GABRIEL network

SPECIAL REPORT

9th INTERNATIONAL GABRIEL MEETING

December 12-15, 2017

Les Pensières Center for Global Health, Veyrier-du-Lac, France

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Special Reports

From December 12th to 15th, 85 researchers, physicians, specialists in laboratory diagnostics, academics and scientists, both from the public and private sectors, arriving from over 22 countries, convened at Les Pensières Center for Global Health in Veyrier-du-Lac, France, for the GABRIEL network's 9th international meeting.

The first day of the assembly was devoted to a symposium on "Flavivirus Diagnostics, Advances and Challenges". As flaviviral infections, such as dengue, Zika, yellow fever, and chikungunya, exhibit high serological complexity and make accurate diagnosis a real challenge, these diseases are inevitably becoming significant for global public health (*or are generating concern for global public health*). The flavivirus symposium was intended to identify the gaps and pitfalls that may hamper accurate and effective diagnosis and to provide a knowledge-sharing platform where improved surveillance strategies and clinical management could be addressed.

The international GABRIEL meetings have become the occasion for members to share experiences, present new collaborative opportunities, review advances in quality assessment, and discuss the latest scientific advances in infectious diseases. This year's program included discussions on respiratory and enteric infectious diseases - particularly pneumonia, tuberculosis and typhoid - and flavivirus infections. The third edition of the "GABRIEL Young Scientist Award" was presented, created to help boost the career development of promising young scientists from low- and lower-middle income countries by giving them the opportunity to present their research at international scientific meetings. Two winners were selected for this year's best presentations.

Proceedings

Dr. Florence Komurian-Pradel, Head Manager of the GABRIEL Network welcomed the participants to the gathering and reiterated the Mérieux Foundation's top priority: its continuing support to scientific research on infectious diseases in developing and emerging countries. The specific mission of GABRIEL is to strengthen the research capacities of local laboratories in member countries and to bring researchers, physicians, and academics from around the world to work collaboratively. To further encourage collaborative research among the members, two working group meetings on pneumonia and tuberculosis discussed new protocols and reviewed present challenges.

The continuing success of the GABRIEL network reflects the commitment of each of its members:

- to participate in on-going collaborative multicenter clinical and epidemiological studies on ARIs, TB, diarrheal diseases, Zika, and other targeted diseases, and
- to strengthen the scientific know-how of local staff through tutorials, workshops, e-learning modules, support to PhD programs, etc.

Two new GABRIEL members were presented, bringing the total to 20 members, located in 16 countries: 1) Laboratoire Microbiologie Santé et Environnement of the Université libanaise in Tripoli, Lebanon, specializing in tuberculosis, respiratory infections and AMR, and 2) the Bangladesh Institute for Tropical and Infectious Diseases, situated in Chittagong and specialized in tuberculosis and enteric infections.

Dr. Hubert Endtz, the Mérieux Foundation's Scientific Director, in his introductory speech, presented the historical context of the Mérieux Foundation, and its long-standing links with the Pasteur Institute.

He described the main thrust of the missions being carried out throughout the GABRIEL network:

- Collaborative research — especially on pneumonia, and tuberculosis through the launch of a working group for each of these diseases, and on mother/child health issues. This research should contribute to the development of innovative ideas and solutions and attract external partners;

- Intercountry technology transfer;
- Professional training and sharing of knowledge worldwide, especially in the fight against antimicrobial resistance. The GABRIEL network is poised to take action on a global scale, not only in the area of medical care, but also in veterinary medicine;
- Continual improvement: this past year, GABRIEL was given an external evaluation that provided an overall assessment of its available resources and capacity for reaching its stated objectives in the areas of professional education, knowledge transfer, support for collaborative research, clinical activities, partnership-building, etc.

SYMPOSIUM ON FLAVIRUS DIAGNOSTICS, ADVANCES, AND CHALLENGES

Session I - Landscape analysis of flavivirus diagnostics

Chaired by Graciela Russomando, Paraguay, and François Simon, France

Overview of flavivirus ecology and diversity

Dr. Juliet Bryant, Director of the Emerging Pathogens Laboratory at the Mérieux Foundation in Lyon, opened the session by presenting a brief overview of viral diversity within the flaviviruses genus. The genus comprises many notorious human pathogens, but her emphasis was on the diversity of lesser-known 'exotic' flaviviruses that have only occasionally been identified and isolated, and whose ecology/transmission is poorly understood.

The major human pathogens within the genus are:

- the yellow fever virus, for whom the genus was named, which was the first virus infectious for humans ever to be identified (in 1898), one of the first viruses to be isolated in cell culture, one of the first viruses for which a vaccine was developed. The YF vaccine has been in continuous use since 1937 and has undoubtedly saved hundreds of thousands of lives, and the vaccine backbone now serves as a 'prototype' for other biomedical developments;
- the dengue viruses, which are the most significant of known arboviruses in terms of disease burden and worldwide distribution, but for which there is still not an effective vaccine, despite >60 years of R&D investments;
- the Japanese encephalitis virus, which for many years was the major cause of pediatric encephalitis in Asia, but is now being effectively controlled by vaccination;
- the tick-borne encephalitis virus, another major cause of encephalitis, but restricted geographically to more northern temperate areas where the tick vectors circulate;
- the West Nile virus, which emerged explosively in North America at the turn of the century,
- and the Zika virus, the most recent high profile flavivirus, which is breaking all the rules of what we believe we understand about the genus.

A better understanding of the distinct ecological and evolutionary transmission cycles of these diverse viruses will be fundamental to understand the risk of future epidemics and the potential spread or emergence of new variants. The concepts addressed in the talk included distinctions between urban and sylvactic transmission cycles, the characteristics of reservoir hosts versus 'dead end' hosts versus amplication hosts, and important constraints to virus evolution in terms of vertebrate host-vector-virus interactions.

Most flaviviruses cause transitory acute febrile infections in their vertebrate hosts, which thus pose important challenges for detection and diagnostics; viremia within infected hosts is usually so short-lived that molecular detection of viral RNA is only feasible within a very brief post-infection window. Therefore, for the purposes of surveillance, and in order to monitor and track flavivirus circulation on a population level, it is imperative to develop novel serological approaches. Bryant advocated for the adoption of new biobanking initiatives within the GABRIEL network in order to enable systematic evaluation of novel high throughput serodiagnostic tools for flaviviruses, such as microarrays and/or Luminex platforms. Core protocols for research and surveillance were briefly introduced for further discussion in the breakout sessions.

Advances in flavivirus diagnostics to support surveillance

Dr. Ann Powers, Lead of the Virology Team in the Arboviral Diseases Branch of the CDC in Colorado, USA, described the optimal traits of diagnostic tests for surveillance of flaviviral diseases. These tests should be sensitive (e.g. should capture every case in pregnant women), definitive, specific, rapid, affordable, applicable on a range of samples, easy to use, and not contingent on specialized equipment.

The current techniques for flavivirus diagnostic testing of serum, urine, and saliva samples by RT-PCR for viral RNA were presented, as well as the tiered algorithms for suspected cases of chikungunya, dengue, and Zika. A CDC algorithm for molecular testing has been devised for use within seven days of onset of Zika symptoms, and another to be used within four days for antibody testing with confirmation by plaque reduction neutralization. Serology for IgM and neutralizing antibodies, PRNT, and immunohistological staining for viral antigens or RT-PCR on fixed tissues are other possible techniques that could be used. It should be noted that extensive cross-reactivity can occur with Zika virus serology and other related flaviviruses (e.g. those causing dengue and yellow fever).

New research is being conducted with the aim of enhancing molecular diagnostic tools. The development of TaqMan array cards, as well as of RT-LAMP and QUASR technology may have the potential for the surveillance of pathogenic viruses. Antigen detection tools are also being improved, as in the case of the development of NS1-antigen based detection systems and the blockade of binding assay.

The Zika IgM Antibody Capture Enzyme-Linked Immunosorbent Assay (Zika MAC-ELISA) is used for the qualitative detection of Zika virus IgM antibodies in serum or cerebrospinal fluid. The plaque reduction neutralization test (PRNT) is used to quantify the titer of a neutralizing antibody for a virus. It has become the gold standard for the Zika virus as it requires no reagents (antigens, antibodies, etc.), just live virus. However, on the downside, completion can take up to 6-8 days (depending on the virus), it is labor intensive, and its cross-reactivity with other viruses may be a problem.

Serological diagnostic tools can be further improved by the development of: a Zika virus multiplex Bead Assay (IgM/IgG); a rapid and specific IgM diagnostic test using mass spectrometry; panels of monoclonal antibodies against ZIKV; and cross-reactive IgG antibody-depletion methods.

Molecular and serological diagnosis of dengue, Zika and chikungunya: epidemiological data in Central and South America

Dr. Graciela Russomando from the Instituto de Investigaciones en Ciencias de la Salud, National University of Asunción, Paraguay, began her talk by describing the clinical features that characterize Zika, chikungunya, and dengue. All three infections share the same vectors and present similar symptoms. In the case of chikungunya, the geographical spread of the disease in the Americas has been steadily widening over the last three years, coinciding with the propagation of the vectors *Aedes aegypti* and *Aedes albopictus* over the last eighty years, yet resulting in a relatively minor number of deaths. Other lineages of the causative virus are distributed in Africa and South East Asia.

As for Zika, although the virus has disseminated around the globe, the number of cases dramatically dropped in 2017. Again, the number of deaths attributable to the virus is relatively minor. The Zika virus can be detected in whole blood (also in serum and plasma), urine, cerebrospinal fluid, amniotic fluid, semen, and saliva. WHO encourages the collection of specimens for confirmatory testing.

Finally, for dengue, the number of severe cases has dramatically dropped in the Americas, even though this virus, too, has spread globally. Secondary dengue virus infections continue to be a problem, however, and this raises issues in properly diagnosing the primary infection. There is considerable overlapping of these three diseases.

Molecular testing for these three flaviviral diseases must strictly follow a prescribed algorithm to properly diagnose and differentiate among them.

Learning from cohort studies: the impact of multiple flavivirus infections on long-term immunity and disease outcome

Dr. Leah Katzelnick from the Division of Infectious Diseases and Vaccinology at the University of California, Berkeley, USA, addressed the question of the extent to which genetically diverse flaviviruses are antigenically distinct or related.

First, it is known that anti-dengue virus (DENV) antibodies can either protect against dengue disease or increase the risk of severe dengue. There is presumed lifelong protection against all strains of the homologous DENV serotype. Yet a prior heterologous DENV infection is the greatest risk factor for dengue, perhaps due to antibody-dependent enhancement, even though many secondary DENV infections are neither severe nor symptomatic. Do antigenic differences within and between DENV1-4 help explain this difference?

While DENV strains are usually located closer to other viruses of the same DENV type, some viruses have greater antigenic resemblance to viruses of a different type than to some viruses of the same type. Antigenic cartography, the method used to make this observation, is useful for the selection of vaccine strains for optimal antigenic representativeness, for the measurement of natural infection and vaccine responses, and for the enhancement of DENV surveillance of genetically diverse DENV strains.

A further question has to do with the relationship between pre-existing anti-DENV binding antibodies (DENV-Abs) and dengue disease severity. Also, does a peak enhancement titer predict severe dengue disease generally? Our studies indicate that DENV-Abs are associated with elevated risk of severe dengue disease in humans. The DENV-Ab titer associated with enhancement of severe dengue is distinct from the titer associated with protection against symptomatic dengue. Our findings suggest that a vaccine that induces DENV-Ab titers, at or near the peak enhancement titer, may place the vaccinated at greater risk of severe dengue than if they had never been vaccinated.

The iELISA test is a simpler assay than neutralization tests and is a promising alternative method for measuring biologically relevant serological responses, but more research is required.

There is possible relevance to ZIKV: high anti-DENV antibodies at some level could protect against symptomatic ZIKV infection, but a peak enhancement titer could increase the risk of severe complications of Zika illness.

In conclusion, antigenic cartography can be useful for determining antigenic relationships among genetically diverse, but related viruses. Cohort studies provide a critical resource for understanding the etiology and epidemiology of specific pathogens, and serve as sentinels for the detection of emerging pathogens.

SESSION II – Diagnostic innovations and patient management

Chaired by Juliet Bryant and Ann Powers

Diagnosis of flavivirus infections in the context of surveillance of acute febrile illnesses in Senegal

Oumar Ndiaye from the Insitut Pasteur in Dakar, Senegal, presented an overview of flaviviruses, stressing that flavivirus disease outbreaks routinely appearing in densely inhabited areas in Senegal are a major health threat. Because other diseases (chikungunya, Rift Valley Fever, malaria, influenza, leptospirosis, leshmaniasis, hepatitis, etc.) can present similar pathologies, it is vital to have the tools on hand to rapidly contain an outbreak of a flavivirus infection.

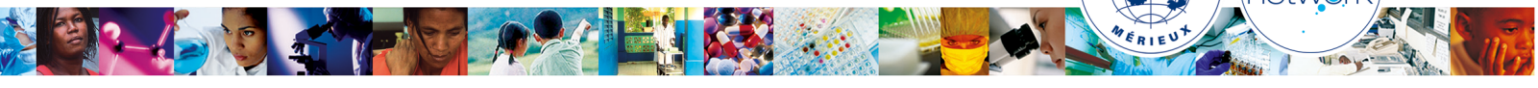
Surveillance must be laboratory-based. Following a diagnostics algorithm, RT-PCR is carried out as a direct method on samples collected before the tenth day of symptom onset, or RPA performed with differential diagnosis (dengue, West Nile, Zika viruses).

These methods each have their advantages and drawbacks.

Virus isolation remains the gold standard for the direct method of assaying. As for indirect methods, the MAC-ELISA screening technique is useful, although it, too, has its advantages and disadvantages (notably in the high level of cross-reactions especially after dengue infection or secondary infection following yellow fever vaccination or previous flavivirus exposure). Finally, the plaque reduction neutralization test (PRNT) has the merit of being highly sensitive, highly specific, and capable of measuring the titer of neutralizing antibodies, although it requires a high level of biosafety and long manipulation time.

Surveillance of flavivirus infections continues to face formidable challenges. For one, correct data from the field is difficult to gather (e.g. YFV vaccination status, date of onset), and some remote areas of the country are difficult to access, which means that some outbreaks may go unnoticed. Electricity may not even be available everywhere to maintain the cold chain for samples and reagents. Because of rapid urbanization, deforestation and climate change, the impact of flaviviruses in Africa will grow in the coming years. Other African flaviviruses transmitted by the *Aedes* mosquito might also emerge (Wesselsbron, Koutango, Ntaya, Spondweni, Usutu, Kedougou).

In terms of prospects at the laboratory diagnostics level, attention has been given to the design of multiplex immunoassays (magnetic beads Luminex-based formats), to the design of point-of-care devices and lateral flow immunoassays, to NS1 protein detection (as an alternative to PCR), and to the design of subunit antigens for use in ELISA assays to overcome cross-reactions. Urine testing should be considered to detect viral genomic RNA as viremia lasts longer in urine than in the blood. There is also a need to design a test to distinguish natural yellow fever antibodies from those that are vaccine-stimulated.



State-of-the-art diagnostics/prognostics for dengue: impacts for patient management

Prof. Bridget Wills from the Centre for Tropical Medicine and Global Health at the University of Oxford introduced the topic of severe dengue by describing its major complications. These comprise increased systemic vascular permeability, potentially leading to hypovolemic shock, hemorrhagic manifestations due to a combination of thrombocytopenia and deranged hemostasis, and severe organ impairment sometimes combined with an underlying disease. Viremia is variable, but is generally higher in more severe forms of the disease and is thought to induce a more aggressive host inflammatory response in these individuals.

Better clinical diagnosis and better risk prediction are key considerations to prevent or reduce the severity of these complications, which occur during the critical phase of the disease, typically at least 3 days after the onset of illness. How can we improve the utilization of public health resources and reduce the economic burden of dengue? How can we diagnose severe dengue early, say on or before day 3, before complications occur?

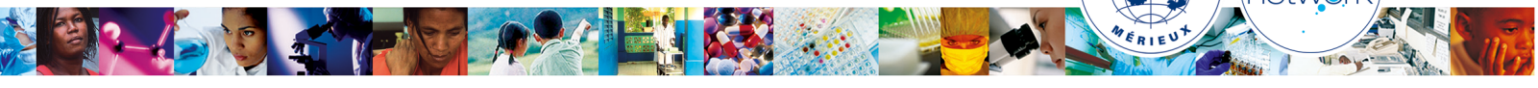
To this end, various studies have been carried out in an attempt to identify risk predictors for severe dengue. One in particular was the IDAMS prospective observational study that recruited 7,500 out-patient participants with suspected dengue within the first 3 days of illness, in eight countries across Asia and Latin America. Study participants were followed daily until resolution of their symptoms, including if hospital admission was necessary, and subsequently classified according to the final severity of their illness using predefined criteria for severe and intermediate severity vascular leakage, bleeding, and organ involvement. There was marked heterogeneity by age and by continent in overall severity, indicating that a straightforward widely generalizable risk prediction model is not presently attainable using clinical features alone. Ongoing efforts are focused on using potential predictors identified from preliminary analyses in the different populations and by sequential day-of-illness analyses. Inclusion of parameters such as viremia, immune status, seasonality and other factors that are continent-specific, and the presence of underlying medical conditions, etc. may help to improve the performance of the prediction models. A number of biomarkers are now being investigated, and could also be extremely valuable.

Viral nervous system infections: flaviviruses and beyond

Dr. James Sejvar from the National Center for Emerging and Zoonotic Infectious Diseases at the CDC, Atlanta, USA, presented the flaviviruses that cause neurotropic diseases affecting the nervous system in humans: Japanese encephalitis virus (JEV), West Nile virus (WNV), dengue virus, Zika virus, Saint Louis encephalitis virus, Murray Valley encephalitis virus, and the tick-borne encephalitis virus. As for the more prevalent diseases, he described their geographic distribution, the number of cases per year, the human diseases caused, the populations at greatest risk, the incidence, and the ratio of symptomatic over non-symptomatic infections.

Japanese encephalitis is the most common infectious cause of encephalitis in Asia with between 30,000 to 50,000 cases reported to WHO annually. It is a devastating illness with a case-fatality ratio of approximately 20 to 30%, resulting in an estimated 10,000 to 15,000 deaths annually. In addition, 30 to 50% of survivors have significant neurologic sequelae. Its clinical features are characterized by a mild fever syndrome, which subsequently leads to encephalitis, particularly in children. For this reason, and because isolated meningeal involvement occurs with a Japanese encephalitis infection, the incidence of the latter illness is certainly underestimated.

The West Nile virus, is another emerging neurotropic virus; while most infections with WNV result in a mild, dengue-like illness, some cases go on to develop neurologic illness – encephalitis, meningitis, and a polio-like myelitis. It has wide distribution throughout Asia, Eastern Europe, and Africa but only



recently has been responsible for large outbreaks, most namely in North America, where it emerged in 1999. Elderly people and persons with an immunosuppressive syndrome are at greatest risk to develop neurologic illness. Patients typically start with nonspecific symptoms of fever, headache and vomiting. Then over the course of several days, they develop encephalopathy and altered mental states such as agitation, delirium, lethargy or coma.

A question has been raised whether the dengue virus causes encephalitis, since there is some evidence both for and against this proposition. West Nile and Japanese encephalitis viruses have been associated with hundreds of thousands of documented cases of neuroinvasive disease worldwide. But reports of dengue virus encephalitis have been relatively rare and uncommon, despite its tremendous burden of illness worldwide. The hemorrhagic manifestations of dengue complicate the diagnostic picture, and so it can often be difficult to interpret the findings of dengue virus or dengue-specific IgM antibodies in cerebral spinal fluid. Finally, some of the more advanced neurodiagnostic techniques that we use to diagnose encephalitis in developed countries are often not readily available in many dengue-endemic countries.

Zika virus illness and Guillan-Barré syndrome (GBS) are exploding in the Americas, suggesting a strong association between the two. Manifestations of Zika are meningitis, encephalitis, myelitis, optic neuritis, but of lesser magnitude than GBS. The Zika virus can cause congenital malformations, whereas GBS is an immune-mediated syndrome, not due to direct viral neuroinvasion.

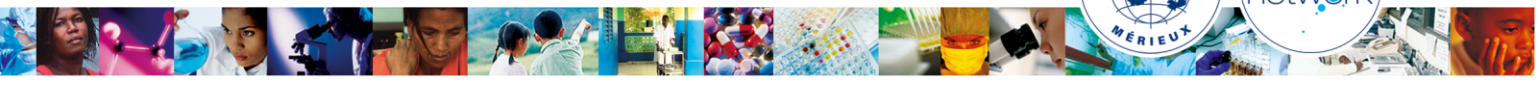
State-of-the-art flavivirus vaccines: evaluating vaccine efficacy and interactions

Prof. Annelies Wilder-Smith, Director of the Partnership for Dengue Control, part of GDAC, began by pointing out that three arboviral diseases, yellow fever, Zika, and dengue, are THE emerging problems of the 21st century. Dengue is the most predominant, having shown a 50-fold increase in incidence over the past decades, with spread to Latin America and Asia, and increasingly now also in Africa. The development of vaccines should eventually reduce the clinical burden of these infections, generate herd immunity, interrupt disease transmission, and result in a population health benefits.

The serious complications of congenital Zika syndrome (CZS) have galvanized the development of a Zika vaccine. Indeed, the technical feasibility of a Zika vaccine appears promising. Multiple vaccine platforms have shown robust protection against ZIKV challenge in animal models.

However, unique challenges will need to be addressed in the clinical development and regulatory pathways of a Zika vaccine, such as the possible association with Guillain-Barre Syndrome, safety concerns for administering a vaccine in women of reproductive age (including pregnant women), and the concern about immune enhancement associated with co-circulating dengue viruses. Current evidence suggests that even asymptomatic infections with presumably low levels of viremia in the mother could result in CZS. It is unknown whether sterilizing immunity and robust T cell response are required to avert transplacental transmission of ZIKV during pregnancy. Answering these questions will be critical for the development of a vaccine that protects against CZS. However, currently the biggest challenge is where any Phase 3 efficacy trials can ever be conducted given the rapid decline in cases globally.

The current yellow fever vaccine is experiencing a shortage: 1.4 billion doses would be required to eliminate yellow fever, however, the annual production is only 80 million per year. To address vaccine shortages during an outbreak where stockpiles have been depleted, WHO endorsed fractional doses (one-fifth of the dose). This is based on the observation that most yellow fever vaccines are overformulated (i.e. contain a higher potency than the WHO minimum standard) and lower doses have shown to be equally immunogenic. However, the duration of protection in different age groups and countries is yet unknown.



The first tetravalent dengue vaccine was licensed in December 2015. In randomized control Phase 3 efficacy trials, the efficacy of the dengue vaccine was shown to vary according to the serotype, the severity of disease, the serostatus and age of the subject. Long-term safety follow-up revealed a higher risk of severe dengue in younger subjects. Sanofi Pasteur reanalyzed the trial data separately in participants classified as seronegative and seropositive to estimate the long-term safety and efficacy of the vaccine by serostatus prior to vaccination using new diagnostic tools. The recently published findings from these additional analyses show that the subset of trial participants who were inferred to be seronegative at time of first vaccination had a significantly higher risk of more severe dengue and hospitalizations from dengue compared to unvaccinated participants, regardless of age at time of vaccination. WHO acknowledges that in high seroprevalence settings, the vaccine still has significant population-level benefits. However, until a full review has been conducted, WHO recommends vaccination only in seropositive individuals.

YOUNG SCIENTIST AWARD PRESENTATIONS

Chaired by Werner Albrich and François Simon

Antiviral susceptibility profile of Influenza A(H1N1) pdm09 viruses in Brazil: identification of resistant strains from 2014 to 2016

Dr. Aline da Rocha Matos from the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, presented the overall objective of her study: to monitor the circulation of resistant influenza A(H1N1)pdm09 viruses to neuraminidase (NA) inhibitors in Brazil between 2014 and 2016. More specifically, this has entailed:

- identifying viruses with genetic markers associated with antiviral resistance in the NA gene,
- evaluating viral NA functional activity,
- analyzing the fitness characteristics of isolated viruses, and
- evaluating the evolution of Brazilian viruses.

This was purely a surveillance study with no quantitative data obtained on viral load.

A series of experiments was conducted to detect NA mutations associated with antiviral resistance using pyrosequencing and Sanger sequencing procedures, as well as tests measuring the inhibition of viral NA activity by antiviral drugs. NA thermostability and replication kinetics of mutant H275Y virus was also run.

The following conclusions were reached:

- NA sequence analysis revealed that some Brazilian influenza A(H1N1)pdm09 viruses contained the H275Y, I223K and S247N substitutions, associated with antiviral resistance profile,
- the 1.1% frequency of the influenza A(H1N1)pdm09 NA mutant virus closely matches the frequency of this virus reported globally,
- the H275Y mutant A/Rio de Janeiro/257s2/2016 virus exhibited reduced fitness when compared to non-mutant viruses, and
- some mutant viruses were identified in patients without prior antiviral treatment, suggesting that community transmission of variant viruses occurs.

Finally, the existence of antiviral-resistant clusters of the virus and resulting sporadic clinical cases very much warrant the surveillance of this virus in high-risk populations.

Characterization of influenza viruses responsible for severe pneumonia in Cambodia

Bonath Ka from the University of Health Sciences, Phnom Penh, Cambodia, spoke about the use of epidemiological surveillance as a means to measure the burden of influenza in Cambodia. The genetic and phenotypic analysis of the influenza viruses can help better describe the disease for improved patient management.

The objective of her study is to investigate the prevalence of causative agents of pneumonia in the ARI population with a focus on the characterization of the influenza virus. This was accomplished through conventional NIC characterization techniques and full genome sequencing from clinical specimens.

Following molecular screening of the samples collected from four surveillance sites in the country, it was found that influenza viruses causes ARI mainly during the rainy season and that the prevalence of influenza in the ARI population is low. Most influenza cases caused by Influenza A viruses have a nearly equal distribution between H1N1pdm09 and H3N2. Further studies will be carried out to characterize the susceptibility of the viruses to anti-viral drugs, to perform full genome sequencing, and to compare the viruses causing severe and non-severe illness.

Molecular epidemiology of Mycobacterium tuberculosis and antibiotic resistance in Lao PDR

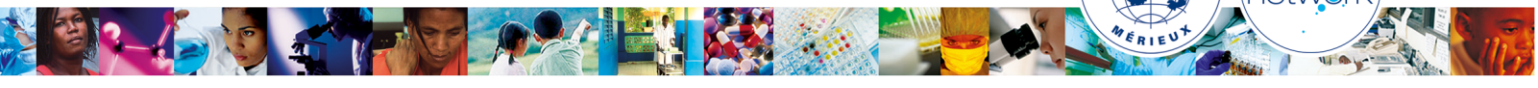
Silaphet Somphavong from the Center of Infectiology Lao Christophe Mérieux, pointed out that genetic data on *Mycobacterium tuberculosis* is at present unavailable in Lao PDR. In response to this, a fist study was launched to characterize the genetic diversity and structure of *M. tuberculosis* in Lao PDR and to examine the microorganism's drug resistance and associated mutations. Through drug-resistance testing and genotyping of samples collected from the first national TB prevalence survey of Lao PDR (2010-1011), from routine analysis of patients suspicion MDR-TB and in three regional hospitals, a map was drawn showing the distribution and transmission of *M. tb* families and the drug-resistance patterns throughout the country. In conclusion, it was found that the EAI families were predominant, followed by Beijing. However, the Beijing strain is associated with drug resistance, as described in neighboring countries. It is at present restricted to the North, but that its spread to the South is possible. Further studies will be necessary to more closely conduct the surveillance of the spread of the various families of *M. tb* in Lao PDR and to track the evolution of drug resistant TB. This data should be useful for large-scale TB control and treatment.

Genomic characterization of a novel human Influenza A(H1N2) variant detected in Brazil

Dr. Paola Resende from the Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, stated that the Influenza A(H1N2) is a virus that has been frequently reported in swine resulting in seasonal epidemics. The emergence of such a virus and its introduction in humans through contact with swine is considered to be a major public health concern. The H1N2 virus was first reported in humans in Japan in 1980 and has caused sporadic cases in various countries, including the USA where 13 cases of illness in humans were reported in humans since 2005. The whole genome of this virus was isolated in Brazil from a patient with an ILI. Through virus genetic sequencing and phylogenetic analysis, it was found that the H1N2 virus detected in Brazil's Southern region has a genomic configuration not previously reported in humans.

It has a swine genetic origin and, possibly, it was introduced in the human population by the direct contact of the patient with pigs.

This virus was most likely a product of successive reassortments of the genetic material that may have occurred in the Brazilian swine population over the years.



Diagnostic yield of active case finding of tuberculosis and HIV at the household level in slums in Haiti

Vanessa Rivera from the GHESKIO Centres, Haiti, spoke about a recently published study on active TB case finding in the Port-au-Prince slums. Haiti has the highest TB prevalence in the Americas with an estimated prevalence of 254 per 100,000 population. The study was funded by the WHO Stop TB Partnership's TB REACH initiative and was conducted in nine slum neighborhoods, where most residents live in extreme poverty, enduring crowded conditions with limited access to basic sanitation and medical services. During the study, 80 community health workers administered a verbal TB screening questionnaire at the household level, querying individuals for chronic cough, or cough sustained for over two weeks. All individuals who screened positive were referred to the GHESKIO clinic for HIV and TB screening. TB screening included same-day chest X-ray, sputum acid-fast bacilli smear and Xpert MTB/RIF tests. Patients diagnosed with TB initiated TB treatment within 5-days of initial visit; patients with chest X-rays suggestive of TB initiated TB treatment the same-day. During the one-year study period, over 104,000 patients were screened in the community of whom 5,598 (5%) reported chronic cough, were >10 years old and included in the analysis. A total of 1,110 (20%) were diagnosed with active TB disease, a prevalence of 1,066/100,000. Of the 5,472 (98%) patients tested for human immunodeficiency virus (HIV), 528 (10%) were HIV-positive; 143 (3%) patients were diagnosed with both diseases. Multivariable regression was conducted to determine predictors of TB among patients with chronic cough. The likelihood of TB infection was higher among individuals infected with HIV, of male gender, between ages 18 to 39 years and of single marital status. Household-level screening for cough with TB and HIV testing for symptomatic patients was a high-yield strategy, leading to the detection of a prevalence of undiagnosed disease, exceeding national estimates by more than four-fold for TB, and by five-fold for HIV.

RESEARCH PROJECTS WITHIN THE GABRIEL NETWORK

Feedback on the GABRIEL research landscape

Dr. Florence Pradel from the Mérieux Foundation's Emerging Pathogens Laboratory in Lyon, France, highlighted the major collaborative research projects that have been carried out by GABRIEL member laboratories since July 2016. Out of a total of 10 ongoing projects involving 11 members, there are

- 5 on ARIs,
  Lebanon (2) – France: pneumonia etiologies among refugees – PEARL
  Cambodia – France: S. pneumoniae typing before vaccine introduction
  Laos – France: active respiratory surveillance in household communities – LACORIS
  India – France: short-period incidence study of SARI
  Mali – China: pneumonia etiology – discovery of new viruses in bats and in rodents
- 3 on tuberculosis
  Laos – France: National TB survey and molecular typing of circulating TB
  Georgia – France: surveillance set-up for drug-resistant TB transmission
  Lebanon – Bangladesh – France: evaluation of markers for anti-TB treatment efficacy and/or detection of disease progression
- 1 on diarrheal diseases: Bangladesh – Brazil – France: characterization of drug resistance in Salmonella enterica serovar Typhi
- 1 on ZIKA/GBS: Bangladesh – Netherlands – France: the role of the ZIKA virus in the etiology of Guillain Barré Syndrome

Only three of these projects involved more than two GABRIEL partners and therefore can be considered as true collaborative studies. This underrepresentation will be remedied by having a greater number of partners work together on a project.

Another priority of the GABRIEL network are professional training courses, thematic workshops, e-learning modules (e.g. on protein synthesis), and support to PhD student programs to help develop research capacities in member laboratories. During the past year, seven workshops were held in Lyon, Phnom Penh, Beirut, Bamako, Beijing, and Zaporozhye.

Finally, as part of our quest for improvement, an evaluation conducted by an independent party highlighted the areas that require greater attention. Feedback on GABRIEL's activities was collected from each member laboratory in the form of a questionnaire. The results were presented during the last session of the GABRIEL meeting.

Fogarty programs for research training: overview of mission and programs

Dr. Guy Vernet from the Mérieux Foundation USA, spoke about the creation of the Mérieux Foundation in the United States in 2011 as a qualified public charity, whose mission is centered on improving access to diagnostics and strengthening local research capabilities. It seeks to establish technical and financial partnerships with US government agencies, corporations, non-profit organizations, foundations, academic centers, and research institutions.

The Fogarty International Center (FIC), as a part of the NIH, has the mission of addressing global health challenges through innovative and collaborative programs for research and research training. Its focus is on building the capacity of foreign institutions to carry out independent research across the full breadth of Global Health. Most FIC-funded projects involve collaborative studies between scientists in the USA and those in low- and middle-income countries in a range of fields that includes mobile technologies and health, HIV and global infectious diseases. FIC also sponsors research training, educational programs, and 3-to-5-year fellowships. At present, FIC supports 6,000 scientists through 500 grants valued at 70 million dollars per year.

The Mérieux Foundation is ready to help GABRIEL scientists who would like to apply for FIC grants by providing advice on the application process, the submission of the grant application, and contacts with universities in the United States.

SESSION III – Tuberculosis and other mycobacterial diseases

Chaired by Delia Goletti and Nestani Tukvadze

Biomarkers for diagnosis and treatment efficacy in tuberculosis

Dr. Delia Goletti from the National Institute for Infectious Diseases, Rome, Italy, described the immunological tools that can assess whether tuberculosis has been cured in a patient. Tuberculosis (TB) remains a devastating disease despite its enormous burden on global health. Tools to control TB are still unsatisfactory and TB Biomarkers (TB-BM) may be useful to measure infection status and predict outcome of infection or therapy. There are several types of TB-BM: Correlates of Infection; Correlates of TB disease; Correlates of increased risk of developing active TB disease; Correlates of the curative response to therapy; and Correlates of Protection. Most TB-BM currently studied are host-derived BM, and consist of transcriptomic, proteomic, metabolomic, cellular markers or marker-combinations ("signatures"). It is crucial to test and validate TB-BM in different well-characterized human TB cohorts, preferably with complementary profiles and geographically diverse populations to ensure their usefulness worldwide. Much research is being carried out to find TB-BM that can be rapidly used in the routine management of TB.

Innovative social enterprise model for increased tuberculosis case detection and treatment in the private sector of Bangladesh

Dr. Sayera Banu, Senior Scientist and Head of the Programme on Emerging Infections of icddr,b, Dhaka, Bangladesh, described a social enterprise model (SEM) as an organization or venture that advances its primary social or environmental mission using business methods.

Its success is determined by both financial outcome and social impact, that is, between sustainability and social welfare. Donors seek social return on their investment, not profit, and low-income earners seek access to markets.

Our implementation research had the aim of observing the sustainability of a social enterprise model in TB service delivery in the private sector. At the same time, we wanted to assess the effectiveness of active screening and an enhanced diagnostic algorithm for improved TB case detection. Our work was prompted by the scarcity of data on TB detection in the private sector. Moreover, TB case detection in the private sector is not quality-assured and is underreported, as it is handled through private laboratories that perform the X-rays and sputum testing.

Therefore, in order to remedy this situation, it was important to establish high-quality diagnostic services through effective public/private partnerships. This was accomplished by establishing TB Screening and Treatment Centres (S&TCs) under an innovative mixed public/private social enterprise model. These S&TCs examined presumptive TB cases referred from the private sector using chest X-rays and GeneXpert testing. More than 12,000 TB cases were detected in this project, of which 400 were resistant. As a result of this icddr,b SEM initiative, the number of TB cases notified by the private sector has grown significantly and the model has been incorporated into the National Public-Private Mix Strategic Plan (2016-2020) for nationwide scale-up.

Prevalence and antimicrobial resistance of Mycobacterium tuberculosis among citizens, non-national residents and refugees in Lebanon: a nationwide study

Dr. Monzer Hamze, Head of the Health and Environment Microbiology Laboratory at the Lebanese University in Beirut, first outlined the situation of the 1.4 million Syrian and Palestinian refugees currently in Lebanon. The purpose of the present study was to gain a more comprehensive overview of the epidemiological situation of TB in Lebanon. In the resident population, the TB burden is relatively low and MDRTB is fully under control. More specifically, given the influx of refugees, the main objectives of the study are to establish more solid diagnostics of TB cases and measure the actual incidence of drug resistance by using phenotypic and molecular testing of the samples collected in the nine TB centers across the country. Patients who tested positive for *M. tuberculosis* were treated in these centers. Of these, 37% were non-Lebanese. The low incidence of TB in the refugee camps is probably underestimated. Drug susceptibility testing was performed on all bacteriologically confirmed isolates of the *Mycobacterium*. Results are still being compiled on the all the positive samples, but so far, 80.2% of TB patients are susceptible to all four first-line drugs. Molecular testing by PCR amplification and MTB genotyping to identify the gene targets associated with resistance in the remaining samples are currently underway.

MDRTB, the new threat: diagnosis and care

Dr. Katey Walsh from the GHESKIO Centres, Port-au-Prince, Haiti, gave an overview of the global burden of TB in 2016. Of the 10.4 million estimated incident cases, 600,000 were detected as MDR, with a case fatality rate of 40%. The current challenge is to improve MDR-TB outcomes through better detection, diagnosis and treatment. Better outcomes are possible, as shown by the almost 90% treatment success seen at GHESKIO.

More effective, less toxic, and shorter treatment regimens are the goal, with a need for the development of new drugs. For this to materialize, sustained and substantial investment and commitment are urgently needed.

An optimal MDR-TB treatment regimen would be no longer than 6 to 9 months in duration, have minimal toxicity, include only oral medications, and be effective for all patients regardless of HIV status or age. The first randomized clinical trial (STREAM) examining a short-course regimen has released preliminary results suggesting a short-course regimen is feasible, although not meeting the non-inferiority criteria and with potentially clinically significant higher mortality in HIV-positive patients. New or repurposed drugs are needed to strengthen the potential success of any future short course options.

Based on prior studies at Weill Cornell Medical College and GHESKIO showing efficacy of nitazoxanide against MTB, GHESKIO is conducting a phase II prospective randomized clinical trial to examine the safety and efficacy of this drug in treatment-naïve adults with drug-sensitive pulmonary TB. If successful, this would represent a successful repurposing of an already available inexpensive drug to combat TB.

Tracking MDRTB transmission in Georgia

Dr. Tsira Chakhaia from the National Center for Tuberculosis and Lung Diseases (NTCLD), Georgia, mentioned that the aim of the current epidemiological study, funded in part by the Mérieux Foundation is 1) to acquire epidemiological evidence-based data to build laboratory capacities for TB transmission tracking, 2) to measure the percentage of direct transmission of TB among household contacts versus independent sources, and 3) to assess contact tracing practices. The study has identified TB household cases from past public health records.

Isolates from household members suspected of direct TB transmission were genotyped to confirm whether transmission occurred within or outside the household. Public health contact tracing procedures were examined through quantitative and qualitative analyses based on face-to-face interviews during field visits and group meetings, followed by proposals for improvement through an infection control plan. Professional training for capacity-building in laboratory techniques in molecular biology has also been carried out.

The current project, “Capacity Building for Diagnostic and Clinical Management of MDR and XDR TB in Civil and Penitentiary Sectors in Georgia”, will run through March 2019 in the context of new anti-TB drug use and surveillance of X/MDR-TB transmission in Georgia. This project is intended to strengthen the capacity of the TB program in Georgia in the diagnostic and clinical management of MDR and XDR tuberculosis in civilian and penitentiary sectors.

Its objectives are to strengthen the capacity of the National Reference Laboratory in phenotypic susceptibility testing for second-line drugs, to boost the capacity of the National TB Program in clinical management and effective treatment observance of drug resistant TB patients countrywide including the penitentiary sector, and to acquire evidence-based data on primary resistance transmission indexes through the identification of the country’s hot spots for M/XDR-TB transmission.

TB outbreak analysis: is MIRU-VNTR enough to understand transmission?

Jean-Luc Berland from the Mérieux Foundation's Emerging Pathogen Laboratory, Lyon, raised one point in the molecular epidemiology of tuberculosis: the comparison between conventional genotyping and whole genome sequencing. Up through the 90s, RFLP and spoligotyping were the techniques employed. MIRU-VNTR appeared in the 2000s, which proved to be useful to investigate population structures and trends, to examine the dynamics of transmission, and to identify spot outbreaks.

In the GABRIEL network, studies using genotyping are currently being carried out in France (MDR epidemiology), Lao PDR (molecular epidemiology of resistance), Georgia (MDRTB transmission in households), Bangladesh (TB transmission in penitentiaries), and Madagascar (*M. leprae* genotyping). Since the 2010s, whole genome sequencing has been used with greater accuracy to provide clinical data on antibiotic resistance and epidemiological data. Despite this greater accuracy, WGS is not being addressed within GABRIEL, thus resulting in missed opportunities to answer TB transmission issues. The talk gave some examples, stemmed from GABRIEL projects, where WGS has given clues to clinical and epidemiological questions.

Assessing drug resistance of Mycobacterium leprae and genotype distribution in Madagascar

Dr. Mala Rakoto Andrianarivelo from the Charles Mérieux Center for Infectious Disease (CICM), Madagascar gave an overview of leprosy, also known as Hansen's disease, caused by *M. leprae* and *M. lepromatosis*. In terms of the burden of the disease, Madagascar is considered to be a priority country with 1,780 new cases reported last year.

The CICM became a national reference laboratory in 2017 for the confirmation of suspected clinical cases of leprosy and for the detection of primary/secondary resistance to dapsone, rifampicin and fluoroquinolones. In seeking to understand the genetic diversity of *M. leprae* in Madagascar, a comprehensive epidemiological study, the first of its kind, was launched to examine the distribution of the microorganism's genotype throughout the country. The study also aims at assessing the occurrence of *M. lepromatosis*-associated leprosy, to share with the Leprosy National Control Program its findings for improved case patient management of the disease. Skin biopsies were collected from 59 patients. PCR genotyping and whole genome sequencing were performed to determine phylogenetic relationships, and a map was arrived at showing the geographical distribution of the various genotypes, consisting mainly of two lineages, 1D (predominant) and 1A (less prevalent). No drug resistance was detected. The comparison of strains of patients under treatment should be helpful to understand the dynamic of *M. leprae* inside the host.

Molecular drug susceptibility testing in the Zaporozhye region (Ukraine): performance of the MTBDRplus assay

Dr. Roman Yasinskyi from the Department of Phthisiology and Pulmonology at the Zaporozhye State Medical University, Ukraine, began his talk by indicating that the incidence of tuberculosis and drug-resistant tuberculosis is higher in the Zaporozhye region than in the Ukraine as a whole. Drug-resistant TB is a public health problem in the Ukraine. The current study was conducted on 131 sputum samples from patients who were examined and treated in dispensaries of the Zaporozhye region in 2017. The results were compared with the GenoType MTBDRplus, v.2 test system, along with standard methods of MBT identification and drug-resistance testing. Results showed that both the GeneXpert test and the GenoType test are extremely accurate when detecting MBT and Rif susceptibility, in spite of the presence of some discrepant isolates. Differences between results may be caused by the absence of phenotypical manifestations of some mutations.

TB activities in BITID, Bangladesh

Prof. M.A. Hassan Chowdhury, Director of the Bangladesh Institute of Tropical and Infectious Diseases - BITID, in Chittagong, spoke in detail about BITID, an institution that was created four years ago. BITID is equipped with a specialized laboratory (biosafety level 2 and biosafety level 3) that began working on suspected TB cases in January 2017. The aim of the laboratory is to boost the regional health system of Chittagong in the field of infectious diseases by improving diagnostic capabilities, especially through prompt and accurate diagnoses. It is equipped to run GenXpert testing and Line Probe Assays, which have both been employed to detect drug-resistant TB. Co-infections among people living with HIV/AIDS are also being analyzed.

As TB in Bangladesh ranks seventh among the 22 highest TB-burdened countries, this laboratory is expected to help improve the nationwide case notification rate and TB treatment success rates.

SESSION IV- ACUTE RESPIRATORY INFECTIONS

Chaired by Marilda Siqueira and Monidarin Chou

IL-1RA polymorphism and levels associated with adverse outcome in severe community-acquired pneumonia in children: a hospital-based study in India

Dr. Shally Awasthi from King George's Medical University, Lucknow, India, described India as a country where one child out of 21 dies before the age of five years. Up to 19% of these deaths are attributable to pneumonia. To better understand the role of interleukin-1 receptor antagonist (IL-1RA) and cytokines in the immune response to community-acquired pneumonia (CAP), a hospital-based, ethically approved study is being conducted to assess the association of IL-1RA gene polymorphism on serum levels of IL-1RA in children under five years of age and hospitalized for CAP. Children meeting the inclusion criteria were recruited for the collection of blood samples. ELISA and PCR tests were conducted to determine the genotype distribution of IL-1RA gene polymorphisms and gene expression in complicated and uncomplicated CAP cases and expired cases.

Resulting IL-1RA levels were correlated with clinical outcomes related to complications and mortality. It was found, in conclusion, that serum IL-1RA levels rise in both complicated CAP and expired cases. Therefore, IL-1RA can be used as a biomarker for severe CAP, and may be useful for differentiating bacterial from viral etiologies of the illness. The potential of newer modalities of therapy using IL-1RA analogs in severe CAP in children can therefore be assessed.

Serotype distribution of clinical Streptococcus pneumoniae isolates in Cambodia

Dr. Youlet By, Mérieux Foundation Cambodia Country Manager, presented a current study that aims at providing baseline data for future monitoring of *S. pneumoniae* serotypes and for the reduction of the pneumococcal disease burden in Cambodia. Stored isolates of the bacterium were recovered from microbiology laboratories across the country along with information on their specific identifying characteristics that include antibiotic susceptibility. The isolates were typed in the Rodolphe Mérieux Laboratory of Phnom Penh, using a multiplex real-time PCR method capable of detecting the 40 most prevalent *S. pneumoniae* serotypes worldwide. A subset of the isolates from the Sihanouk Hospital Center of Hope was serotyped by the latex agglutination method with Quellung confirmation of ambiguous results. Antimicrobial susceptibility between PCV13 isolates and non-vaccine isolates were compared. The study was approved by the National Ethics Committee in Cambodia.

Etiological study on adult pneumonia: a multicenter study in China

Prof. Jianwei Wang from the Chinese Academy of Medical Sciences pointed out that the lack of global prevalence data on respiratory viruses hinders the development of antiviral drugs and vaccines. The understanding of the global genetic diversity of respiratory viruses is a means to prepare to respond to new diseases. The China Pneumonia Research Network has been studying the etiology and the frequency of respiratory viruses in adults with ARIs. In one study, among 1,877 adult patients with pneumonia, 59% had at least one pathogen detected during enrollment and the follow-up period. Bacterial pathogens (35%) were detected more frequently than viral pathogens (32%), and 22% of patients had more than two pathogens. Co-detection of viral and bacterial pathogens was identified in 18% of patients. The prevalence of pathogens was comparatively examined in different age groups, clinical settings, and clinical outcomes. Microbial results of adult patients with pneumonia between deceased patients and surviving patients, as well as between ICU-admitted patients and non-ICU patients were also compared.

Deep-sequencing methods combined with neutralizing antibody detection provided evidence that rare HRV genotypes such as HRV-A21 and B91 are pathogens leading to severe community-acquired pneumonia. The combination of metagenomic analysis and conventional pathogenic testing methods will enhance the diagnostic ability for “unknown” pathogens in severe community-acquired pneumonia, facilitate the identification of new pathogens, and thus improve clinical management and surveillance of the infections.

Pneumonias’ Etiology Among Refugees and the Lebanese population (PEARL): preliminary results

Dr. Thomas Kesteman from the Mérieux Foundation, Lebanon, presented the PEARL (Pneumonias’ Etiology Among Refugees and the Lebanese population) project, whose main objective is to estimate the proportion of community-acquired pneumonia (CAP) attributable to specific viral and bacterial pathogens in the refugee and national populations of the country.

This multicenter case-control study will determine the fractional distribution of each causative microorganism according to time period, age of patient, and site. A total of 1,500 individuals from all age groups with CAP meeting the inclusion criteria of the study will be recruited at four primary health care centers. Biological testing is carried out on samples from urine, blood, nasopharyngeal swabs or sputum.

The preliminary results, from a population of 236 cases and controls, reveal that 21% of cases present more than one criterion for severe CAP, but only 1% was hospitalized, 46% of the individuals are less than 18 years of age, and 91% are Syrian refugees.

The range of etiological bacterial and viral agents has been identified with a predominance of viral etiologies (influenza, RSV, rhinovirus, hMPV, parainfluenza virus), and a low or null fraction attributable to *S. pneumoniae* or other bacteria. It is difficult to evaluate at present the effectiveness of vaccination from this dataset.

In the long run, the study should lead to undertaking steps to reduce CAP morbidity rates, and guide population-based health interventions, such as immunization campaigns or adapted CAP treatment guidelines for Syrian refugees.

Pneumococcal serotypes among children less than 5 years of age in developing and emerging countries: descriptive analysis from the GABRIEL pneumonia multicenter study

Cédric Danaché, a pharmacist at the epidemiology unit at the Hospices Civils de Lyon, France, described the GABRIEL multicenter prospective pneumonia case-control study that was carried out between 2011 and 2014. Hospitalized children meeting inclusion criteria for pneumonia were recruited over a one-year period from the eight participating sites located in nine countries. Samples of whole blood, serum, urine, and from nasopharyngeal swabs were collected from patient cases, and only nasopharyngeal swab samples were collected from patient controls. Pneumococci detected were categorized according to country of sampling, serotype of the pneumococcus, and were then tested by molecular analysis.

The results show that there is great heterogeneity in the prevalence of pneumococci in both cases and controls among the different countries. Further research will be conducted to determine the interactions between pneumococci serotypes and other microorganisms and the association between co-carriage/co-infection and severity.

Respiratory infectious disease among cohorts in Laos (LaCoRIS)

Dr. Valentina Picot from the Mérieux Foundation, France, presented LaCoRIS (longitudinal surveillance of respiratory infectious diseases in metropolitan Vientiane, Laos PDR), an ongoing prospective community-based cohort active surveillance study that should ultimately lead to cross-country comparisons of disease burden and epidemiology. Its objectives are to measure the incidence rates of acute respiratory diseases and identify the causative pathogens within the established catchment area in metropolitan Vientiane. The promotion of laboratory diagnostics capacity building for disease surveillance in Laos is an essential part of the study objectives.

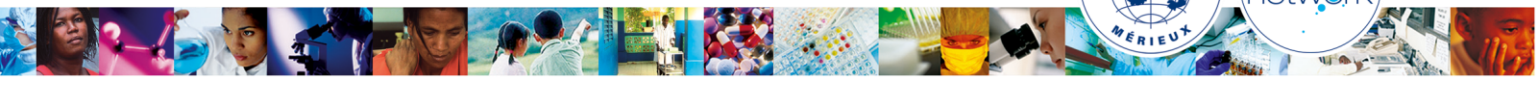
The catchment area of the LaCoRis Phase 1 population included 996 households from 25 villages whose 4,965 household members consented to having nasal and throat swabs and sputum samples taken. The Lacoris Phase 2 (ongoing) population comprises 1,082 households with 5,383 members. Microbial pathogens are identified in all the samples according to their estimated incidence, the patient's age group, and co-infection frequency. Preliminary data shows that *S. pneumoniae* (serotype distribution determined), *Chlamydia pneumoniae*, and the influenza A virus are the most often encountered.

When complete (project extended to 2019), the surveillance data should provide a better understanding of the types of pathogens at the onset of respiratory diseases that go undetected in the current passive surveillance systems in Vientiane. This should help establish a framework for systematic evaluation of future public health interventions in well-defined catchment areas.

Feedback from an external evaluation of GABRIEL

Dr. Hubert Endtz presented a summary of the responses received from the evaluation questionnaire sent to all GABRIEL member laboratories. The purpose of this questionnaire was to evaluate the overall performance of laboratory practices and to identify the quality assurance priorities that must be taken into account.

The structure of this evaluation was broken down into modules dealing with the GABRIEL network's objectives, its resources (staffing, equipment, funding), its accomplishments in training, technology transfer and research, plus its role in clinical activities, promotion of scientific careers, sharing of know-how, and partnership development. The results were categorized in a SWOT analysis.



All of the feedback from the questionnaires is available for consultation upon request. The overall recommendations from the GABRIEL respondees are summarized as follows:

- **Research:** continue to focus on and prioritize thematic topics, make SEED money available for projects, prioritize collaborative multi-centric studies, emphasize applied and operational research, shift from a lab-based to a clinical-based type of surveillance, promote greater LPE staff presence in the field (resources now concentrated in Lyon), incorporate PhD programs in new projects (up to 50);
- **Partnerships**: seek long-term institutional partnerships with global health centers of excellence, academic partners, and international consortia/research networks, use core SEED money for partnership-building;
- **Training**: map out existing research skills throughout the network, map out current needs in professional education, teach clinical and epidemiological skills at the local level, develop the role of LPE staff as initiators, coordinators, facilitators, and instructors, make greater use of advanced e-learning modules;
- **Communication**: disseminate more information on the network's key activities and reemphasize the network's mission through greater communication;
- **Management:** develop a 1-to-3 year overall GABRIEL strategic plan, decentralize network management, place greater emphasis on joint North-South leadership, retard the construction of new Rodolphe Mérieux Laboratories and consolidate existing ones;
- **Financing:** reach out to major donors, develop a global Rodolphe Mérieux Laboratory budget, shift funds allocated for construction to research and surveillance activities.

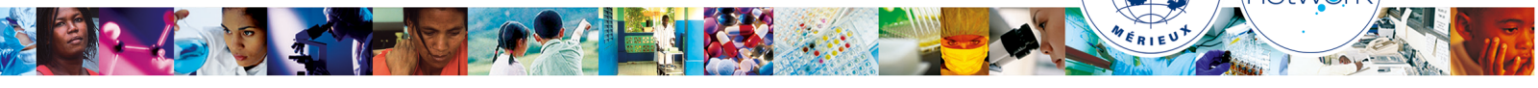
GABRIEL members have expressed their full appreciation for this evaluation. The next step is to prioritize these recommendations and also integrate the recent recommendations of the Mérieux Foundation Scientific Advisory Board, and turn them into an action plan for 2018 and beyond. Both core funds from the Foundation and external funding sources will be required to fully implement these various actions.

Session V: OTHER RESEARCH INITIATIVES WITHIN GABRIEL

Chaired by Antoine Andremont and Luc-Hervé Samison

Typhoid fever: its propensity in Bangladesh and the future ahead

Dr. Firdausi Qadri, from the Infectious Diseases Division of icddr,b, Dhaka, Bangladesh, described typhoid fever as globally reaching over 20 million cases and over 200,000 deaths each year, mainly in South Central Asia, Southeast Asia, and areas of southern Africa. *S. paratyphi* A currently accounts for 1 in 5 cases of enteric fever in Asia. Typhoid fever is very common in children from 6 months to 2 years of age. An enteric disease surveillance study is currently ongoing in nine locations in Bangladesh to determine the age distribution of positive cases, the antimicrobial susceptibility of the pathogens, and ultimately, to build local diagnostic capacity. In numerous countries, typhoid fever vaccine trials are underway, notably through the Strategic Typhoid Alliance Across Africa and Asia (STRATAA), whose goal is to study the microorganism's transmission and antibiotic resistance, so that diagnostic and vaccine strategies can be devised. In Bangladesh, the objective is to characterize the burden of enteric fever in the urban area of Mirpur, Dhaka, through a passive surveillance study of febrile patients from different healthcare facilities over a 2-two year period. In addition, there is a healthcare utilization survey and a household contact study underway to better understand the incidence and mode of transmission of the disease. A serosurvey and a study of antibiotic susceptibility patterns of isolated *S.* Typhi are also being carried out.



In addition to STRATAA, TyVac Bangladesh, a typhoid conjugate vaccine trial is presently assessing the impact of a vi-polysaccharide conjugate vaccine to prevent childhood typhoid, as compared to a control vaccine (Japanese Encephalitis Vaccine Live).

In the next few years, data from STRATAA and the TyVac study should eventually lead to the development of a typhoid conjugate vaccine. One that can protect against *S.* Paratyphi will require further research to prevent morbidity from all types of enteric fever.

Characterization of the antimicrobial resistance in Salmonella enterica serovar Typhi isolated in Bangladesh

Arif Mohammad Tanmoy from the Child Health Research Foundation, Bangladesh, described the characteristics of typhoid fever caused by the *Salmonella enterica* serovar Typhi that is especially rampant in developing countries, such as Bangladesh. However, the emergence and rise of antimicrobial resistance (AMR) is alarming and has become a major public health threat worldwide. AMR is encoded on a number of different chromosomal and extra-chromosomal genes that can be identified by Next Generation Sequencing (NGS).

Our current study seeks to identify resistant genes and mutations in *Salmonella* Typhi by whole genome sequencing (WGS), and to correlate those with resistance phenotypes. It also explores the prevalence of plasmids and genomic/pathogenic islands, as well as the genetic diversity of the microorganism in Bangladesh by phylogenetic analysis.

Clinical and epidemiological data collected by an NGS approach have thus far successfully detected genotypic resistance patterns that match laboratory-confirmed phenotypes for most *S.* Typhi isolates. Most mutations on *gyraseA/B* and *parC/E* genes cause interim resistance to ciprofloxacin. The evolution of *Salmonella* genomic islands 11 variants needs further study and the association of the bacterial efflux pump still needs to be explored. The final results of this study should lead to the possible identification of biomarkers for typhoid fever.

Epidemiology and antimicrobial resistance patterns of Salmonella Typhi and Paratyphi – possible impact of a typhoid conjugate vaccine

Dr. Samir Saha from the Child Health Foundation in Dhaka, Bangladesh, stressed the importance of conducting comprehensive epidemiological surveillance of enteric fever to guide treatment and prevention policies. This entails tracking the age distributions of typhoid and paratyphoid cases at the hospital and in the community: in other words in out- and in-patients. Comprehensive enteric fever surveillance is also important to understand patient antimicrobial susceptibility in a hospital and community environment. Without proper surveillance, drug resistance may be misunderstood as it varies between out- and in-patients, leading to inappropriate antibiotic therapy. Our study reports that none of the Paratyphi isolates showed multi-drug (ampicillin, cotrimoxazole and chloramphenicol) resistance. Although no significant increase in ciprofloxacin and ceftriaxone MIC has been observed, there are sporadic outbreaks with resistant strains, as recently seen in Pakistan. Third generation cephalosporin is the most recent antibiotic and it is commonly administered in Bangladesh. Both *S*. Typhi and Paratyphi are important vaccine-preventable pathogens, and a typhoid vaccine (not bivalent) is expected to be introduced soon.

New initiatives in vaccine prevention of typhoid fever

Dr. Robert Heyderman from the Division of Infection & Immunity, University College London, spoke about the high worldwide burden of typhoid fever that is increasingly seen to affect young children as well as teenagers and adults. In Africa, the typhoid situation is complex and less well-defined, as surveillance centers are scattered across the continent. In the past few years in Malawi, for example, there have been two outbreaks characterized by greater antibiotic resistance. Throughout Africa, typhoid has particularly gained momentum in urban centers. New conjugate typhoid vaccines have been tested to combat the current worldwide epidemic, but their full effectiveness has not been comprehensively evaluated. One of these vaccines is now being administered to children in Nepal as part of a randomized trial, and will be tested in Bangladesh and Malawi as well.

The TyVac consortium is a coordinating body for typhoid-related research and control activities that supports countries in decision-making and preparation for the introduction of a sustainable typhoid vaccine. It also provides data on impact, effectiveness, appropriate vaccination strategies, and associated costs, and it ensures typhoid and TCVs are recognized as global, regional, and national health priorities.

Typhoid diagnostics for measuring disease burden in three African countries

Stéphane Pouzol from the Mérieux Foundation's Emerging Pathogens Laboratory in Lyon, France, reviewed the current status of typhoid fever and its global incidence. The current study carried out in three African countries is an extension of a typhoid study that was developed in Bangladesh. In its initial phase, *S.* Typhi*, S.* Paratyphi A, and *S*. *enterica* from clinical samples were identified by real-time PCR directly from 1ml of whole blood but results were poor. In a second phase, the assay was evaluated in Bangladesh on larger volumes of blood samples (2/3ml) and compared to blood culture. This current third phase of the study consists in validating the assay in an African context (in Malawi, Burkina Faso, and Ghana) and of strengthening the knowledge of the typhoid fever burden in those regions. Preliminary results indicate that confirmed cases of typhoid fever are below expectations in the three countries. Similarly to Bangladesh, an increased detection of cases, mainly due to the identification of non typhoidal *Salmonella*, was observed and no *S*. Paratyphi A has yet been identified at any of the sites.

With sensitivity > 90% and a Negative Likelihood ratio at 0.08, the assay meets the criteria to rule out a diagnosis, but is not sufficient to confirm a typhoid diagnosis. In addition, the comparison of the performance with a PCR competitor or other diagnostic methods highlights the usefulness of this assay in surveillance or vaccine efficacy studies.

In conclusion, we have developed and validated a method to identify, directly from a limited volume of whole blood, pathogens responsible for enteric fever. The next step is to develop a strategy to disseminate this novel technique. Additional work is needed to benchmark PCR and others assays in order to define a Composite Reference Standard Method that may be useful to correctly evaluate the performance of methods in the field.

Retrospective study on the surveillance of hepatitis

Dr. Phimpha Paboriboune, Scientific Director of the Center of Infectiology Lao Christophe Mérieux (CILM), Laos, spoke about the high prevalence of persistent HBV infection in Southeast Asia. In Laos, despite the use of the HBV vaccine under the Expanded Program of Immunization since 2004, HBV infection rates continue to rise. In addition, hepatocellular carcinoma (HCC) is the most prevalent cancer in Laos in terms of incidence and mortality. In the absence of national guidelines, patients are subject to different treatment regimens and follow-up protocols.

The CILM is committed to the surveillance of infectious diseases in the Lao population, especially viral hepatitis. A retrospective study was carried out in a hospital setting between 2010 and 2016 to characterize the profile of HBV-infected patients testing for a HBV viral load. Blood samples were collected from these individuals across the country and were submitted for molecular tests. A second study is underway to identify specific biomarkers through metabolomic analysis. Results are pending.

The outcome of our study indicates that the number of HBV-infected patients at CILM has steadily grown every year. This may reflect a dramatic increase of the number of new infections. The fact that men are twice as likely to be infected as women is not unusual and this is commonly noted in most studies on HBV prevalence. There is also a higher prevalence of HBV chronic carriers among males than females. HCC affects more men than women.

The high level of HBV VL is significantly associated with patients less than 40 years of age, who are male, and who have an abnormal ALAT/ASAT. HBV DNA quantification represents the best marker of viral replication associated with HCC risk.

The picture drawn from this study is alarming in that over 60% of the patients have a very high HBV VL with aggravating factors: high ALAT/ASAT and a critically low level of treatment. All of the factors are in place for a continuing escalation of HCC in the near future.

In Laos, as equipment and skills are currently limited, it is urgent to reverse this disastrous trend through large-scale national vaccination campaigns. In addition, biological and medical therapeutic management of at-risk infected patients must become systematic and dynamic, with better coordination between medical and laboratory staff, improved up-to-date online surveillance networks, and more affordable diagnosis and treatment.

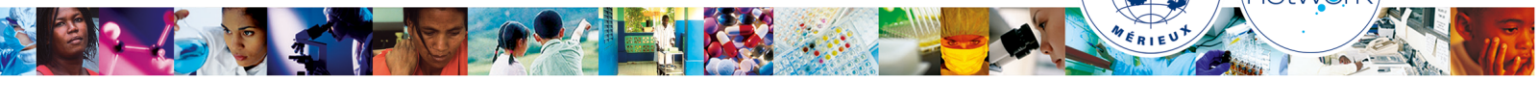
Bacterial and viral causes of fever in children less than 15 years of age in two hospitals in Bamako, Mali

Dr. Bourema Kouriba, from the Charles Mérieux Center for Infectious Disease in Mali, spoke about the paucity of information on the causes of febrile illnesses in Mali. The current practice of presumptive antibiotic treatment for febrile illnesses not only does it not convey benefit to most patients, but causes a number of problems: undesirable side effects and development of antibiotic resistance to pathogens. A better understanding of the locally relevant causes of fever could improve clinical decision-making and guide public health programs.

In our study, we recruited febrile patients from the pediatric wards of the Hôpital Gabriel Touré and Hôpital du Mali in Bamako. Blood samples from children meeting the inclusion criteria were submitted for laboratory diagnostic testing. We established an active hospital surveillance system for the following viral and bacterial agents that cause fever and, in most cases, flu-like symptoms: a) viral agents: Ebola virus, Lassa virus, Rift Valley fever virus, Crimean-Congo hemorrhagic fever virus, dengue virus; b) bacterial agents: *Salmonella* Typhi, non-typhoidal *Salmonella enterica* ssp., *Leptospira* spp., *Brucella* spp., *Streptococcus.*

This study is ongoing and should reach 1,000 children and provide additional screening for viruses causing chikungunya and yellow fever. Molecular detection of AMR should follow.

The results should lead to improved management of fever cases in pediatric wards, and better epidemiological surveillance to predict outbreaks of diseases. As Malian physicians encounter infections that they are unfamiliar with, many of these diseases often go unrecognized and therefore remain untreated.



SESSION VI: Quality initiative

Chaired by Lili Ren and Graciela Russomando

How to implement a Quality Initiative in a research laboratory? Challenges and main improvement at the Emerging Pathogens Laboratory

Mélina Messaoudi from the Mérieux Foundation's Emerging Pathogens Laboratory, Lyon, spoke about the quality assurance initiative that was launched in the GABRIEL network in 2015. Its objective is to have members qualify for the ISO 15189 accreditation that applies to medical laboratories for the provision of clinical diagnostic services. The laboratory in Lyon is participating in this quality initiative, but at the level of ISO 17025 accreditation that applies to reference and research laboratories seeking to be recognized for their competence in novel assay development and calibration of methodologies. Changes in our internal organization and management systems should contribute to having us deliver and validate qualitative novel innovative solutions in the detection of pathogens.

A quality management audit of the laboratory was performed by the COFRAC (COmité FRançais d'ACcréditation) in December 2016. It identified several components of the laboratory management system that require upgrading: document control, sample inventories, processes for stock/consumables management, equipment maintenance. Based on the audit report, an action plan was developed in 2017 with nine working groups to take corrective action on the non-conformities so that the laboratory can qualify for ISO 17025.

The Quality Initiative: results and perspectives

Dr. Nicolas Steenkeste, from the Mérieux Foundation, France, gave an overview of the different ISO standards that concern medical laboratories:

- ISO 15189:2012 - Medical laboratories: requirements for quality and competence
- ISO 17025:2005 - General requirements for the competence of testing and calibration laboratories
- ISO 15190:2003 - Medical laboratories: requirements for safety

Laboratories from developing countries are encouraged to first meet their national standards, and then progressively phase-in the requirements of the internationally-recognized ISO standards.

The Laboratory Quality Stepwise Implementation (LQSI) tool from WHO is based on 12 quality system essentials: facilities & safety, organization, personnel, equipment, purchasing & inventory, process management, information management, documents & records, customer focus, assessment, non-conformity management, continual improvement.

At the Mérieux Foundation, a working group on quality has come up with a flowchart designed to guide laboratories from the initial need for quality improvement to the final accreditation step.

These processes involve an evaluation and the completion of a checklist, a roadmap, so to speak, with indicators that are to be progressively validated in four phases.

Five GABRIEL member laboratories are in the process of obtaining accreditation: Instituto de Investigaciones en Ciencias de la Salud in Asuncion, Rodolphe Mérieux Laboratory in Vientiane, Rodolphe Mérieux Laboratory in Chittagong, Rodolphe Mérieux Laboratory in Bamako, and Rodolphe Mérieux Laboratory in Port-au-Prince. The program is now starting at Rodolphe Mérieux Laboratory in Beirut.

The program is also developing e-learning tools for knowledge sharing on Quality Management and is also creating a YouTube channel with quality experts.

YOUNG SCIENTISTS AWARD CEREMONY

Dr. Juliet Bryant presented the GABRIEL 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 our future leaders in the fight against infectious disease.

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

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

- ***Aline da Rocha Matos** for her work on the antiviral susceptibility profile of flu viruses in Brazil, and
- ***Silaphet Somphavong** for her research on the molecular epidemiology of *M. tb,* and on antibiotic resistance in Lao PDR.

The two winners were congratulated for their outstanding contribution to science.

CLOSE OF THE GABRIEL MEETING

Benoît Miribel, Director General of the Mérieux Foundation, brought this year's edition of the GABRIEL international meeting to a close with warm thanks extended to all the participants for their contribution. In its ten years of existence, the GABRIEL network has always moved forward with new ideas, testable hypotheses, and project designs. Now is the time to apply certain specific audit recommendations, to take the network to a new level.

We must never lose sight of our mission: to share knowledge, to keep the laboratories at the center of our actions and in our line of vision, to make a true impact in terms of health, scientific innovation, relief to populations, and scientific career opportunities for the youth of the developing world. The commitment of each individual member of our network, the time spent to share and work with others is part of the fundamental purpose of our network. By working collectively we become more efficient. We believe that being together is one facet of our battle against infectious diseases. We should be proud of our achievements and we should look forward to our next phase of accomplishments.