotti P, et al. Prevalence of hepatitis E virus infection among blood donors resident in the Apennine mountains, central Italy, 2014. Oral presentation at the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), Stockholm, Sweden, 5–7 November 2014.
3. Lucarelli C, Luzzi I, Trezzi L, Busi L, Passera M, Kärki T, et al. 'An outbreak of *Campylobacter jejuni* in a kindergarten in the north of Italy, November 2013'. Accepted as poster presentation at AMCLI 2014, National Conference of Clinical Microbiologists, Rimini, 4–7 November 2014.
4. Lucarelli C, D'Ambrosio F, Monaco M, Ingrosso L, D'Ancona F, Pantosti A and the laboratories of AR-ISS network. 'Evaluation of the EQA performance of the Italian laboratories participating in the AR-ISS during the years 2009–2013. Accepted as poster at AMCLI 2014, National Conference of Clinical Microbiologists, Rimini, 4–7 November 2014.

\* Participation funded by ISS

## E. Abstracts accepted for presentation at ESCAIDE 2015

1. Ingrosso L, Toma L, Prignano G, Lucarelli C, Fortunati M, García-Fernández A, et al. 'Healthcare infection of *Ralstonia mannitolilytica* in an oncological day ward: characterisation of a new pathogen for vulnerable patients'. Accepted as poster presentation.

## F. Selection of other presentations

1. Lucarelli C. *Ralstonia* spp., an 'old' pathogen among healthcare associated infections: an outbreak in a day hospital oncological unit. Oral presentation delivered during period spent at ECDC, Stockholm, Sweden.

2. Lucarelli C. *Ralstonia* spp. an 'old' pathogen among healthcare-associated infections. *Ralstonia* outbreak in oncological patients with a central venous catheter (CVC) attending the day hospital oncological unit at IRE hospital. Presentation during the meeting of the IRE hospital's committee for the control of healthcare-associated infections, to present and discuss the results obtained. March 2014, IRE, Rome, Italy.
3. Lucarelli C. Seroprevalence of hepatitis E virus among blood donors in a district of central Italy. Oral presentation delivered as a component of the Initial Management in Public Health module, European Centre for Disease Prevention and Control, Stockholm, Sweden, February 2014.

## 9. EPIET/EUPHEM modules attended

- EPIET/EUPHEM introductory course, Spetses, Greece (three weeks)
- Computer tools in outbreak investigations, Robert Koch Institute, Berlin, Germany (one week)
- Initial management in public health microbiology, ECDC, Stockholm, Sweden (one week)
- Biorisk and quality management, ECDC, Stockholm, Sweden (one week)
- Multivariable analysis module, AGES, Vienna, Austria (one week)
- Vaccinology, Public Health England, London, UK (one week)
- Rapid assessment in complex emergencies, National Institute of Public Health, Athens, Greece (one week)
- Project review module, ECDC, Stockholm, Sweden (one week)
- Project review module, Faculdade De Medicina Da Universidade De Lisboa, Hospital de Santa Maria Lisbon, Portugal (one week)

## 10. Other courses

- Ethics in the scientific research: clinical, population and genetic studies, Istituto Superiore di Sanità, Rome, Italy (one week)\*
- Period spent at ECDC, Stockholm, Sweden (four days)
- Workshop on outbreaks in healthcare settings, NIZP-PZH, Warsaw, Poland (two days)

\* Funded by ISS

# Discussion

## A. Coordinator's conclusions

Claudia was the first EUPHEM fellow in Italy. She was a Member State fellow who had been working at ISS on food and waterborne diseases before EUPHEM. The EUPHEM Member State Track pathway is a unique and an excellent opportunity for all Member States to train their own scientists and medical specialists as public health microbiologists and thus strengthen communicable disease surveillance through integrated public health microbiology and epidemiology networks. The added value of the Member State track training is that the trained person remains within the host organisation, contributing to the work of the host organisation and passing on the skills and competencies gained within their institute and beyond. This has been successfully demonstrated in Italy by their first Member State track fellow (as described in this portfolio). It has been possible to strengthen multidisciplinary approaches to epidemiology in outbreak investigations and improve surveillance, while ensuring the validity of diagnostic evidence in projects where epidemiology and microbiology need to go hand in hand, such as the outbreak investigation of *Campylobacter jejuni* and the outbreak of *Ralstonia mannitolilytica*.

During the fellowship Claudia showed a great interest for EU-wide and broad public health microbiology projects. Her work on EARs-Net and European Union IBD projects and the European case definition of hepatitis B and C demonstrate the fellow's contribution to European investments.

Projects involved different professional groups, such as physicians, laboratory technicians, epidemiologists, statisticians, government officials and public health officers, strengthening the fellow's ability to work in a multidisciplinary team.

Activities 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 educational and public health relevant outcome, with results communicated in scientific journals and at conferences.

The EUPHEM coordinator team concludes that the fellow has succeeded in performing all her tasks to a high standard and with a professional attitude. In addition, the fellow demonstrated strong leadership and communication skills.

## B. Supervisor's conclusions

As the first Member State-track fellow in the EUPHEM site at Istituto Superiore di Sanità, Claudia Lucarelli has shown a remarkable capacity to comply with the challenges of a demanding training programme, combining modules, wet-lab research projects and epidemiological investigations in collaboration with EPIET fellows. In this two-year programme Claudia has greatly improved her skills in microbiology, her competencies in public health and, in addition, the capacity to deal with stressful situations. She particularly enjoyed networking with the other fellows and the international exposure which, together with the programme's requirement for multi-tasking and flexibility, have greatly contributed to her professional and personal growth.

In particular, her training was an opportunity for the department to develop a novel cross-sectoral approach to public health, combining microbiology with field epidemiology. The EUPHEM programme is creating a professional competency that has no precedent in Italy. Claudia is the first of a new generation of public health specialists with a deeper understanding of microbiology and epidemiology who will be able to perform public health activities in our country in the context of a broad European collaboration.

## C. Personal conclusions of the fellow

The EUPHEM programme provides an unprecedented and unique opportunity to receive professional training and experience in public health microbiology and the field of communicable diseases. Over the course of the past two years I have had the opportunity to improve my competencies in bacteriology and gain experience in virology and parasitology, topics on which I had never worked before EUPHEM. All these experiences have been gained while taking a very useful 'learning by doing' approach.

The programme also improved my ability to multitask and manage stress and gave me the opportunity to develop my leadership skills by leading and coordinating most of the projects.

In addition, the constant interaction with people with a multidisciplinary background (epidemiologists, statisticians, clinicians, microbiologists) and the modules attended gave me the possibility to experience various aspects of field work and develop a new perspective to my work.

A key objective of the EPIET family of programmes is to improve working relationships between disciplines, especially epidemiology and public health microbiology. Throughout the fellowship, my projects and activities have incorporated both these elements and I have also worked jointly with epidemiology training fellows on two projects.

These experiences have also served to further strengthen interdepartmental relationships in the institute and increase my knowledge of activities at the Istituto Superiore di Sanità.

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