www.gabriel-network.org

February 2014 | Issue 14

Special Report
6th Annual GABRIEL Meeting
10-13 December 2013, Les Pensières, Annecy, France

From 10-13 December 2013, more than 90 researchers, physicians, laboratory diagnostics specialists, university professors, and scientists, from both the public and private sectors, traveled from over 20 countries to gather at the Pensières Conference Center near Annecy, France, for the 6th annual meeting of the GABRIEL Network. It was the occasion to share experiences and discuss the latest scientific advances in the fields of acute respiratory infections, tuberculosis, typhoid, fever biomarkers, meningitis, neglected tropical diseases, emerging pathogens, and influenza surveillance.

Proceedings

In his opening remarks, **Benoît Miribel**, Fondation Mérieux's Director General, reminded participants that one of the foundation's top priorities is supporting scientific research on infectious diseases in developing and emerging countries. He also introduced Fondation Mérieux's and the GABRIEL network's new scientific director, **Prof. Hubert Endtz**.

Dr. Florence Komurian-Pradel, who oversees the GABRIEL network, reviewed its objectives and presented an update on its efforts to strengthen local research capabilities and to develop and evaluate new laboratory tests for the detection and identification of pathogens in developing countries.

A New GABRIEL Member in 2013

The Zaporozhye State Medical University in Ukraine is the newest laboratory to join GABRIEL

Programs conducted with support from GABRIEL span training and technology transfer, collaborative studies with local hospitals on infectious disease monitoring, and support to research in the areas of epidemiology and pathogen surveillance. The AFRICARAMI project was highlighted. Its objective was to strengthen the skills of the scientific community in Africa and foster scientific communication. The training courses received extremely positive feedback for their effectiveness.

A recently published booklet, "Fostering Research Collaboration in the Field of Infectious Diseases", provides a comprehensive overview of GABRIEL's mission and activities.

Program Highlights

Individual Presentations

Session I: Advances in Tuberculosis multidrug resistance, chaired by Dr. Delia Goletti of the INMI, Rome, Italy

TB genotyping in the EPL: main accomplishments in 2013

Jean-Luc Berland, a researcher at the Fondation Mérieux Emerging Pathogens Laboratory (EPL), spoke about the various tuberculosis (TB) diagnostic and genotyping techniques that can be used by GABRIEL laboratories to conduct molecular epidemiological surveys of MDR-TB. Typing the strains of M. tuberculosis is vital to track sources of infection and monitor the prevalence of reinfection, risk factors, and links to virulence. QIAxcel Advanced system, in particular, provides an automated solution allowing easy acquisition of MIRU-VNTR patterns. However, whole genome sequencing was shown to be the ultimate genotyping method for identifying person-to-person transmission in a cluster of TB cases, and also to detect known mutations that confer resistance.

Next generation sequencing genotyping:

case contact transmission in Georgia and patient follow-up in Haiti

Marie Gauthier, a researcher at the Fondation Mérieux EPL, described her current work on TB drug resistance mechanisms and transmission. In a follow-up study carried out on patients in Haiti, whole genome sequencing has been used to detect mutations conferring drug resistance to TB and their evolution during treatment monitoring. A second investigation, a cohort study in Georgia, is attempting to measure the extent to which patients infected with MDR-TB transmit the infection to other household members. Whole genome sequencing has proved to be invaluable for strain genotyping and is superior to conventional methods. It makes it possible to exclude direct transmission between patients while current typing methods such as MIRU-VNTR associated with spoligotyping were not discriminant enough. The results of current research are encouraging and should help national TB programs to better control infections.

The TB situation in Bangladesh, and a prison cohort study

Dr. Sayera Banu, a senior scientist at the Center of Communicable Diseases, International Centre for Diarrhoeal Disease Research, Bangladesh, described the current TB situation in the largest prison in Bangladesh and in the country's general population. She also singled out important MDR-TB diagnosis and management activities that severely lack resources. GeneXpert® is expected to be rolled out soon in 20 districts in the country. Plans are underway to further develop MDR and XDR TB surveillance programs and TB diagnostics. The transmission of TB in urban slums is presently being evaluated. The results of the prison cohort study have yielded data on drug resistance patterns of *M. tuberculosis* and on the risk factors of TB transmission in a prison environment. Future projects are underway to extend the study to other prisons and to scale up the screening, detection, and management of the disease.

An overview of TB in Ukraine: ongoing research projects

Dr. Olga Konakova and **Dr. Nataliia Kolisnyk** are associate professors at the Zaporozhye State Medical University, which has recently become a GABRIEL network member. They described the structure of the health system in Ukraine and, more specifically, the national TB program based on the Stop TB strategy. The incidence, the prevalence, and the mortality rates of TB were presented, as well as the epidemiology of TB in the Zaporozhye region. Most laboratories have inadequate funding and lack modern equipment, but new tools have been recently introduced, such as the BACTEC™ MGIT™ 960, GeneXpert®, and GenoType® MTBDR*plus*. These acquisitions should help laboratories obtain greater information on the genotypic characteristics of M. tuberculosis. The comparison of genomic data with epidemiological data should provide information on tuberculosis risk factors. One

of the major challenges in Ukraine is to get a better insight on the transmission of TB within and beyond households settings, to develop optimized TB control strategies and to improve TB patient treatment.

Trends of TB in the West Department of Haiti: data on resistance testing, typing, epidemiology, and next generation sequencing

Dr. Jean William Pape and **Dr. Oksana Ocheretina** from the GHESKIO Centers in Haiti described the current TB situation in the West Department of Haiti and in the country at large, with specific data on the incidence of TB in the refugee camps and the surrounding slums of Cité de Dieu. The number of TB cases has risen dramatically since 2009, including MDR-TB as more diagnostic tools are now available in the country. The daily administration of MDR-TB DOT (Directly observed therapy) is difficult anywhere in the world because patients are reluctant to take the prescribed drugs that are associated with serious side effects. GHESKIO has developed a comprehensive community MDR-TB DOT plan that includes the use of mobile teams on motorcycles to visit the patients daily, GPS to locate the patient's residence, smartphones to take the patient's photo while taking the drugs. Resistance testing has been carried out over the last four years to establish a resistance profile of MDR-TB strains. The findings should help to better counter the spread of the disease and monitor the impact of public health measures in Haiti. Whole genome sequencing, in fact, appears to be the most promising technique for the future in this regard and is expected to replace current methods within several years.

Molecular TB surveillance in France: what is the next step?

Dr. Wladimir Sougakoff from the medical faculty of the Pitié Salpêtrière hospital in Paris is currently conducting molecular typing of a population of MDR-TB strains in France, and is constructing a dendrogram analysis from the results of his epidemiological and phenotypic investigations. He has succeeded in identifying the main lineages among MDR strains, showing their relationships to the Euro-American and Beijing families. The study also suggests that genotyping by MIRU-VNTR and spoligotyping must be completed by analysis of the drug-susceptibility profiles of the strains and the patients' history. Globally, spoligotyping clearly lacks the discriminatory power needed for epidemiological studies, while MIRU-VNTR is a good phylogeographic marker but lacks discriminatory power for specific lineages and is time consuming and technically demanding. The availability of whole genome sequencing at a reasonable cost offers a high-value alternative for molecular investigation of TB outbreaks, since it is capable of identifying SNPs in a chain of transmission, as well as the mutations that impact the development of drug resistance and the ability of bacteria to transmit and cause disease.

Software package for next generation sequencing data analysis

Dr. François Rechenmann, a senior researcher at Inria and the CEO of Genostar, conducts collaborative research projects with Fondation Mérieux and works in partnership with GABRIEL network laboratories. Genostar has developed a web-based integrated software application for the analysis of information obtained from new genotype sequencing. This application conducts complete analyses, ranging from sequences to metabolic reactions of pathways, both comparatively and differentially. This is especially useful for laboratories who handle large quantities of data from the genomic characterization, not only of *M. tuberculosis*, but of other microbial pathogens as well, such as Salmonella, provided that the genome is stable. Plans are underway to extend the software to the situation in Africa.

Towards a regulatory map of the *M. tuberculosis* genome

Dr. Claudia Sala, a researcher at the Ecole Polytechnique Federale de Lausanne, Switzerland, described the projects she is currently conducting on global transcription regulation in M. tuberculosis. The first concerns the characterization of the distribution of RNA polymerase (RNAP) and NusA throughout the *M. tuberculosis* genome in the exponential and stationary phases of bacterial growth. The data generated resulted in a catalogue of gene expression that will support further applications, including refining the genome annotation with the discovery of new genes as well as generating knowledge and tools for applications in the drug discovery process. Another project concerns targeting virulence in *M. tuberculosis*. Dr. Sala is examining the transcriptional regulation carried out by PhoP and EspR, DNA-binding proteins implicated in virulence with the second being a nucleoid-associated protein. A genome-wide mapping of binding sites should have applications in the discovery of drugs against TB and in the neutralization of virulence factors. Finally, ChIP-seq studies on PhoP dissected virulence networks in *M. tuberculosis*. PhoP controls 20% of *M. tuberculosis* genes and has a specific regulatory function. Understanding the hierarchy of transcription regulators should provide an insight into the molecular basis of attenuation and pathogenesis.

Challenges and new advancements in the diagnosis of TB in children

Dr. Giovanni Delogu, from the Insituto di Microbiologia of the Università Cattholica del Sacre Cuore, Rome, spoke about the burden of TB in children, which is believed to be underestimated and underreported. Childhood TB can develop asymptomatically in 23% of cases and can progress differently from adults. There is a need for early, reliable diagnosis of childhood TB, but the tools are lacking to detect infections. In terms of immunological diagnoses, TST and IGRA do not have high accuracy in predicting TB, and discordant results between the two tests cannot be dismissed as differences in

specificity and sensitivity. QFT-IT performed on children has been useful to detect TB, but its prognostic value is uncertain. Dr. Delogu is presently carrying out a further study on the T-cell response against *M. tuberculosis* antigens in TB-infected children, and the results of these investigations are promising.

Session II: Overview of field research initiatives, chaired by Hubert Endtz and Arnold Monto

Typhoid fever in Bangladesh: from infection to protection

Dr. Firdausi Qadri, Director of the Centre for Vaccine Sciences at the icddr,b, Bangladesh, spoke about the worldwide incidence of typhoid fever with a special focus on the situation in Bangladesh. There is a need to understand the mechanisms of survival of *S. typhi* and *S. paratyphi* within infected humans, as well as the pathogens' novel antigens and virulence factors. The study of the gene expression profile in the host is complicated by the fact that the organisms are present in low numbers in infected blood and that their prokaryotic RNA is generally unstable and undergoes limited polyadenylation. Immunoscreening techniques and immunoproteomic analyses are used to investigate the pathogeneses in typhoid fever. SCOTS, a sensitive RNA analysis method based on cDNA and PCR amplification, has been successfully applied. As for the diagnosis of *S. typhi* and *S. paratyphi* infections, the results of the TPTest used on patients with bacteremia are reliable. Children under two years of age do not respond well to available vaccines. Work is underway to improve the immune response to oral typhoid vaccine in children from 2 to 5 years of age. A surveillance program is currently underway in various areas of Bangladesh to determine the prevalence of enteric fever as well as to determine the antimicrobial sensitivity pattern of major bacterial organisms in hospital surveillance sites.

Multiple modalities to explore typhoid in children: implications for immunization and treatment policies

Dr. Samir Saha, Executive Director of the Child Health Research Foundation in Dhaka, began his talk with an overview of the multicenter hospital surveillance program for invasive bacterial diseases that is being carried out in Bangladesh. The incidence of typhoid fever in urban and rural communities and the distribution of the disease among different age groups in children were measured. Unlike what was expected, young children under the age of 5 are more severely affected. Typhoid has a higher prevalence in an urban setting and this is having a major impact because urbanization in Bangladesh is rapidly increasing. Also, since 1992, there has been a progressive epidemic of microbial resistance to second-line drugs such as Ciprofloxacin. Vaccination is problematic because there is no vaccine for children under 2 years of age. A conjugate vaccine must be developed for this age group, and current research in this area has yielded promising results.

Typhoid diagnostics for measuring disease burden

Dr. Jean-Noël Telles, a researcher at the Fondation Mérieux EPL, spoke about a project funded by the Bill & Melinda Gates Foundation on the development of a molecular assay to detect *S. typhi*, *S.paratyphiA* and *S.spp* directly from blood samples. Specific focus was given to the work being carried out with other research institutions, such as the Pasteur Institute and Fast-track Diagnostics, as well as the in-site evaluation in Bangladesh. Tests in Bangladesh have been conducted on different volumes of blood samples from children with suspected typhoid and controls. The results have been compared with those obtained by blood culture and have been extremely encouraging thus far. Plans are underway to extend the typhoid multi-center study to other countries on other continents.

A general introduction to malaria

Pr. Ogobara Doumbo from the Malaria Research and Training Center in Mali described the changing epidemiology of malaria as a result of bacterial co-infections and adaptations in the interactions between the mosquito, the plasmodium, and the human host. Malaria has had a major impact on the education of school-aged children and on their intellectual development. Two population groups in Mali, the Fulani and the Dogon, are currently being studied to explore possible protection mechanisms against the disease. It has been found that Seasonal Malarial Chemoprotection given at monthly intervals to children is effective. A long forgotten form of malaria caused by Plasmodium vivax has re-emerged as a new threat in Africa. The organism is more virulent, more drug-resistant, and more greatly affects Duffy-negative populations. It is now spreading to sub-Saharan Africa where it was not previously endemic. A whole new generation of malaria vaccines is being developed in an effort to achieve an efficiency rate of 80% and for a duration of at least 12 months.

Fever biomarkers in children living in malarial endemic regions

Dr. Eustache Paramithiotis from Caprion Proteome, Canada, explained the current medical need to improve the diagnosis of overlapping symptoms of childhood ALRI (Acute Lower Respiratory Infection) and malaria in malaria-endemic regions. This is the basis for a collaborative research project with Fondation Mérieux. At present, due to this overlap, malaria cases are overestimated, resulting in the overuse of anti-malarial drugs, which further encourages anti-malarial drug resistance. The objective of Dr. Paramitiotis' current project is to identify blood-borne protein biomarkers that can be used to distinguish the cause of unexplained fever in children under the age of 5, and establish an algorithm for the relationships between biomarkers and disease. Following proteomic analysis on serum samples collected from children in Madagascar, host proteins, whose differential expression are associated with the infections, have been

identified. Candidate biomarkers that can accurately classify the infections have also been found. Further work is in progress and it is hoped that the results can strengthen understanding of the host response to multiple infections and provide the basis for advanced testing applications.

Meningitis in developing countries: new vaccines, new challenges

Dr. Jennifer Moïsi from the Agence de Médecine Préventive, France, presented an overview of the epidemiology of meningitis and a description of its main bacterial etiologies (pneumococcus, Hib, and meningococcus). Polysaccharide vaccines against meningitis-causing pathogens are based on capsular antigens and stimulate the production of B lymphocytes and antibodies. However, these vaccines are not immunogenic in children and therefore not effective for public health programs. Newer vaccines are based on the conjugation of capsular polysaccharides to a carrier protein and induce a T-cell response that enables production of memory B cells, including in infants. Conjugate vaccines are safe and immunogenic, prevent naso-pharyngeal carriage, and generate herd immunity. The introduction of the Hib vaccine in developing countries experienced considerable delays due to the cost of the vaccine and insufficient knowledge of disease burden. In order to overcome these barriers to introduction, the Hib Initiative (funded by the GAVI Alliance) promoted surveillance and research on Hib disease and advocated for Hib vaccine use. Similarly, the PneumoADIP helped accelerate pneumococcal vaccine introduction through coordinated activities including surveillance, advocacy and communication, in addition to vaccine funding. MenAfriVac, a vaccine designed especially for the meningitis belt, was developed by SII with support from the Meningitis Vaccine Project and is currently being rolled out across the region.

Neglected tropical diseases

Dr. Nathalie Strub-Wourgaft from the Drugs for Neglected Diseases Initiative (DNDi), based in Switzerland, spoke about the response to the needs of patients suffering from neglected disease. DNDi is a non-profit organization with the objective to develop 11 to 13 new treatments for some specific neglected diseases by 2018 (having already succeeded for 6). Sleeping sickness, leishmaniasis, Chagas disease, onchocerciasis, and pediatric HIV are among the 49 diseases considered to be neglected. These diseases have been relatively overlooked by research institutions. They affect populations with low visibility and little political voice, cause stigma and discrimination in women and girls, and have a high impact on morbidity and mortality, despite the means available for control and prevention. DNDi works to create regional disease platforms through partnership building, to strengthen research capacities in disease-endemic countries. One of major roles of these platforms is to conduct clinical studies for the research, translation, development, and implementation of specific drugs. Numerous challenges remain. Problematic ethical considerations, the lack

of pre-existing gold standards, the difficult access to vulnerable populations, regulatory barriers, and the search for sustainable funding are among the issues that must be addressed.

Session III: Current status of the pilot multi-center case-control pneumonia study, chaired by Werner Albrich and Ron Dagan

Roadmap of the pilot multi-center case-control pneumonia study

Dr. Valentina Picot, Scientific and Research Advisor at Fondation Mérieux, presented the actual timeline for the Pilot Multi-center Pneumonia Study. The study began the conception phase in mid-2010 and was formally launched at the end of 2010. Today, the study is in the process of closure and is scheduled to end in the second quarter of 2014. Each of the GABRIEL sites taking part in the study are moving forward to meet the four remaining roadmap challenges: ethical and regulatory, laboratory, clinical, and documentary closure. The entire process has proved to be very complex. Processing laboratory specimens is dependent upon the capacities of each individual site. During the course of the study, decisions were made with regards to compliance, informed consent, the planned vs. the actual place of recruitment, specimen custody, inclusion criteria, case-control matching, deviation and underreporting, raw data interpretation, document completeness, recordkeeping, data management systems, etc. All issues for each component necessary for closure are being addressed, including the quality control of all related variables to enable data polling.

Country updates on the pilot multi-center case-control pneumonia study

Local country presentations were given by:

- Dr. Marilda Siqueira, Fiocruz, Brazil
- Dr. Monidarin Chou, Rodolphe Mérieux Laboratory, Cambodia
- Dr. Lili Ren, Christophe Mérieux Laboratory, China
- Dr. Vanessa Rouzier, GHESKIO Centers, Haiti
- Dr. Shally Awasthi, King George's Medical University, India
- Dr. Ashish Bavdekar, KEM Hospital, India
- Prof. Luc Samison, Charles Mérieux Infectiology Center, Madagascar
- Dr. Bréhima Traore, Charles Mérieux Infectiology Center, Mali
- Dr. Graciela Russomando, IICS, Asunción, Paraguay

Data processing and preliminary pooling analysis

Pr. Philippe Vanhems and **Dr.Thomas Bénet** reviewed the objectives of the Pilot Multi-center Case-Control Pneumonia Study:

- to identify the causative bacterial and viral agents of pneumonia in children,
- to assess the predictive values of CRP

and procalcitonin regarding the severity of disease,
- and to assess the factors associated with infection by a specific pathogen.

Available clinical and microbiological data from the participating GABRIEL sites have been merged to create an overall database with cross-validation and quality controls. From this, a flowchart will be built for the clinical description of pneumonia patients, and a comparison of the microbial burden between cases and controls by site, as well as the typology of co-infections. Potential selection bias can be an issue, due to the different process of case and control selection among the sites. Proper interpretation of the results will depend on the quality of the data received from each site. Additional information will be collected from each site via a questionnaire on demographics, patients' socio-economic levels, etc. A final report per country and a pooled descriptive analysis should be ready by 2014.

Novel diagnostics for pneumococcal pneumonia: focus on the urine serotype-specific Assay

Dr. Werner Albrich, a physician at the Kantonsspital, St. Gallen, Switzerland, spoke about the mistaken assumption that S. pneumoniae (the pneumococcus) is disappearing as a major respiratory pathogen. Pneumococci are actually underdiagnosed using traditional methods. In the absence of a specific diagnostic gold standard, a variety of diagnostic assays are nevertheless available. Serological assays (e.g. anti-PsaA) are mainly applicable to research and epidemiological studies. Molecular testing for pneumococcal pneumonia with lytA real-time PCR on blood and nasopharyngeal specimens is more sensitive than blood culture. The immunochromatographic assay for the pneumococcal capsular C- polysaccharide (Binax®) in urine has suboptimal sensitivity, but has good specificity in adults (but not in children). The Luminex technology-based multiplex urinary antigen detection assay (UAD) can simultaneously detect 13 serotypes of *S. pneumoniae* included in the 13-valent Pneumococcal conjugate vaccine (PCV13). Its advantage is that it is cost- and labor-efficient, provides etiologic and serotype information and can be performed in a single well with small sample volumes. UAD has been successfully tested on mainly HIV-negative Dutch and US adults and also on HIV-positive adults with CAP (Community-Acquired Pneumonia) in a current project on a cohort in South Africa. For the PCV13 serotypes, it has much higher sensitivity than the urine Binax and therefore is a very promising assay, particularly if its serotype coverage can be expanded.

Introduction on perspectives: phylodiversity, biobanks, co-infection

Dr. Glaucia Paranhos-Baccalà, Research Director at the Fondation Mérieux EPL, spoke about the creation of a biobank from the human biological material and associated clinical information collected from the Pilot Multi-center Case-Control Pneumonia Study. One arm of this project deals with viral phylodiversity. Its purpose is to determine

the local and global dissemination and the evolution of viruses and establish a profile of diversity through the countries which participated in this study. This is achieved through the monitoring of circulating strains, the comparison of viruses in cases and controls, and the characterization of the molecular evolution of respiratory viruses and their link to virulence. Nasopharyngeal aspirate or swab samples taken from cases and controls in the study are tested by sequencing and subjected to data analysis at different laboratory sites. The second arm of the project deals with co-infections. Nasal aspirate and serum samples collected from mono- and co-infected subjects are tested for synergistic modulators in an attempt to identify biomarkers.

Study of viral and bacterial biodiversity: from next generation sequencing to bioinformatic analyses

Dr. Ana Teresa Ribeiro de Vasconcelos, Research Coordinator at the National Laboratory of Scientific Computation, Brazil, gave a presentation on the metagenomic analysis of nasopharyngeal samples collected from patients with severe respiratory syndromes caused by bacterial and viral co-infections with the flu virus. Following DNA sequencing and assembly using SABIA the results obtained offer a pipeline for different software applications, which will enable a comparative and a taxonomic analysis.

Viral and bacterial interactions in the upper respiratory tract

Jonathan Hoffmann, a PhD student at the Fondation Mérieux EPL, spoke about the worldwide burden of acute respiratory infections, and more specifically, about the symptoms and etiologies of pneumonia. Mixed viral and bacterial respiratory co-infections are very common and represent a major public health burden. The Influenza A virus pandemics of the 20th century (1918, 1957, 1968 and 2009), clearly demonstrated that most fatalities were not due to the viral infection itself but to the secondary bacterial infection, predominantly caused by the pneumococcus. The viral infection facilitates the progression of the bacteria from a commensal organism to a potentially fatal pathogen. The viral infection damages the respiratory tract epithelium, permitting bacteria to adhere, colonize, and invade tissues and the blood stream. The host's innate immune response is strongly deregulated during mixed viral and bacterial infections, probably explaining the acute lung inflammation and disease severity. For example, it has been shown that primary viral infection of leukocytes (monocytes or macrophages) can impact their capacity to engulf and kill secondary bacteria, allowing them to propagate through the body. The innate immune modulation following bacterial and viral infection is being studied at the EPL through an *in vitro* observation of inflammatory signalization pathways, the expression of specific cellular markers, and modulation of host cytokines and chemokines released during mixed infection. Biomarkers will help

assess the severity of mixed infections and help to come up with therapeutic strategies to reduce inflammatory responses.

Emerging respiratory viruses: what's new?

Prof. Ab Osterhaus, head of the Virology Department at the Erasmus MC in Rotterdam, reviewed the history of newly-identified human respiratory viruses over the last 15 years. In our changing and globalizing society, such "new" viruses are appearing with increasing frequency in animals and humans due to a complex mix of predisposing factors. Also, more advanced molecular techniques are available to detect new influenza viruses, many of which are regularly introduced from birds and swine. The new paramyxovirus, hMPV, discovered in 2001, can cause severe respiratory infections in young children, the immunosuppressed, and the elderly. The SARS-CoV, a coronavirus declared as the causative agent of SARS, appeared in 2003, and is being studied in an attempt to clarify its routes of transmission, and to develop diagnostic tools. The novel human coronavirus, MERS-CoV, also termed hCoV-EMC, first reported in 2012, has been identified, and its genome has been characterized. Candidate vaccines against this virus have been developed and are being tested for their immunogenicity. Finally, the epidemiology and the drug-resistant patterns of the various new flu viruses causing seasonal influenza, avian influenza, and pandemic influenza were compared. The key for their control lies in multidisciplinary research and international cooperation.

Detection of MERS-CoV infection among pilgrims with Ili and routine influenza surveillance in Bangladesh

Dr Tahmina Shirin, principal Scientific Officer of the Department of Virology at the IEDCR in Dhaka, presented an update on MERS-CoV infections. The greatest number of cases has appeared in the Middle East. Initiatives to control the disease have been put into place in Bangladesh. In addition, the IEDCR is conducting routine influenza surveillance and has established seasonal variations of outbreaks of circulating strains.

Pneumococcal infection in Bangladesh

Dr. Dilruba Ahmed, Head of Clinical Microbiology at the icddr,b in Dhaka, reviewed the epidemiology of invasive pneumococcal disease (IPD) in Bangladesh, one of the countries where over one half of the world's pneumonia cases occur in children under 5 years of age. The icddr,b is conducting hospital- and community-based studies on IPD in both urban and rural settings. Pneumococcal Etiology Research in Child Health (PERCH) is a multi-country study that is investigating *S. pneumonia* serotype distribution. This should lead to the selection of an appropriate pneumococcal conjugate vaccine for Bangladesh.

Etiological features of the influenza virus H7N9

Dr. Jianwei Wang, Director of the Christophe Mérieux Laboratory in Beijing and Deputy Director of the Institute of Pathogen Biology, Chinese Academy of Medical Sciences, China, spoke about the progress in understanding the etiology of the novel H7N9 avian influenza virus. He described the epidemiology and the geographical distribution of human infections in China. The virus normally affects avian populations, but transmission to humans from poultry has been demonstrated. Closing poultry markets have effectively decreased the incidence of cases. Most patients had severe pneumonia. Mortality can be attributed to ARDS and multiple organ failure. As the infection does not produce symptoms in poultry, monitoring the disease and investigating the evolution of the virus is difficult. There is evidence that the virus undergoes mutations that allow it to infect mammalian hosts, which has been demonstrated in animal models. It is important to monitor the emergence of resistant variants of the virus and evaluate their sensitivity to neuramidase inhibitors. Antiviral resistance appears to be conferred by the NA R292K mutation. A vaccine is being developed, but H7N9 has not extended far enough to merit widespread vaccination.

GABRIEL and the Influenza Network

Dr. Florence Komurian-Pradel, head manager of GABRIEL, spoke about the Global Influenza Hospital Surveillance Network (GIHSN). It was initiated through funding from Sanofi-Pasteur in 2011, and now includes four members: Spain, Turkey, Russia, and France. It operates in partnership with Fondation Mérieux and GABRIEL. The objectives of GIHSN are to document the burden of severe influenza leading to hospitalization, to measure the burden of disease associated with each viral strain, and to evaluate vaccine effectiveness. Each country site is led by its own public institutions and health authorities coordinating a pool of hospitals. Standardized core protocols apply to all the sites, but there is local adaptation based on national priorities and feasibility. GIHSN has provided data to complete SARI surveillance, and this has been important to evaluate the global public health impact of influenza. In 2014, Brazil will start SARI surveillance by involving three hospitals located in Fortaleza (Northeastern part of the country), Rio de Janeiro and Porto Alegre (Southeastern part of the country). Other country sites such as Mali, Laos, and Mongolia, are being considered for membership.

Flu survey in Brazil

Dr. Marilda Siqueira, researcher at FIOCRUZ, Brazil, described the sentinel surveillance program of influenza-like illnesses in Brazil that was launched in 2000 with a total of 59 sentinel units until 2011. This was a heterogeneous system providing restricted information for influenza surveillance and control measures. In 2009, during the influenza pandemic, the lethality rate for SARI was 4.6%. To obtain more comprehensive influenza data in Brazil, the

MoH proposed, in 2012, an improvement on influenza and other respiratory virus surveillance. This system now includes 128 sentinel units for ILI and 74 for SARI.

Influenza cases occur in seasonal patterns at different times of the year depending on regional distribution. Plotting a map of seasonal outbreaks is important to determine the pathogenicity and the virulence of respiratory viruses, so that appropriate therapy for both severe and mild cases can be developed.

The influenza vaccine is offered annually, since 1999, for individuals 60 years or older, health workers and, more recently, for children from 6 months to 2 years old, pregnant and post-partum women and indigenous populations. Vaccination has reached around 85% of the target population. Further studies are needed to evaluate the efficiency of vaccination, and its impact on disease mortality and on the circulation of the various virus strains.

Principles for quality management in scientific research

Dr. Valentina Picot from Fondation Mérieux provided an overview on the principles of quality management required to conduct research in human subjects. She emphasized that research is a systematic and contained process to effectively and truthfully answer the intended research question(s); while handling human subjects and their community in an ethical manner and ensuring their safety. Maintaining quality protects the rights, safety, and welfare of individual subjects and is a safeguard for the accuracy and reliability of the data generated. A system of quality standards must be compliant with research guidelines and regulations.

Essential instruction on quality and ethical standards can be found in the Good Clinical Practice (GCP) guidelines.

Quality initiative

Dr. Nicolas Steenkeste, from Fondation Mérieux, described the Laboratory Quality Management System (LQMS). Its benefits are to ensure accurate and timely diagnosis, reduce costs, provide faster responses to public health events, minimize the wasting of resources, and give laboratories greater confidence in their results. One quality management application is WHO's Laboratory Quality Stepwise Implementation (LQSI), which translates ISO 15189 into practical steps. It is a web-based, freely accessible tool designed to assure the technical competency of testing, implement quality control measures, create traceability, establish a policy cycle with proper management, leadership and planning, and finally, to assist laboratories, regardless of size or location, in meeting international quality standards. Its structure is based on the one used by another quality management program, Global Laboratory Initiative (GLI), which is a stepwise plan to guide TB laboratories to ISO 15989 accreditation. A lot of other tools are available, such as the quality manual from WHO or a e-learning program on quality management in the

biology laboratory available on the Globe website.

Workshop 1: Tools for genome sequencing and database analysis, chaired by Jean-Luc Berland and Ana Teresa R. de Vasconcelos

The purpose of the workshop was to allow participants to exchange their experiences and their views on genome sequencing. About a third of the laboratories represented have the capacity to perform in-house sequencing, and the remaining laboratories rely on subcontractors. All recognize that there is a great need for further training in the interpretation and analysis of data for potential use in clinical applications and the prevention of disease.

Workshop 2: Food-borne diseases in GABRIEL countries chaired by Jaap Wagenaar and Jean William Pape

During this workshop, the participants discussed the burden of specific food-borne diseases in their respective countries. The aim was to identify the capacities for diagnosis of diarrheal illnesses among GABRIEL members, and discuss possibilities for coordinating resources to avoid the overlapping of work. There is a need for greater collaboration between epidemiologists and microbiologists in this area. Information on disease burden in many countries of the world is lacking and GABRIEL needs to move forward to fill this gap.

GABRIEL Research Committee and GABRIEL Steering Committee meetings restitution

Dr. Delia Goletti summarized the points that were discussed at the GABRIEL Research Committee meeting. The committee commented on the significant work accomplished by the GABRIEL network.

Regarding the pneumonia multicentric pilot study, the committee suggested using the biological specimens (biobank) for testing to answer unresolved questions. For example, to know the real impact of the presence of multiple pathogens in the swabs and aspirates, it would be crucial to quantitate those pathogens; moreover, all results should be correlated with biomarker data. Before any formal data analysis is performed, the committee suggested defining the severity of the diseases by scores (University of Genève) and a careful determination of whether patients had received antibiotics or not. They also suggested whole genome sequencing to evaluate the presence of multiple mutations and thus try to discriminate between carriage and disease.

For the tuberculosis projects, the committee suggested exploring the potential of PCR to evaluate *M. tuberculosis* drug resistance. It could be useful for determining the single *M. tuberculosis* drug resistance genes; better defining clinical cohorts (in particular the TB-HIV cohorts) and assessing if contact tracing is needed to reduce the impact of MDR-TB transmission.

To show the public health impact of the research performed by the GABRIEL network, the added value of the new methods discovered or implemented by the network could be assessed in the context of a vaccination campaign. For example, the prevalence of a disease before and after the campaign could be tested to evaluate the benefit of the vaccine. The PCR test for Salmonella developed by the Emerging Pathogens Laboratory could be used in this way.

Dr. Florence Komurian-Pradel summarized the points that were discussed at the GABRIEL Steering Committee meeting that was attended by its seven members: Monidarin Chou (Cambodia), Hubert Endtz (France), Florence Komurian-Pradel (France), Jean William Pape (Haiti), Luc Hervé Samison (Madagascar), Marilda Siquiera (Brazil), and Jianwei Wang (China).

Participants reviewed the three applications for GABRIEL membership in 2014, and two applicants, The International Centre for Diarrheal Disease Research, Bangladesh, and The National Laboratory for Scientific Computing, Brazil, were approved as new members.

It was also decided that there will be no call for applications for 2015, as the GABRIEL network has grown significantly in recent years and it is time for a periodic reevaluation of its strategy with current members before expanding further. In the meantime, the membership agreement is expected to be revised to more clearly distinguish industry members from the other types of members.

Organized by the Fondation Mérieux and sponsored by Fast-track Diagnostics, QIAGEN, bioMérieux, IMACCESS and Luminex