

Special Report 7th Annual GABRIEL Network Meeting

14-17 December 2014, Les Pensières, Annecy, France



From 14 to 17 December 2014 more than 80 researchers, physicians, specialists in laboratory diagnostics, university academicians, and scientists, both from the public and private sectors, arriving from over 20 countries, convened at Les Pensières conference center near Annecy, France, for the 7th annual meeting of the GABRIEL network. It was the occasion to share experiences, present new opportunities for collaboration, review progress in quality assessment, and discuss the latest scientific advances in infectious diseases.

The “GABRIEL Young Scientist Award” was added to this year’s program. This award was created to help boost the career development of young scientists from low- and lower-middle income countries by giving five pre-selected young scientists the opportunity to present their research data. Two winners were selected for the best presentations.

Proceedings

Mr. Benoît Miribel, Director General of Fondation Mérieux, welcomed the participants to the gathering and spoke of Fondation Mérieux's top priority: its continuing support to scientific research on infectious diseases in developing and emerging countries. The continuing success of the GABRIEL network reflects the commitment of each of its members.

Dr. Florence Komurian-Pradel, Manager of the GABRIEL network, reviewed the missions of the GABRIEL network:

- to enhance laboratory capacity for infectious disease surveillance through the transfer of technology,
- to develop and strengthen human resource capacities in member laboratories,
- to conduct multi-center clinical and epidemiological studies on targeted diseases.

She also presented the main activities of the GABRIEL network:

- collaborative research: especially on pneumonia, tuberculosis, and typhoid, through the launch of a working group for each of these three diseases to generate innovative solutions and expand partnerships,
- technology transfer,
- training and knowledge-sharing: in 2014, the GABRIEL education program included a workshop on bioinformatics and genomic data analysis in Dhaka,

Bangladesh, a course in Bioinformatics in Beijing, and training at the Emerging Pathogens Laboratory in Lyon and at the Charles Mérieux Infectiology Center in Laos,

- quality assurance to help GABRIEL member laboratories meet the ISO 15189 accreditation standards.

This past year, member laboratories received an evaluation questionnaire, and the overall results highlighted the strengths of the network: the capacity to conduct research projects, to strengthen the research potential of laboratories with limited resources and know-how, and to provide training and technology transfer. It also identified some weaknesses: the need for increased funding, for better representation of laboratories in Europe, Africa, and North America, and a greater number of multi-center studies and articles published as a network.

The new members of GABRIEL were presented: the International Centre for Diarrhoeal Disease Research Bangladesh (icddr,b), the Institute for Developing Science and Health Initiatives (ideSHi), Bangladesh, and the National Laboratory for Scientific Computing (LABINFO), Brazil.

Program Highlights

Session I: Portfolio of research projects and new collaborative opportunities in GABRIEL, chaired by Luc Samison and Prof Traoré

Etiology of pneumonia with unknown origin, with the integration of NGS

Dr. Jianwei Wang, Director of CML in Beijing, reviewed the progress and challenge to epidemiology of acute respiratory infections together with recent data. He pointed out that the most important unresolved problem is the inability to detect the causative organism of ARIs. Negative results from routine molecular tests and cell culture are indicative of novel, mutant, or unknown pathogens that have evolved or adapted. Next-generation sequencing (NGS) is a powerful tool being used to identify new viruses and mutants. New genomic technologies can provide real-time insights into a pathogen's origin, transmission dynamics, and evolution. This should eventually lead to the development of novel diagnostic techniques and surveillance approaches, and should help develop vaccines and appropriate therapeutic approaches. Genomic surveillance has elucidated, for example, the origin of the Ebola virus and its transmission during the 2014 outbreak. Virome research, now in its infancy, will be extended in the future through various NGS platforms in an attempt to discover the etiology of infections and to possibly find a link between new viruses and the severity of disease.

Molecular epidemiology of human rhinovirus infection in Mongolia

Prof. Pagbajabyn Nymadawa of the Mongolian Academy of Medical Sciences described the current study on the molecular epidemiology of the human rhinovirus (HRV) in Mongolia. Samples collected from patients at the influenza sentinel surveillance sites located throughout the country were screened for viral infections by multiplex real-time PCR. Viral RNA was then extracted from all HRV-positive samples. A phylogenetic analysis was carried out, followed by an analysis to determine the seasonality of HRV, the monthly distribution of HVR types, and the incidence of HRV infections by different age groups over the study period from 2008 to 2013. In summary, HRV was detected in 11% (295/2,689) of the nasal swabs tested. Among 170 HRV strains typed using VP4/VP2 and 5'UTR regions, 80 (47.1%) were HRV-A, 76 (44.7%) - HRV-C, and 14 (8.2%) - HRV-B. In addition, 28.8% of patients with HRV were co-infected with other respiratory viruses. HRV was detected throughout the year with peaks in late summer/autumn.

The molecular behavior of the influenza A virus during and after the pandemic event in Brazil

Mrs. Paola Cristina Resende, from FIOCRUZ, Brazil, reviewed the epidemiology and the seasonality of the influenza A (H1N1) virus between 2009 and 2014, and proceeded to describe the objectives of her study: to investigate the phylogenetic relationship of H1N1 viruses to viral variants in Brazil, to study the viral polymorphisms

associated with increased virulence, and to identify virus resistance markers for the anti-viral drug, Oseltamivir. The investigation included an analysis of geographic clustering, a phylogenetic analysis, whole genome sequencing, and the detection of polymorphism associated with Oseltamivir resistance. The findings of the study revealed that, five years after the pandemic, H1N1 continues to circulate in the Brazilian population, that variants are regularly replaced by new phylogenetic groups over time, and that there are distinct patterns of circulation of the virus in each geographical region. In Brazilian strains of the virus, the mutation G222 of the HA gene is associated with more severe illness. But as many severe cases cannot be explained by these polymorphisms, other considerations, such as genetic host factors, may enter into play.

The Haiti research-based model of international public health collaboration: the GHESKIO Centers

Dr. Jean Willam Pape, Director of the GHESKIO Centers in Haiti, and Professor of Medicine at Weill Cornell Medical College, NY, USA, described the GHESKIO Centers as a model of success for future collaborative public health programs. With its major international partners, Cornell University and Fondation Mérieux, the GHESKIO has marked significant milestones in the last three decades in HIV-related research in Haiti with important worldwide applications. It was also the first to evaluate the impact of primary and secondary isoniazid prophylaxis on the incidence of active TB in dually HIV- and TB-infected populations. GHESKIO has

been on the forefront of post-earthquake relief through the provision of global health services to thousands of displaced persons, including the establishment of an acute care hospital for the injured with rehabilitation services, primary and vocational schools, and the extension of microcredit programs. To address the cholera epidemic, GHESKIO developed a model for cholera prevention and care with the provision of WASH, coupled with the first introduction of oral cholera vaccine. It now studies the epidemiology of HPV infections in Haiti, the interaction of cholera and chikungunya with HIV, carries out research on TB and MD-RTB, and monitors nutrition in women and children and the impact of global health on slum communities. Its actions have resulted in a 2/3 decrease in both national infantile mortality and HIV seroprevalence. GHESKIO Centers is addressing the challenges of better implementing HIV discoveries and of providing global health services to Haitians, thanks to the commitment of the thousands of health workers, MDs, RNs, lab technicians, rural providers, and community health workers it has trained.

National collaborative efforts on enteric and respiratory infections

Dr. Firdausi Qadri, from the icddr,b in Bangladesh, described how the icddr,b has carried out disease surveillance for over 40 years in selected hospitals and field sites. The main thrust of her collaboration with national and international institutes, together with the Institute of Epidemiology and Disease Control Research (IEDCR), the Institute for Developing Science and Health Initiatives

(ideSHi), as well as with the Bangladesh Institute of Tropical and Infectious Diseases (BITID), has been centered on understanding enteric disease prevalence in the country. The aims are to estimate the frequency of hospitalizations due to specific enteric pathogens for future vaccine interventions, and to determine the antimicrobial sensitivity patterns of major bacterial microorganisms in the hospital surveillance sites. Currently, the research and surveillance at icddr,b on respiratory disease burden concern:

- influenza and other viruses (PERCH)
- bacteria and other pathogens (PERCH)
- diagnostics (Erasmus, Sanofi-Pasteur)
- pilot surveillance on pneumonia, diarrhea and febrile illness in children under five (Gates Foundation)
- vaccine studies on flu and pneumococcus
- bacteria and other pathogens in government hospitals in Dhaka (BMRC funds at ideSHi).

The Paraguay experience in research projects

Dr. Rosa Guillén from the Instituto de Investigaciones en Ciencias de la Salud, Paraguay, spoke about the wide range of research activities being conducted in the Molecular Biology department of the IICS, namely:

- the molecular characterization of congenital Chagas disease
- the identification of *Leishmania* species by multiplex PCR
- the genotyping and molecular

epidemiology of the causative agents of viral gastroenteritis

- the detection of the pandemic influenza virus H1N1 in Paraguay
- the phylogenetic analysis of the human adenovirus isolated from children
- the pneumonia multi-centric pilot study
- the detection and multiplex PCR identification of atypical mycobacteria and enterobacteria

In 2014, the Molecular Biology laboratory received an Honorable Mention as part of the 2014 National Science Award of Paraguay in recognition for the excellence of the laboratory's work on pneumonia.

These accomplishments have been made possible thanks to the commitment of the research team and to the valuable support received from international research groups.

Bi-seasonal environmental detection of pathogens in inhabited regions of Tonle Sap Lake, Cambodia

Dr. Monidarin Chou, Director of the Rodolphe Mérieux Laboratory of Cambodia, described the study being carried out on the presence of human pathogens in various environmental matrices of Tonle Sap Lake in dry and wet seasons. The lake provides 60% of the fish for the country and serves as a source of livelihood for one-third of the population. Due to limited hygiene and sanitation infrastructures, inhabitants are continually exposed to infection by microbial pathogens in the environment. In the study, samples were collected from

water, soil, and bivalves in the lake and were analyzed by DFA, PCR, and examined by electron microscopy. The results indicate that pathogens are less prolific during the wet season, suggesting that flooding has a diluting effect on environmental pathogens in the lake. These findings provide evidence for the appropriate health authorities to implement the necessary healthcare measures that will prevent these human pathogens from crossing the environment and inflicting their infections on Cambodians living in the Tonle Sap flood areas.

Further work is necessary to establish the link between the pathogens and the cause of gastrointestinal illnesses.

An update on molecular tests

Mr. Milen Milenkov, researcher at Fondation Mérieux's Emerging Pathogens Laboratory (EPL), presented an overview of the development of molecular methods for the identification of bacteria responsible for meningitis. *Neisseria meningitidis*, in particular, can be identified by Gram stain, blood agar culture, and latex agglutination on CSF and blood samples. These techniques, however, are time-consuming. Molecular methods for the serotyping and identification of *N. meningitidis* are being developed with the use of end-point PCR, real-time PCR, and multiplex PCR-Luminex. Ideally, the aim is to develop assays that yield quick results on the specificity and sensitivity of the pathogen so that clinical treatment can be improved. Work is currently being carried out in collaboration with the EPL in Lyon and the Charles Mérieux Infectiology Center of Madagascar to analyze PCR

results and clinical data.

Detection of *Neisseria meningitidis* and *Streptococcus pneumoniae* by molecular assay in Madagascar

Dr. Mala Rakoto-Andrianarivelo, from the Charles Mérieux Infectiology Center in Antananarivo, gave his talk on the detection of *N. meningitidis*, *S. pneumoniae*, and *H. influenzae b* by the newly-developed PCR method, from CSF specimens of suspected bacterial meningitis cases detected through the surveillance sentinel site of the capital city. The presenter reported that well-prepared and effective planning resulted in the smooth implementation of the new PCR technique. The results were compared with those from culture and emphasized the great advantage of using the PCR method in terms of diagnostic capabilities. During the 2-year study period, no *N. meningitidis* was detected, but a high proportion (17%) of *S. pneumoniae* and a low circulation of *H. influenzae b* were reported among the 294 specimens tested. The absence of *N. meningitidis* should be confirmed by further study. The various serotypes of *S. pneumoniae* and their role in meningitis were also discussed. In conclusion, this study should help provide better healthcare for patients with meningitis, improve response to outbreaks, assess the impact of vaccination, and define and monitor the strategy for disease control. There needs to be continued surveillance of meningitis and a translation of research results into the development of public health policies.

Evaluation of a molecular assay for the diagnosis of typhoid fever in Bangladesh

Dr. Jean-Noël Telles, researcher at Fondation Mérieux's Emerging Pathogens Laboratory, spoke about the study funded by the Bill and Melinda Gates Foundation on the development and validation of a pilot molecular assay for the direct detection and identification of *Salmonella typhi* and *Salmonella paratyphi* A in the blood of patients with suspected enteric fever (typhoid). The final aim is to monitor disease burden in developing countries. The project first consisted in comparing PCR techniques and blood culture used for the identification of *Salmonella* on 5 ml of suspected typhoid blood samples collected from patients at the Sishu hospital in Bangladesh. Further studies were carried out on 3-ml blood samples (n=1,000) gathered at various other collection sites in the country and tested in different laboratories to compare blood culture, PCR, and a third assay. The preliminary results on the first 100 samples showed that the PCR assay yields a 52% increase in the detection rate of *Salmonella* present in blood as compared to blood culture. These results have been extremely encouraging thus far and need to be confirmed on the 1,000 blood samples. The Gates Foundation wishes to broaden the scope of the study in mid-2015 as a multi-center project to include at least three countries in Africa (Kenya, Malawi, Nigeria), and to run a pilot program that should introduce the assay in several Asian countries (Bangladesh, India, Nepal) for surveillance purposes.

Session II: Young scientist presentations, chaired by Werner Albrich and Glaucia Bacçalà

Human papillomavirus infection and cervical cancer in HIV-infected women in Haiti

Dr. Cynthia Rivière, clinical research site coordinator at the GHESKIO Centers, Haiti, presented the objectives of her study: to define the epidemiology of HPV infection and cervical cancer in HIV-infected women, and to evaluate the impact of antiretroviral therapy (ART) on the natural history of HPV infection and related cervical lesions. HIV-infected women, enrolled in a clinical trial comparing early initiation of ART (at a CD4 cell count between 200 and 350 cells/mm³) to delayed initiation of ART (at a CD4 cell count of 200 cells/mm³ or less), were evaluated for HPV infection and high-grade cervical lesions. The progression from HPV infection to high grade lesions according to various high-risk HPV subtypes was also studied. It was found that 11% of HIV-infected women eligible to initiate ART had high-grade cervical lesions. Also, early initiation of ART, when compared to delayed ART, decreased the incidence of cervical HPV, and indicates a trend for a lower persistence of HPV. Finally, early ART may retard the progression of the infection caused by HPV subtypes other than HPV 16 and 18.

Introducing the burden of acute respiratory and enteric infections among children under five: Bangladesh perspective

Mr. Golam Sarower Bhuyan, Research Fellow at the Institute for Developing Science and Health Initiatives (ideSHi), Bangladesh, presented his study, whose aim is to help determine the prevalence of acute respiratory infections (ARI) and enteric infections, identify the etiological agents of these infections, and study the antibiotic-resistance patterns of the isolated pathogens. Blood samples, nasal swabs, and rectal swabs, collected from selected patients at the study site in Dhaka, were tested for the presence of bacterial and viral pathogens by culture methods and real-time PCR techniques, respectively. Antibiotic resistance-sensitivity patterns of isolated pathogens were compared. It was found that the TP (Typhoid-Paratyphoid) test is more specific and more sensitive than the blood culture method, and that it can be used as a quick and reliable method for diagnosing enteric fever. Also, as a matter of concern, antibiotic-resistant *Klebsiella* species are seen to be emerging. It is hoped that, in the future, the early detection of the causative agents of ARI and enteric infections, followed by proper treatment, will reduce the percentage of under-five mortality and morbidity in Bangladesh.

A PCR-based strategy for the diagnosis of chomoblastomycosis and sporotrichosis in Madagascar: a study of 31 cases

Mr. Tahinamandranto Rasamoelina, from the Charles Mérieux Infectiology Center in Antananarivo, began his talk by explaining

the epidemiology of the two subcutaneous fungal infections examined in his study: chomoblastomycosis and sporotrichosis. For each infection, he described the causative agents, the clinical features, the diagnosis, and the treatment. The purpose of his study was to demonstrate that these mycoses are still endemic in Madagascar, and to develop molecular tools to diagnose and identify the causative agents. Samples from 31 infected patients were collected and underwent histopathological and mycological analyses, as well as molecular testing. From the data obtained, the epidemiological and clinical features of the 31 suspected cases were mapped out. Reference strains of the causative agents were characterized and a decisional algorithm was set up through the development and validation of molecular methods of identification. The next step in this research will be to extend the clinical study to include a greater number of patients and to perform an extensive environmental study that will describe the spread of the causative agents in the environment so that steps can be taken to prevent the spread of contamination in Madagascar.

Human papillomavirus (HPV) prevalence and probability of unknown or rare HPV subtypes in Mongolia

Mrs. Binderya Ganzorig, from the “Gyals” medical center, Mongolian Academy of Medical Science, presented the research being conducted to establish the prevalence of HPV in Ulaanbaatar, and to identify a possible regional-specific subtype of the virus. Cervical cell samples were collected from 872 women who were grouped as

being symptomatic for HPV, asymptomatic, or who had cervicitis. Following molecular testing and identification of HPV serotypes, the prevalence of HPV among all the women was determined and correlated to age. The prevalence of high risk and low risk infections according to HPV subtypes was also established. Research is expected to move forward with amplification and sequencing in order to classify the subtypes and the variants of the virus.

Molecular characterization and bioinformatic analyses of viruses detected in children with acute lower respiratory infection in Paraguay

Dr. Emilio Espínola from the Instituto de Investigaciones en Ciencias de la Salud, Paraguay, reviewed the research that has been carried out on the genomic analysis of the influenza virus H1N1pdm09. He also spoke about viruses and bacteria responsible for co-infections in children with ARIs. The adenovirus was specifically studied and new genotypes have been identified. As for rhinoviruses A, B, and C, and the respiratory syncytial viruses A and B, phylogenetic trees were established. This work has given us a model that can be used to predict the severity of disease and, ultimately, create vaccines.

The Young Scientist Award ceremony, hosted by Hubert Endtz, Scientific Director of Fondation Mérieux, and the GABRIEL Research Committee

In his introductory remarks, **Dr. Hubert Endtz** presented GABRIEL's Young Scientist Award as an important initiative for the future of science and an incentive for young investigators. Countries throughout the world are in need of talented scientists who should be recognized as future leaders in the fight against infectious disease.

The two winners of the award were selected on the basis of the value and relevance of their research, and the quality of their presentations.

After careful deliberation, GABRIEL's Research Committee decided to award the two prizes to:

- **Tahinamandranto Rasamoelina** for his work on the diagnostic techniques for two subcutaneous fungal infections in Madagascar,
- **Cynthia Rivière** for her research on human papillomavirus infection and cervical cancer in Haiti.

Mr. Alain Mérieux, President of Fondation Mérieux, gave concluding remarks congratulating the two winners for their outstanding contribution to science.

Session III: The pneumonia study and new perspectives, chaired by Ron Dagan, Samir Saha, and Werner Albrich

Data analysis of the multi-center pneumonia study

Prof. Philippe Vanhems, from the Université Claude Bernard, and Dr. Thomas Benet, from the Hospices Civils of Lyon, spoke about the preliminary pooled analysis of GABRIEL's multi-center case-control study on the etiology of child pneumonia. The objectives of the study are to identify the causative agents of pneumonia and to assess the individual and microbial factors associated with the risk of severe pneumonia. Children under five years of age, representing incident cases (presence of pneumonia) and controls (absence of pneumonia) from the ten participating GABRIEL sites were enrolled in the study, and were classified according to country of origin, median age, gender, PCV vaccination status, and Hib vaccination status. The cases and controls were examined for the presence of antibiotics in urine, clinical manifestations of pneumonia, and presence of pathogens. Patterns of infection based on the results from blood cultures, PCR testing, the level of hypoxemia, and the measure of other variable characteristics were subsequently drawn up. A final descriptive report related to each participating country conclusions is expected to be issued early in 2015. It will fully describe the clinical, radiological, and biological characteristics that were observed in the study populations, as well as the

association between the etiology, the clinical features, and the gravity of the disease. At the end of the presentation, it was agreed that all the participating GABRIEL sites will be able to review the final report before it is released.

Session IV: GABRIEL and links with other networks, chaired by Marilda Siqueira and Souleymane Diallo

Funding initiatives for research and capacity building

Dr. Léticia Lobo Luppi from Fondation Mérieux reported on the full extent of the activities carried out by the members of the GABRIEL network in the area of respiratory infections (namely, pneumonia and tuberculosis) and fevers, food- and water-borne diseases, and neglected diseases. These activities, range from molecular assay development, and epidemiological studies, to research in biomarkers, immunopathogenesis, and antimicrobial resistance. The number of projects that have been submitted and accepted, has risen at least three-fold in the last three years and the amount of funding received has tripled in the last year alone. She also spoke about the consortium, Global Research Collaboration for Infectious Disease Preparedness (GloPID-R), a network of research funders that was launched in 2013. It strives to improve the global research response to a potential outbreak of new or re-emerging infectious diseases through research and policy development during inter-epidemic periods. The GloPID-R secretariat is coordinated by Fondation

Mérieux and the University of Oxford. Its missions include facilitating the exchange of information between funders, connecting existing and future research networks, and addressing scientific, logistical, legal, regulatory, ethical, and financial challenges. Finally, she presented the COMPARE project (Collaborative Management Platform for detection and Analyses of (Re-) emerging and foodborne outbreaks in Europe), a collaboration of institutions with hands-on experience in outbreak detection and response. It serves as a global platform for the sequence-based rapid identification of pathogens.

TDR strategic directions for research capacity strengthening

Dr. Dermot Maher, from the WHO in Switzerland, described the Special Program for Research and Training in Tropical Diseases (TDR), hosted by the WHO, as a program that plays a pivotal role as catalyst, facilitator, and advisor in the global health research debate. It seeks to improve the health and well-being of those burdened by infectious diseases of poverty through research. It fosters an effective global research effort on infectious diseases and promotes the translation of innovation to health impact in disease-endemic countries. Research capacity strengthening activities address issues related to the control of neglected tropical diseases, tuberculosis, and malaria. These activities include IMPACT grants aimed at equipping researchers to address disease control program priorities, and training grants, which are competitively awarded to help nationals from low- and middle-income

countries acquire postgraduate qualifications and training for careers in health research. They also facilitate mentorship and “re-entry” of LMIC researchers in their home countries/institutions to initiate independent research careers. In addition, Career Development Fellowships have been granted to enhance the skills of 42 scientists from 24 countries in the area of clinical trials. TDR supports regional training centers across the globe with a view to disseminate the training courses and materials which are relevant to strengthen capacity in implementation research. TDR’s learning approach combines skill-building with research activities. A global web-based system is under development to link up the TDR grantees, donors, etc., to track careers, evaluate the impact of grants, and to promote collaboration and research cooperation in developing countries.

Towards better Ebola diagnostics

Dr. Viktor Volchkov, from the Human Virology Department of INSERM, France, described the factors that contribute to the high pathogenicity of the Ebola virus (EBOV): the virus’ high efficiency of replication, its pantropic character, its prime targets being immune cells, and its role as an antagonist of innate immunity. The molecular basis of the virus’ high pathogenicity is related to the expression strategy of its glycoprotein (GP) gene. This gene regulates the expression of glycoproteins on the surface of the virus that, in turn, block the host’s virus-neutralizing antibodies and prevent phagocytosis of the virus by the host’s cells. No vaccine or treatment is available for humans against the EBOV. Control is effectively achieved

by isolating infected and suspected-for-infection patients, limiting the circulation of the exposed population, decontaminating waste, providing safe burials, and conducting full case findings. Current research revolves around anti-GP antibodies and their capacity to recognize various forms of GP, soluble GP, and different epitopes. The only diagnostic tools available for Ebola fever are limited to isolation of the EBOV, the detection of host antibodies, the detection of viral antigens, and real-time PCR. Further diagnostic techniques that do not require extensive training must be developed. They must be fast, sensitive, affordable, applicable in low-resource settings, specific, and safe.

Field experience on Ebola Virus Disease

Dr. Christophe Longuet, Medical Director of Fondation Mérieux, spoke about his field experience with the West African Ebola epidemic that currently numbers 18,000 cases and 7,000 deaths. During his mission in Guinea for the opening of the Ebola Treatment Center of Macenta, he participated in the care of people affected by the Ebola virus disease (EVD). In the first three weeks of activity at the Macenta Ebola Treatment Center (ETC), there were 104 admissions, of which 59% were confirmed cases of Ebola. Of these, 51% died. Among the difficulties being faced in the control of the epidemic, there is the adequate and rapid diagnosis of EVD. The clinical algorithm based on the WHO guidelines used to suspect EVD and admit the patients is very sensitive (with a strong negative predictive value), but poorly specific (with inadequate positive predictive value), and a patient with fever isolated in

the ETC may actually have malaria. About one third of the individuals hospitalized in the ETC of Macenta for suspected EVD did not have the Ebola virus disease. RT-PCR, the molecular diagnostics of reference, is a high-performance solution, but requires a high-level biosafety cabinet, and extremely well-trained laboratory professionals. Several hours are required to obtain results. More rapid and simpler diagnostic tests are needed. In Conakry, a new molecular test and a rapid test are about to be evaluated in the viral laboratory at Donka Hospital. If validated, these new tests will lead to faster diagnosis and contribute to the control of the EVD epidemic.

Presentation of the European Virus Archive goes global (EVAg) network

Prof. Jean-Louis Romette, Scientific Coordinator of EVA, presented a new project called EVAg, for European Virus Archive goes Global, which is a consortium of 30 international virus collections. EVAg is a non-profit organization arising from the coordination of European and non-European laboratories. Its mission is to define and establish best practice quality guidelines, improve QMS standards, and guarantee the supply of authenticated and quality-controlled resources to users. End-users include research infrastructures, the pharmaceutical industry, academia, and government agencies. EVAg incorporates extensive expertise in virology and compiles a wide range of infectious human and animal viruses. In the case of the Ebola epidemic, for example, EVA has participated in the West African emergency response with

the deployment of mobile laboratory units. EVAg partners send teams of scientists to support local authorities in the diagnosis of suspect cases. Support includes the development and production of reagents and protocols for molecular and serological detection of emerging pathogens, as well as the validation of protocols for the collection of viruses, genomes, antibodies, antigens, and clinical samples. The EVAg project has established a web-based catalogue to provide an easy and user-friendly access to the viruses in its collection, as well as to virus-derived material. In addition, EVAg can produce associated reagents upon request to laboratories in Europe and worldwide. EVAg's ultimate objective is to develop the largest repository of quality-controlled virus stocks and associated metadata, worldwide.

Session V: Tuberculosis, chaired by Warren Johnson, Delia Goletti, and Stewart Cole

South-East Asia circulating TB strain: a collaborative study

Mrs. Marie Gauthier, from Fondation Mérieux's Emerging Pathogens Laboratory, spoke about a new multi-center project on TB lineages circulating in South-East Asia. The project is to develop approaches for the study of the molecular epidemiology and spread of TB in the Mekong region. It intends to reinforce the connection between National Tuberculosis Control Programs and research institutes, as well as the coordination of activities between Vietnam, Laos, and Cambodia. The project investigates the

distribution of TB lineages and genotypes, and temporal trends in the three countries. The transmission of TB and the level of drug resistance are also being studied, as well as the risk factors associated with the transmission of TB and MDR-TB. In regions of high transmission of the disease, it is vital to design appropriate interventions to halt the spread of TB and MDR-TB. In a first step, the characterization of TB lineages was carried out in the EPL, Lyon, on 224 isolates obtained from a national TB survey in Laos. Preliminary results on 100 isolates show a prevalence of 74% for the EAI lineage and 20% for the Beijing lineage. Spoligotyping remains to be done for additional typing information, and the genes associated with drug resistance are now to be sequenced. The results of this project should ultimately help to discover the correlations between lineages and disease outcome, between lineages and mutations conferring drug resistance, and between lineage prevalence and clustering rate and socio-demographic factors. The findings will be compared with those from Vietnam and Cambodia.

TB in Dhaka prison: from transmission tracing to intervention

Mrs. Marie Gauthier, from the EPL of the Fondation Mérieux, outlined the objectives of the prison TB study: to provide data concerning the rate of TB transmission among the inmates of a prison in Dhaka, compared to the rate of transmission in the population outside the prison, and to trace the routes of TB transmission and risk factors in the Dhaka prison setting. The findings of this study are aimed at providing

evidence of transmission among inmates to help public health authorities to design and to monitor efficient interventions. Samples collected from actual cases were subjected to MIRU-VNTR typing and to whole genome sequencing in order to infer the transmission of TB and detect mutations conferring drug resistance. Results of this investigation indicate that TB in a prison setting is highly prevalent. In a specific 59-prisoner cohort, 8 TB clusters were discovered. For these clusters, it is difficult to track the source as originating from inside or outside the prison, as TB can remain latent and show clinical symptoms after undetermined periods. Additionally, drug-resistance mutations were detected in 5 patients, two of which were MDR-TB cases. None of the resistant strains was included in a cluster. Further work needs to be carried out on the prevalence of drug resistance and associated mutations on a larger sampling.

MDR- and XDR-TB surveillance in Bangladesh

Dr. Sayera Banu, senior scientist at the Tuberculosis and Leprosy Research Group of icddr,b, Bangladesh, reviewed the MDR-TB situation in South-East Asia. The surveillance study being conducted in Bangladesh is designed to assess alternative approaches that can improve the accuracy and speed of diagnosis of MDR-TB among MDR-TB and TB patients hospitalized without complications. The surveillance sites include public and private health facilities located countrywide in both urban and rural settings. Participants were carefully selected for enrollment into the MDR-TB and XDR-

TB studies according to specific inclusion and exclusion criteria. Sputum samples were collected and subjected to culture and molecular testing. Drug resistance was also measured. The results of the study indicate that the rate of MDR-TB is 3% in primary cases and 13.2% in retreatment cases. GeneXpert® proved to be a good marker for MDR-TB. Plans are underway to sequence drug-resistant strains and to further investigate the types of strains that prevail in Bangladesh. This should lead to a better understanding of the transmission dynamics of drug resistance.

Ongoing TB/MDR-TB research activities

Dr. Nestani Tukvadze, head of the research unit at the National Center for Tuberculosis and Lung Diseases (NCTLD) in Tbilisi, Georgia, reviewed the epidemiology of TB in Georgia. Georgia is one among 27 high MDR-TB burden countries and the case detection rate was 78% in 2013. Laboratory-confirmed MDR-TB prevalence was 11.2% among new cases and 38% among retreatment cases. The percentage of XDR-TB among MDR-TB was 20% in 2013, which represented a two-fold increase over the prior year. NCTLD research unit is interested in transnational and operational research. One translational research project was recently conducted to study the effect of high-dose vitamin D in patients with pulmonary tuberculosis in Georgia, as it has been found that vitamin D generates a positive effect on the human immune system (e.g. upregulation of specific anti-microbial peptides). The study was designed as a controlled, randomized, double-blind, intent-to-treat clinical trial that

tests the efficacy and safety of high-dose oral vitamin D3 versus placebo in pulmonary TB patients who were receiving standard TB treatment. The study showed that a high dose of vitamin D was safe to use, although there was no additional efficacy in clearance of *M. tuberculosis* between the vitamin D arm and the placebo arm.

Diagnostic issues of MDR-TB in Ukraine and the Zaporozhye region

Dr. Olga Konakova, associate professor at the Zaporozhye State Medical University, Ukraine, stated that although the incidence of tuberculosis in Ukraine and the Zaporozhye region has been in steady decline since 2002, there is mounting concern for the increasing rate of MDR-TB and XDR-TB in the population, as well as for the increasing rate of TB combined with an HIV infection. In a recent study, samples taken from newly-diagnosed TB patients were subjected to drug susceptibility testing. It was found that 23% were MDR-TB positive. Among TB patients who had been previously treated, the rate was 53.5%, and among those who failed to respond to treatment, 43.5%. In Ukraine, there is an increasing need to develop the capacities of biological laboratories for the early detection of MDR-TB and follow-up of inter-patient transmission. Line Probe Assay technology must be further developed. Despite the complicated economic and political situation in Ukraine at this time, the local healthcare system must strengthen its capacities in the fight against TB.

Working group: the design of a new TB multi-centric study

In order to sustain the TB Working Group to be established in 2015, a workshop was set up to gather GABRIEL members and key opinion leaders. The objective of this workshop was to brainstorm on proposals, to re-inforce horizontal South-South initiatives, trigger and develop innovative ideas and solutions, design new collaborative research projects, and find ways to acquire external funding.

Mr. Jean-Luc Berland, from Fondation Mérieux, led the general discussion with GABRIEL members to generate feedback, and collect expectations from the field, and advice from key opinion leaders. Suggestions from the participants follow:

- We need to work on diagnostic tools - children, point of care - with our ultimate priority in mind: to get patients on appropriate therapy.
- In Bangladesh, the diagnosis of TB is a major concern, especially in children. Cases need to be detected early on. A new treatment regimen needs to be found because injectable drugs can cause side effects, such as hearing loss. This is a problem. We need to find ways for more rapid treatment. Studies have been carried out with the University of Virginia on reducing the duration of therapy with injectable drugs to one or two months. A trial on 9-month short treatment of MDR-TB (led by Fondation Damien) has been ongoing, but it has not yet been endorsed by NTP and WHO. However, we lack the techniques to ascertain whether the

patient is subsequently still infectious or definitely cured. The transmission study in Bangladeshi prisons is of value: it is very important to give clear evidence to NTP so that it can put a national program into place.

- All MDR-TB patients cannot be hospitalized in Bangladesh before being tested by culture that may be negative. Also, injectable drugs can have very undesirable side effects. Does the duration of treatment need to be that long?
- We need tools to diagnose TB latency, as they are not generally available. However, in high burden regions more than 50% of population may be infected by TB. Preventive treatment regimen for MDR contacts (latent TB) has not yet been endorsed by WHO.
- In Georgia and in Central Asia, the policy is quite different. TB patients are put into isolation for several months, but the exact diagnosis that prompts this measure is not conclusive.
- In Ukraine, the principle concern has to do with the movement of people from the eastern to the western part of the country. We are seeing increasing rates of TB in the west and decreasing rates in the east. The high rate of transmission, the lack of disease control, and the greater numbers of MDR cases are huge problems. Proper diagnosis and epidemiological indicators are important in this regard.
- In Haiti, we provide same day diagnosis with smears, X-rays, clinical observations, and GeneXpert system. All have proven to be extremely useful, as 30% of the people who come in GHESKIO with a cough have active TB. WHO, TB Reach, as well as TDR, should be interested in this approach.
- In Madagascar, we are ready to take part in the multi-centric TB study. In addition, apart from TB, other infectious diseases, such as leprosy, are of concern. Individuals may have been exposed to leprosy and may be carrying a latent form of the disease.
- To what extent is it really necessary to hospitalize TB patients? We need to investigate the transmission of TB in ambulatory patients.
- We need to share our views on the development of biomarkers that can identify active and asymptomatic TB. Treatment must be shortened. We should investigate the presence of chemokines that are useful for diagnosis.
- We would like to know to what extent a TB patient transmits the disease once therapy has been administered. We need more rapid diagnostic tests.
- An evaluation with GeneXpert appears to be the best: GHESKIO experience: One GeneXpert is better than three sputum AFBs. We should eventually move away from smears.
- Results from GeneXpert can be very rapid, but the problem in rural, outlying areas is that communicating the results to the physician may take weeks due to a lack of transportation and reliable infrastructures.

- We are very interested in comparing the consistency of results obtained from different molecular methods.
- One dogma says “TB must be treated for 6 months”. Patient management is easier with standardized treatment but what is the real efficiency? Also, drugs cause side effects and many patients are cured before 6 months. Reducing the duration of treatment would improve outcome and treatment withdrawal rate. More longitudinal work must be done. Establish cohorts to monitor outcome and/or latent TB over time. There is much to do: immunoassays, and not only on blood, cytokines and chemokines are described in saliva, sputum, urine.

Dr. Florence Komurian-Pradel concluded by saying that GABRIEL members will be contacted at the beginning of 2015 for further discussion on this subject. The members of this working group must be actively engaged.

Session VI: Quality Initiative, chaired by Antoine Pierson and Asadulghani

The main actions in 2014

Dr. Nicolas Steenkeste, from Fondation Mérieux, spoke on the significant benefits of adopting a Laboratory Quality Management System (LQMS): to reduce costs, better respond to the needs of patients, provide accurate and timely diagnoses, meet regulatory safety requirements, reduce waste, improve turnaround time, and help the decision-making process of clinicians. ISO 15189 is the internationally-accepted standard that applies to biomedical/clinical laboratories. Laboratories in countries with limited resources are encouraged to first

meet their national standards, and progressively advance in stages toward the requirements of the internationally-recognized ISO standard. At Fondation Mérieux, a working group on quality has been created. It has come up with a flowchart designed to lead laboratories from the initial need for quality improvement to the final accreditation step. The process involves an evaluation and the completion of a checklist, a roadmap, so to speak, with indicators that are to be progressively validated in four phases.

Experience sharing from Paraguay, Haiti, Mali, Laos

Dr. Rosa Guillén, from the Instituto de Investigaciones en Ciencias de la Salud, Paraguay, gave a report on the quality initiative that was launched in the Molecular Biology department of the IICS. The Quality System Essentials, such as facilities, organization, personnel, equipment, etc. are gradually being phased in to meet quality requirements. Slides comparing the laboratory facilities before and after the launch were displayed. Work on quality improvement is still ongoing.

Mr. Xavier Elie, Quality Assurance Manager at the GHESKIO Centers, Haiti, stated that when the laboratories were first evaluated 15 years ago in the context of HIV vaccine trials, quality control materials, written SOPs and other documentation were lacking. Since then, quality initiatives have made a tremendous leap forward to the extent that, in 2014, the laboratory launched the ISO 15189 accreditation process. Phase 1 is expected to be 100% complete by March 2015, and full accreditation in 2016. The biggest challenge remaining is to convince lab personnel that this process is beneficial.

Mr. Abderrhamane Maiga, Quality Manager at the CICM in Mali, spoke about the quality initiative taken at the CICM since 2005. A Quality Manager has been appointed, and a quality policy has been adopted complete with a quality manual and written SOPs. In 2014 a quality assurance team was created, whose job is to assure the implementation of quality initiatives, train staff, and follow maintenance activities. Also, in 2014, the process to meet ISO 15189 accreditation requirements began. This has moved forward with the acquisition of new equipment, the update of quality documents, and the writing of new SOP's. The Quality System Essentials are being gradually phased in.

Dr. Laurent Malato, from the Fondation Mérieux in Laos, gave an update on the quality audit that took place last June at the CICML. The CICML laboratory had taken certain quality initiatives before the audit, but subsequently after the audit, the assessors released their findings and reported that further quality improvements were necessary. Key responsibilities and position needed to be defined, safety of personnel was a concern, documentation was scarce or not up to date, stock management and inventory were below average. Since then, remedial measures have been applied to overcome these problems. Further action in terms of maintenance, internal assessment, SOPs, training, etc. is underway or is planned for 2015.

Results Laboratory Quality Management System (LQMS) implementation in Cambodia

Dr. Youlet By, from Fondation Mérieux,

Cambodia, spoke on the upgrading of biological laboratories in Cambodia. The program, Strengthening Laboratory Management towards Accreditation (SLMTA) was introduced to Cambodia in 2011 thanks to the support of the U.S. Centers for Disease Control and Prevention. It promotes training and mentoring through workshops and on-site visits to laboratories. In 2013, Laboratory Quality Stepwise Implementation (LQSI) was introduced to Cambodia with the support of the WHO. It provides in-service mentorship to help lead laboratories stepwise through the four phases of the interactive quality roadmap. Nearly 30 laboratories have undergone the quality assessment process and the findings show that the national laboratory system, in terms of microbiology testing capacities, human resources, governance, etc., obtained the average score of 36%. In terms of laboratory facilities, between 40% and 80% of the goals have been reached.

Presentation of the action plan for 2015 and QA self-evaluation questionnaire results

Dr. Nicolas Steenkeste presented a summary of the responses received from the self-evaluation questionnaire sent to the GABRIEL member laboratories in 2014. The categories of the analysis were divided into laboratory description, quality assurance, training, biosafety, equipment, and purchase and inventory. Moving forward into 2015, all member laboratories will be making further progress toward completing the requirements of LQSI, beginning with Phase 1.

Workshop 1: External Quality Assessment, chaired by Antoine Pierson and Xavier Elie

The purpose of the workshop was to allow participants to share their experience and exchange their views on external quality assessment (EQA). The question was asked: What kind of EQA are you performing in your laboratories? Some laboratories stated that they receive no EQA, except when one is conducted through the GABRIEL network at irregular intervals. Other laboratories said that they take part in their country's national program. All agreed that a minimum of two EQAs must be conducted each year for the process to be worthwhile, and that EQAs conducted at quarterly intervals are would be the best.

Participants were also asked: For which analyses do you have trouble finding EQA? The responses were variable and wide-ranging: bacteriology, hepatitis serology, leprosy microscopy, and HIV.

The problem with choosing an EQA provider often lies with the cost and transportation issues. Inter-laboratory comparisons would be helpful.

Workshop 2: Biosafety, chaired by Asadulghani and Magdi Samaan

Biosafety in the context of the Ebola epidemic is a topic of concern. There are questions on the transportation of specimens and on the proper training of professionals in terms of packaging and preparation of shipments. Ideally, one individual per laboratory should be especially trained for this task. Who should take full responsibility for the shipment? It was agreed that responsibility is to be shared between the laboratory and the carrier, but this is an issue that must be investigated on a per country basis.

The sharing of responsibility may not be possible in all countries.

The need for training on infectious substances in accordance with international regulations was also a topic of discussion. The application of guidelines regarding the LQMS-certified transport of infectious substances needs to be strengthened.

Steering Committee Report

Dr. Jianwei Wang began the report by welcoming the two industrial partners that have joined GABRIEL: Bioaster Technology Institute and Fast-Track Diagnostics. Fondation Mérieux is in the process of finding additional industrial partners.

With the view of improving network organization, three working groups, composed each of four to five people, are planned to help generate ideas and set up collaborative studies in the areas of tuberculosis, pneumonia, and typhoid.

The Steering Committee is reviewing the GABRIEL membership applications received from King George's Medical University in Lucknow, India, and the Pasteur Institute in Cameroon. The Committee will soon be voting on their application. The Committee has also decided on the provisions of membership status. New entrants are "associate members" until they successfully pass an evaluation after a five-year trial period. Every three years, all members will need to pass an evaluation on their activities and involvement in the network to retain their full membership in GABRIEL.

As for the Steering Committee itself, it has been suggested that there be one rotating member each year. This year, Dr. Monidarin Chou will be

leaving and will be replaced by a new member chosen by the Committee. Dr. Chou was thanked for his contribution as a member. The Committee will be meeting more often, most likely once every six months or as the need arises.

The 2015 GABRIEL budget allocated to working groups, grants for equipment, training, quality assurance, communication/management was compared to that of 2014.

Research Committee Report

Dr. Glauca Bacçalà announced the arrival of two new Committee members, Philippe Vanhems and Samir Saha.

It was agreed that Committee meetings need to be scheduled more frequently and that members be more greatly involved. The Committee will be participating in the activities of the new GABRIEL working groups and stronger interaction will develop from that. The Committee will also begin to validate the publications to be released from the multi-center pneumonia study.

Closing Words

Dr. Hubert Endtz brought this year's edition of the GABRIEL meeting to a close with thanks to all the participants for their contribution, as well as to the industrial and financial partners for their support. This year's GABRIEL meeting was all about new ideas on testable hypotheses, project designs, and fund raising, all generated in a stimulating environment. The sessions on quality assurance were most valuable and thought-provoking, particularly given the impact quality has on clinical biology and research. Testimonials from the speakers on their real-life experiences with infectious diseases in the field were especially enriching.

The next GABRIEL meeting is planned for Spring 2016.

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