



SPECIAL REPORT

8th ANNUAL GABRIEL NETWORK MEETING July 5-8, 2016

Vientiane, Laos

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From July 5 - 8, 2016, more than 100 researchers, physicians, specialists in laboratory diagnostics, university academicians, and scientists, both from the public and private sectors, arriving from over 27 countries, convened in Vientiane, Laos, for the GABRIEL network's 8th annual meeting. The first two days were set aside for the Symposium on Antimicrobial Resistance at which experts and representatives from Southeast Asia presented the latest updates on AMR surveillance and public health interventions in their respective countries. The GABRIEL meeting has become the time-honored occasion to share experiences, present new collaborative opportunities, review advances in quality assessment, and discuss the latest scientific developments in infectious diseases. This year's program also included the "GABRIEL Young Scientist Award", created last year as an initiative to help boost the career development of promising scientists from low and lower-middle income countries by giving young researchers the opportunity to present their research. Two winners were selected this year for the best presentations.



PROCEEDINGS

Dr. Florence Komurian-Pradel, Manager of the GABRIEL network 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 has been a reflection of the commitment of each of its members to:

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

The two new members of GABRIEL were presented: King George's Medical University in Lucknow, India, a tertiary care hospital and teaching institution, and the Centre Pasteur du Cameroun (CPC), an establishment of the Ministry of Public Health located in Yaounde, Cameroun.

Prof. Hubert Endtz, Scientific Director of Fondation Mérieux, in his introductory address, welcomed participants to this year's gathering. He made special mention of this year's two-day Symposium on Antimicrobial Resistance as an opportunity for specialists from Laos, Cambodia, Thailand, and Vietnam to report on national policies and government interventions that have been set up to help combat the ongoing threat of antimicrobial resistance to antibiotics.

In presenting the historic roots of Fondation Mérieux, he described the main thrust of the work being carried out in the GABRIEL network:

- Collaborative research — especially on pneumonia, and tuberculosis through the launch of a working group for each of these diseases. This should help develop innovative ideas and solutions and bring in new partners,
- Technology transfer,
- Training and knowledge-sharing worldwide, especially in the fight against antimicrobial resistance. The GABRIEL network is poised to take action on a global scale, not only in the areas of diagnostics and research, but also veterinary medicine and antibiotic stewardship.

Madame Claudine Ledoux, French Ambassador to Laos, an honorary guest, reiterated France's full commitment to scientific research, and its readiness to reaffirm the responsibility of the State to ensure that vulnerable populations have access to medical services. This represents a huge challenge. Public health services must lay the foundations. To do this, they need the support and contribution of the entire scientific community, especially in fighting antimicrobial resistance. We need to encourage the appropriate use of medication in humans and animals, without forgetting the economic and social impact of the strategic use of drugs.

Dr. Bounkong Sihavong, the Minister of Health of Laos

In his opening remarks, Dr. Bounkong Sihavong expressed his government's full dedication to research in the fight against infectious disease in low-income countries. Antimicrobial resistance is ubiquitous, a matter of global concern, one of the world's most pressing health issues. He considers this symposium to provide an opportunity to find some answers.



INTRODUCTORY SESSION

Lecture on the ZIKA Virus

Dr. Monica Chan from the Institute of Infectious Diseases and Epidemiology of the Tan Tock Seng Hospital, Singapore, gave an overview on the Zika virus (ZIKV) infection currently spreading, and described the symptoms of the infection, comparing them with those of dengue fever and chikungunya. In terms of epidemiology, as of June 29, 2016, 61 countries and territories reported continuing mosquito-borne transmission, mostly in Central America, South America, and Southeast Asia.

Regarding the preparedness for Zika virus infection in Singapore, the Ministry of Health (MOH) of Singapore issued a circular on January 27, 2016, providing treatment guidelines for suspected and confirmed Zika cases. This document included advice on clinical symptoms, laboratory testing, hospital admission, confirmed case management, and discharge criteria for patients. Revisions are being made periodically to reflect updates in the ZIKV situation.

The current techniques for ZIKV diagnostic testing of serum, urine, and saliva samples by RT-PCR for viral RNA were presented. Serology for IgM and neutralizing antibodies, PRNT, and immunohistological staining for viral antigens or RT-PCR on fixed tissues are other possible techniques that can 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). Hence, plaque reduction neutralizing antibody testing (PRNT) may be helpful to distinguish between cross-reacting antibodies in primary flavivirus infections.

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. WHO also has come up with its own algorithm.

Sexual transmission of ZIKV is also a research topic and the MOH circular provides advice on the prevention of this mode of transmission.

Case control studies have been carried out on the association of Zika virus infection with the Guillain-Barré syndrome in French Polynesia. The association of ZIKV with birth defects is also a major cause of concern.



SYMPOSIUM ON ANTIMICROBIAL RESISTANCE

July 6, 2016

Session I: AMR SURVEILLANCE

Chaired by Erika Vlieghe, Belgium, and Ly Sovann, Cambodia

Antimicrobial Resistance in Laos: ESBL Infection

Prof. Valy Keoluanghot, Head of the Infectious Disease Center of the Mahosot Hospital, Laos, spoke about the epidemiological challenge of antimicrobial resistance in Laos. Many species of *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBL) are resistant to multiple classes of antibiotics. ESBL organisms are also a major point of concern both in terms of community-acquired and nosocomial infections, and they appear to be carried in healthy people in remote communities. These microorganisms represent an increasingly important cause of human infections worldwide and are associated with severe adverse clinical outcomes, such as septicemia. However, predicting patients who are likely to contract ESBL septicemia is difficult, as diagnosis depends on having a high index of suspicion and the capacity to perform high quality microbiology diagnostics. But in Laos, only one laboratory, at the Department of Microbiology at Mahosot Hospital, is equipped to provide cost-free blood culture and quality assured susceptibility testing. It routinely runs ESBL detection on isolates of *E. coli* and *K. pneumoniae*. Results are showing an increasing number of infections that are difficult to treat due to resistance to cephalosporins (widely used empirically), and often to gentamicin and fluoroquinolones. Given the widespread improper use of antibiotics in Laos, it is important to promote “Antibiotic Stewardship”, and to seek affordable alternative antibiotics. There is also the danger that empirical therapy being used is compromised. Antibiotic resistance in Laos has lagged behind neighboring countries. Control strategies are urgently needed to fight the major and growing problem of resistance in Gram-negatives, such as *E. coli*, and *K. pneumoniae*.

Challenges in AMR Surveillance, Cambodian Provincial Hospital Perspective

Joanne Letchford, Cambodia Country Director of the Diagnostic Microbiology Development Program (DMDP), described Cambodia’s National Strategy to Combat AMR developed by the Ministry of Health of Cambodia in collaboration with partners. The strategy comprises the following components: a commitment to a Master Plan to combat AMR; the strengthening of laboratory capacity and of AMR surveillance; uninterrupted access to essential medicines of assured quality; regulated and rational use of medicines; greater infection prevention and control; and innovation and research and development of new tools.

Ms. Letchford listed the possible solutions to ten major challenges facing the country in laboratory capacity and AMR surveillance:

- 1) Lack of adequately trained laboratory staff has been addressed by long-term onsite mentoring and capacity-building activities, and the development of a sense of professional responsibility and professional ethics through role modeling by mentors (low salaries have been a barrier),



- 2) Training in the appropriate use of microbiology services (e.g. collection of good quality specimens, understanding appropriate diagnostic testing, and understanding microbiology reports),
- 3) Quality media production and distribution by the creation of a central media-making facility (supported by the Diagnostic Microbiology Development Program) that ensures availability of quality controlled media for diagnostic testing in national and provincial hospital labs,
- 4) On-site mentoring in accurate pathogen identification and Antibiotic Susceptibility Testing (AST) using sustainable basic diagnostic testing with attention to internal quality control,
- 5) Implementation of international standards for AST (guidelines prepared, networking with other labs, onsite mentoring and training workshops),
- 6) Access to quality microbiology supplies (the Ministry needs to know what labs need, laboratories need to know how to manage supply inventory, storage, and procurement),
- 7) Laboratory Quality Management (LQM) (via training in LQM alongside increasing technical capacity, implementation of regular internal quality control, participation in External Quality Assessment and regular internal audit),
- 8) Coordination of activities to avoid competing priorities (communication and coordination of activities with the many partners working in strengthening laboratory capacity in Cambodia),
- 9) Reliable data entry and management through standard reporting and data review before data dissemination,
- 10) AMR surveillance through the development of national AMR surveillance of four priority blood culture pathogens and implementation of monthly microbiology activity reports (monthly reports of pathogens and susceptibility patterns are submitted to the Ministry of Health).

In response to a question raised by the audience, Ms. Letchford acknowledged that internal quality control is indeed a challenge. Mentors assigned to the laboratories remain on site and encourage and guide staff to develop good work habits and accept responsibilities.

Antibiotic Stewardship and HCAI Surveillance at Angkor Hospital for Children

Dr. Pasco Hearn, clinical microbiologist at the Cambodia Oxford Medical Research Unit (COMRU), spoke about Healthcare Associated Infection (HCAI) as being one of the most common preventable adverse events worldwide. He discussed the value of point prevalence surveys, but highlighted the increased accuracy and utility of prospective surveillance, producing HCAI incidence rates. He is currently involved in HCAI surveillance, alongside teaching medical students and junior doctors, producing antibiotic guidelines and helping to provide a clinical infection service to the wards of Angkor Hospital for Children (AHC) in Siem Reap. Surveillance throughout 2015 found an HCAI incidence rate at the hospital of 4.6 per 1,000 patient-days (95% confidence interval (CI) 3.8–5.6) and an attack rate of 3.1 HCAI per 100 admissions (95% CI 2.5–3.8). Respiratory infections made up 52.9% (54/102) of cases and Gram-negative organisms made up the majority of clinically significant isolates 13/16 (81.3%). Antimicrobial resistance was widespread, with resistance to third-generation cephalosporins in 12/16 (75%) isolates. While the incidence was lower than expected, the need for more expensive antibiotics and prolonged admissions meant that HCAI cases produced a huge cost, estimated to be close to \$300,000. HCAI reduction can be promoted by introducing education programs, hand hygiene



monitoring, infection guidelines and antibiotic stewardship. The encouraging results so far demonstrate that simple, affordable measures can be effective. Ongoing HCAI surveillance can help gauge the efficacy of ongoing infection prevention and control programs.

WHONET Implementation and the AMR Surveillance System in Thailand

Aekkawat Unahalekhaka from NIH, Department of Medical Sciences, Thailand, and **Dr. John Stelling** from WHO Collaborating Centre for Surveillance of Antimicrobial Resistance, Boston, spoke about the Surveillance Network being set up at the National Antimicrobial Resistance Center, Thailand, with the support of WHO. The objectives of the Surveillance Network are 1) to monitor the magnitude and trend of antimicrobial resistance among bacteria isolated from humans, 2) to disseminate information on a regular basis, and 3) to standardize lab techniques and strengthen support for quality assessment in the clinical microbiology laboratory. Achieving these objectives entails the collection and analysis of data on antimicrobial susceptibility, followed by dissemination of this information via workshops on new techniques, AMR detection, WHONET, and report systems. In addition, a material support system has been set up that supplies culture media, antimicrobial susceptibility test discs, E-tests, multiplex PCR detection of resistant genes, etc. An external quality control is run twice a year and corrective action can be taken, if needed. In 2016, there were 88 participating hospitals in Thailand.

WHONET is cost-free Windows-based database software developed for the management and analysis of microbiological and clinical data with a special focus on antimicrobial susceptibility test results. The use of common software supports local, national, regional, and global collaboration and analyses to support: 1) recognition, tracking, and containment of emerging threats, 2) cost-effective patient care, and 3) public health policy and interventions. Over 2,300 labs worldwide now use WHONET as well for quality control, geographic tracing of outbreaks, cluster alerts, creation of AMR scatterplots, etc. WHONET also provides the tools for a global microbial sensor network, whereby data is collected, centralized, and redistributed to selected databases. WHONET forms part of a quality control strategy.

Beta-lactam Resistance Among *Enterobacteriaceae* in Cambodia: the 4-year Itch and Presentation of the BIRDY Project

Dr. Alexandra Kerléguer, head of the Medical Biology unit at the Pasteur Institute, Cambodia, described the challenges related to AMR surveillance and laboratory capacity in Cambodia. The provision of uninterrupted access to essential quality medication, as well as regulation and guidance on the rational use of antibiotics in human health are other areas that must be addressed. Cambodia urgently needs robust information on the extent of AMR to guide adapted public health interventions. The surveillance study carried out between 2012 and 2015 focused on the characteristics and progression of ESBL *E. coli* and *K. pneumoniae*. Resistance of these bacteria to fluoroquinolone, cotrimoxazole, gentamicin, and aminoglycosides was tested. The results showed a gradual and statistically significant increase of ESBL. In addition, carbapenemases, first identified in 2014, are on the rise and imipenem-resistance in *Acinetobacter* and *Pseudomonas* needs to be closely monitored.



Interventions to Reduce Antimicrobial Use and Antimicrobial Resistance in Animal Production Systems in Southeast Asia

Dr Juan Carrique-Mas from the Oxford University Clinical Research Unit (OUCRU), Vietnam, presented ViParc, the Vietnamese Platform for Antimicrobial Reduction in Chicken Production. Increased AMR/AMU in animal production, now undeniably present, has appeared due to several factors. First, rapid urbanization and economic growth has stimulated per capita consumption of animal protein (by nearly three-fold), and more intensive and more specialized production systems generate an incentive for export of meat products. There is a lack of veterinary supervision and an absence of expert veterinary advisors. Antimicrobial compounds in medicated animal feeds, often unlabeled, enter into a complex environmental cycle that eventually contributes to infections in humans through leakage of antibiotics and chemicals. This has been observed not only in Vietnam, but also in Laos and Cambodia. An AMR study has revealed a carryover of AMR from flocks to wild animals, such as rats, due to a lack of disinfection.

The aims of the ViParc project are to quantitatively reduce antimicrobial usage by 33 to 50% by providing farmers with a locally-adapted veterinary support system, and to elucidate the relationship between antimicrobial usage, farming practices, and antimicrobial resistance.

In terms of more overall aims, ViParc seeks to:

- 1) provide the government with practical advice on reducing antimicrobial resistance in chicken farming systems by using the results of socio-economic analyses,
- 2) strengthen the veterinary advisory and diagnostic capacity for improved surveillance, treatment, and prevention of poultry diseases in Vietnam by mapping out the diseases commonly affecting chickens in the Mekong Delta.

A farmer training program, one key factor in achieving these goals, should teach chicken farmers good farming practices and recordkeeping, should explain prevention and control of diseases, and should convey the usefulness of proper waste management and environmentally sustainable practices. The ViParc project is ultimately expected to characterize the drivers of AMU/AMR in poultry farms, map out bacterial diseases, measure the economic impact of infectious diseases and AMU/AMR, help develop veterinary and laboratory expertise, provide guidelines on disease surveillance, and render advice to the government on these topics.



Session II: PUBLIC HEALTH INTERVENTIONS

Chaired by John Stelling, WHONET

Antibacterial Resistance: an Ecologically Complex Public Health Threat

Prof. Antoine Andremont, from Bichat Hospital, France, spoke about antibacterial resistance as an ecologically complex public health threat, giving as an example the trend of the rising incidence of the ESBL burden of disease at the Bichat university hospital in Paris. Despite the application of hygiene programs in the hospital, the rate of incidence of community-acquired (often food-borne) AMR diseases has increased dramatically. On a global scale, the ease of international travel, the use of antimicrobials in the chain of animal food production, and the presence of active pharmaceutical ingredients in waste effluents are some of the many factors that have contributed to the AMR resistance patterns being witnessed. Bacterial resistance has become a major threat. It is ecological in nature with no region of the planet being spared. The fight against AMR entails greater awareness, innovation, money, and brains.

National Policy to Combat AMR

Dr. Ly Sovann from the CDC presented Cambodia's first National Policy to combat antimicrobial resistance that was approved by the Ministry of Health in March 2015. It has been formulated to mobilize resources and educate key stakeholders and the general public on the emergence and spread of AMR. It is designed to enhance laboratory capacity for detecting and monitoring AMR, as well as to ensure uninterrupted availability of essential medicines for disease management. Its intent is to regulate and promote the rational use of medicine in humans and in food-producing animals, as well as to enhance the prevention and control of infections. Research and development are key factors to achieve these objectives. Coordination at the national and subnational levels must be strengthened and good governance and accountability relative to these measures must be secured. This National Policy has already made progress in reaching its objectives. A coordinated mechanism has been set up, which includes monthly AMR working group meetings with all parties concerned.

In terms of surveillance, monthly reports on antibiotic resistance are being submitted from six government laboratories. A standard antibiotic resistance form is currently being developed for laboratories to report cases of antibiotic resistance. A national campaign has been launched to raise awareness of AMR and promote behavioral change by the sharing of knowledge via the media and internet, and AMR has become a priority in the National Public Health Emergency Plan. These are positive steps in the right direction.

There is still much to be done. Coordination and collaboration between the various health sectors must further develop to ensure commitment from all stakeholders. The surveillance system must continue to monitor levels of antibiotic resistance and antibiotic utilization. The success of awareness campaigns needs to be evaluated and communicated to all interested parties.



AMR in Vietnam: Extent of the Problem and Government Response

Prof. Nguyen Van Kinh, from the National Hospital of Tropical Diseases, Vietnam, gave his talk on effective government processes to develop and implement policies and guidelines for controlling AMR in Vietnam. The Global Antibiotic Resistance Partnership (GARP) has carried out research projects on topics dealing with AMR in 15 hospitals, as well as on the range of antibiotics being dispensed in the community, the use of antibiotics in agriculture, antibiotic resistance in *V. cholerae*, AMR in *Acinetobacter baumannii*, and NDM-1 in hospital environments.

Vinares, the Vietnam Resistance Project, is designed to strengthen national control of the use of antibiotics in the country. Vinares has been monitoring antibiotic consumption and antibiotic resistance in major hospitals in Vietnam.

Government-funded research projects have been studying the application of molecular technology to determine AMR patterns in common pathogens in Vietnam. The government's response to the findings from these studies has led to the development of a national plan for the period 2013-2020 to combat drug resistance. This plan is designed to 1) improve public awareness and inform health professional about drug resistance, 2) strengthen the capacity of national surveillance system to measure antibiotic use and resistance, 3) ensure that quality, essential drugs are sufficiently dispensed, 4) improve rational and safe use of medication, 5) improve infection control, and 6) improve the rational and safe use of antibiotics in agriculture.

Translation of Surveillance Findings into Treatment Guidelines and Educational Activities

Dr. Kruey Lim from the Sihanouk Hospital Center of Hope (SHCH), Cambodia, described SHCH, created in 1996, as one of the leading non-governmental hospitals in Cambodia, managed by a US-based organization, HOPE. Its mission is to provide for the advanced education and clinical training of medical professionals, while delivering high-quality, free medical care for the poor and disadvantaged in Cambodia. The study, Blood Stream Infection (BSI) Surveillance, has been running since 2007, and performs routine blood cultures on all patients presenting SIRS (Systemic Inflammatory Response Syndrome). The identification of key bacterial pathogens and their resistance patterns, and evidence obtained from surveillance findings has resulted in the redaction of practice guidelines for improved patient care. The forthcoming challenges lie in the translation of findings to clinical care and the proper application of these guidelines. This should lead to the rational use of antibiotics and improve infection control.

Interventions to Reduce AMU/AMR in ICUs

Lan Nguyen-Phu from the Microbiology Department of the Hospital of Tropical Diseases, Vietnam, began by presenting an overview of common multidrug-resistant organisms (MDRO) in intensive care units (ICU) and the risk factors associated with different bacterial pathogens. The most common pathogens are *Acinetobacter baumannii*, *P. aeruginosa*, MRSA, *Stenotrophomonas maltophilia*, and *K. pneumoniae* ESBL. Strategies to combat MDRO involve infection control (e.g. hand hygiene, environmental cleaning, shortened ICU length of stay, and avoidance of biofilm burden), and antibiotic management (antibiotic guidelines and restrictions, use of narrow-spectrum antibiotics, antibiotic cycling and scheduling changes, and optimization of pharmacokinetic and pharmacodynamics principles). The Hospital of Tropical Diseases has recently issued antibiotic guidelines.



Session III: ROUNDTABLE

Moderated by Antoine Andremont, France, and Thong Phe, Cambodia

Restitution of the AMR Survey Results

Prof. Antoine Andremont, from Bichat Hospital, France, reported on the results of the AMR questionnaire that had been sent to 64 participating laboratories. Despite the 50% response rate, the findings were significant, as the main objective of the survey was to raise awareness. In summary, almost all of the labs report that they conduct surveys related to AMR, mostly at an individual level. As for surveillance in the consumption of antibiotics, 10 said they did and 13 said they did not. In response to the question on whether the official bodies of each laboratory's country have a surveillance plan for AMR, 50% said yes. One half of the labs said that they were unaware of any manufacturing of antibiotics in their country. Almost all laboratories expressed interest in taking part in an international survey on AMR.

One Health Approach to Controlling AMR

- **Stopping AMR is Everybody's Business**

Professor Visanu Thamlikitkul from Mahidol University, Thailand. Thailand's antimicrobial resistance (AMR) containment and prevention program has been carrying out several activities and taking a "One Health" approach since 2013: (i) horizontal actions i.e. estimating national AMR burden; establishing dynamics of AMR chains to understand how AMR in Thailand develops and spreads; developing national infrastructure of AMR containment and prevention, laboratory and information technology systems for surveillance of AMR, antibiotic use and hospital-acquired infections, AMR containment and prevention package; regulating distribution of antibiotics to be used in humans and animals; generating local evidence for promoting responsible use of antibiotics and efficient practices for infection prevention and control in the local context; raising awareness campaigns on AMR and AMR containment and prevention; conducting research and development on diagnostics, therapy and prevention of AMR infections, and (ii) vertical action i.e. implementing AMR containment and prevention in selected pilot communities to determine the needed resources, benefits, key success factors, obstacles and barriers of such implementation. The main campaigns focus on stopping the development of antimicrobial resistance by promoting responsible use of antibiotics, and stopping AMR infection and transmission by promoting good sanitation and hygiene, and compliance to infection control and prevention practices.

The program will be evaluated according to local, national and international indicators and targets. Once the program's success is confirmed, the plan is to expand it nationwide.

- **The Thai Ministry of Public Health's Point of View**

Dr. Suriya Wongkongkathep from the Ministry of Public Health, Thailand, gave the Ministry's point of view on the fight against antimicrobial resistance. The WHO Global Action Plan on AMR and the Global Health Security Agenda initiative, to which Thailand contributes, have successfully established a political platform for a global response to AMR. Under the Global Action Plan, each member state must develop an action plan as a matter of priority. This is the case, for instance, in Cambodia and AMR has become an ASEAN health priority. On a national scale, under Dr. Wongkongkathep's responsibility, an action plan has been launched in Thailand and should run through 2018. The drafting process started in 2014



with a landscape report of AMR key findings that determined, for the time being, several limitations, such as lack of a coordinated working mechanism against AMR, inadequate political engagement, and poor awareness among policymakers and the general public. As a result, an integrated framework was devised to tackle these problems.

The core principle of Thailand's national AMR action plan is to establish a national policy and a collaborating mechanism, taking a One-Health approach to address AMR, and to carry out sustained and effective measures against AMR.

The ultimate goals of the action plan are to create greater awareness of AMR, to stimulate partnership and engagement of stakeholders, to invoke political and policy engagement, and to encourage internal and global collaboration and support. Multi-sectoral meetings have already been held and leveraged to help shape political agenda. A further, but critical, step is to propose the establishment of a dedicated policy committee.

Questions raised by the GABRIEL meeting participants:

Q: Once legislation has been passed and funds allocated by the government, how are priorities determined in terms of spending?

A: It will be a hard task to arrive at a compromise because so many parties are involved: FDA, medical and veterinary services, government administration, etc.

Q: It seems that the whole process is taking too long, despite all the funds that are available. Time is of the essence because AMR is life-threatening. Is there any indication that concrete actions have been carried out? Have the authorities applied recent legislation on this matter?

A: We are moving forward. There have been many accomplishments during this past year. Momentum is building. The best approach is to invest in infrastructures for rapid testing, providing access to diagnostic equipment and keeping labs open late - and these are examples of measures that help reduce the needless prescription of antibiotics.

Q: Are we sure? Sanctions need to be applied, but public support is needed. In France, for example, doctors get a bonus for prescribing fewer antibiotics. The Far East is not at that stage yet.

A: Thailand is aiming at increasing public awareness by 20%, which is ambitious enough. Instead of enforcement of possible sanctions, Thailand is aimed at collaboration, because multiple agencies are involved.

- **The Veterinary Services' Point of View**

Dr. Juan Carrique-Mas from OUCRU, Vietnam, first mentioned that antimicrobial use (AMU) in animal production responds to economic drivers. AMU and AMR in animal production have impacts on animal/human health, on the environment, and on farm productivity. He listed some of the proposed actions needed to move forward in AMR surveillance: in animal production systems, there should be farmer training programs on the treatment and control of animal diseases, an increased AMR awareness campaign, and the development of vaccines that reduce the incidence of endemic bacterial diseases in animal production. In terms of AMU, veterinary pharmacists should have a better understanding of antimicrobial prescription practices and receive training. In addition, licensing procedures for antimicrobial drugs should be not only be tightened, but the quality of feed and antimicrobial products should be policed. Laboratory and veterinary advisory capacities should be developed with the farmer in mind. The range of antimicrobial products available for different production systems should be limited.



As for the environment, the impact of farm waste on ecosystems should be measured, solutions should be found to reduce the spillover of antimicrobials and AMR bacteria from animal waste into the environment, and farmers should be trained on environmentally sustainable farming practices and waste management. A Government body needs to be set up to oversee AMU and AMR in animal production systems with clearly defined targets and a timeline. A surveillance system of AMU must be instituted beginning with compulsory notification by industry.

The default inclusion of antimicrobials in animal feed must be eliminated, farming techniques using fewer antimicrobials should be promoted, a regulatory framework for farms with respect to biocontainment and antimicrobial residues must be strengthened, and a farmer accreditation scheme with a strong component on prudent AMU should be introduced.

Comparing animal, human, and food chains is difficult because animal antibiotics are for prevention - not treatment - and are a business component. Farmers are reluctant to change their habits since they are driven by productivity. Farmers must understand that their production is vulnerable to resistant infections, but as farmers do not see any infections, it's hard to make that case.

In summary, the priorities are 1) the development of basic surveillance systems for AMU and AMR, 2) government initiatives to improve veterinary advisory capacity for AMU and animal diseases, and 3) the tightening of legislation and enforcement of use of antimicrobials.



GABRIEL NETWORK MEETING

July 7–8, 2016

Feedback on the GABRIEL Research Landscape

Dr. Florence Komurian-Pradel from the Emerging Pathogens Laboratory (LPE), Fondation Mérieux, France, reviewed the GABRIEL network's missions in 1) collaborative research — especially on acute respiratory infections, food-borne diseases, tuberculosis and typhoid fever, 2) technology transfer — diagnostic tools, typing of tuberculosis, pneumonia and meningitis, and 3) training and knowledge-sharing.

GABRIEL collaborates with other global networks. In 2014, GABRIEL joined the Global Influenza Hospital Surveillance Network (GIHSN), as a coordinating partner, to study and identify strains of the influenza virus and evaluate the effectiveness of vaccination in three Brazilian hospitals. GABRIEL has also worked with the African typhoid network. In addition, GABRIEL is a member of COMPARE, a collaborative management platform that seeks to improve the identification of emerging infectious diseases and food-borne outbreaks and to identify pathogens and resistant genes for epidemiological purposes with the use of molecular technology (NGS/WGS). COMPARE aims to establish a database of clinical isolates and to create a framework for sequence-based identification and characterization of clinical isolates.

GABRIEL is also involved with SPRINT-SARI (Short PeRIod IncideNce sTudy of Severe Acute Respiratory Infection) the objectives of which are to establish a research response capability for future epidemics/pandemics through a global observational SARI study. It seeks to describe the clinical epidemiological and microbiological profiles of patients with SARI.

GABRIEL has worked with EVAg (European Viral Archive goes global). It is a European-funded program that mobilizes a global network devoted to the characterization, conservation, production, and distribution of biological materials in the field of virology.

GABRIEL's other area of focus is training and the organization of workshops in immunology, molecular biology, biosecurity, and quality assurance. Specific courses on molecular tests for tuberculosis have been taught at the LPE for participants from Laos, Georgia, and Bangladesh. Courses in molecular tests for typhoid detection have been run at the LPE for participants from Malawi. Upcoming courses on molecular tests for the detection of respiratory pathogen and typing are being planned.

GABRIEL is also very interested, in the future, to participate in an initiative of the WHO Advisory Group, AGISAR, that is conducting a global survey on ESBL-producing *E. coli* as part of a One Health approach.

Impact of a Research Network in a Developing Country

Dr. Graciela Russomando, from the Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Asunción, Paraguay, spoke about the benefits of laboratories belonging to a research network such as GABRIEL from a developing country's perspective:



What do we expect, as a developing country, from a collaborative research network? A research network must focus on important and relevant health problems by providing: a platform to discuss common health issues, access to new information and methodologies, and technology transfer (to avoid having to “reinvent the wheel”). It must open opportunities for professional education and create opportunities for the development of leadership skills. Also, there is a “direct benefit”, because jointly planned research has a better chance of achieving success with widespread problems than an isolated effort.

A research network for developing countries must focus on:

- standardized protocols that are common to all,
- sites in different regions with similar health problems,
- evaluating low cost interventions,
- sustainable interventions to improve public health,
- building local research capacity and infrastructures,
- promoting a partnership between the universities, the Ministry of Health, and NGOs.

To ensure its success, a research network in a developing country must set clear objectives, have a solid base of good quality data, and have a comprehensive, qualitative and quantitative knowledge of the issues and their impact. In most developing countries, one of the problems we face has to do with the long distances between health centers and difficulties for access to diagnosis to provide proper treatment, especially of infectious diseases. We must promote research protocols inserted in “a system” to identify gaps between science and practice and disseminate research findings for local, national and regional health policies.

Paraguay participated in the multicenter case-control study on pneumonia through the GABRIEL network. This provided a unique opportunity for us to reap multiple benefits, most of them mentioned above. The partnership between the university and the Ministry of Health was a success: we standardized a multiplex qPCR test for the surveillance system of 27 respiratory pathogens. *S. pneumoniae* serotypes carried in healthy children were demonstrated before the pneumococcal vaccine was introduced, PCV10 (Sinflorix), in March 2012. The circulation of non-common respiratory viruses, either alone or in coinfections with other respiratory viruses such as CoVs, BoV, hMPV and EV, was described for the first time. Since 2016, the Pediatric Hospital of the Ministry of Health and laboratories of the private sector have been using multiplex qPCR reactions in cases of respiratory infections.

In conclusion, we can say that funding and technology are important, but are not the sole keys to success. To belong to a network implies having goals, and not just a list of questions, and most importantly requires true leadership from people who are personally committed



Session I: ACUTE RESPIRATORY INFECTIONS

Chaired by Werner Albrich, Switzerland and Ron Dagan, Israel

Relationship between Etiology and Severity in Children under 5 with Pneumonia in Developing Countries

Dr. Glaucia Paranhos-Baccalà, from bioMérieux, Brazil, gave an update on the study of under-five hospitalized children with pneumonia suffering from hypoxemia and living in developing countries. She pointed out that in this population, the relationship between microbiological findings and mortality has seldom been previously described. The objectives of the study were to assess the microorganisms associated with hypoxemia, to identify the clinical and para-clinical predictors of hypoxemia, and to assess the risk factors associated with mortality in this population. The study aimed to examine the etiology, host markers, and severity of pneumonia in children, and it was set up to prospectively collect harmonized data using advanced molecular diagnosis in all hospitalized patients. The results presented came from 5 hospitals located on three continents.

The results of the study indicate that the respiratory syncytial virus (RSV) and the human metapneumovirus (hMPV) are associated with an increased risk for hypoxemia, whereas the bacteria detected in respiratory samples are not to any significant degree. However, detection of *S. pneumoniae* in blood samples appeared to be linked to a higher rate of in-hospital mortality. An elevated procalcitonin concentration and hypoxemia were straightforward predictors of death in children with pneumonia, and the contribution of hypoxemia to the risk of death was independent from the presence of pneumococci in blood samples.

Interpretation of the study results was limited due to incomplete information on disease exposure before the children's hospital admission. It was also difficult to differentiate infections (past or existing) from colonization in the respiratory tract using molecular assays. And finally, there were a limited number of samples for survival analysis.

In conclusion, in hypoxemic patients, viral etiology might be considered, while in very severe pneumonia, pneumococcus might be considered as the primary source of infection, even in the absence of hypoxemia at hospital admission. Preventive measures, such as vaccination of children, and the use of oxygen therapy in hypoxemic patients, would reduce the burden of death. PCT values at hospital admission should be taken into account (antibiotic therapy, septic shock, and intensive care).

Etiology and Factors Associated with Pneumonia in Children Less than 5 Years of Age in Mali

Dr. Bourema Kouriba from the Charles Mérieux Infectiology Center, Mali, spoke about the study in Mali (as part of the overall multicenter pneumonia study) designed to assess the etiology and determine the factors associated with community-acquired pneumonia in hospitalized children under 5 years of age in Mali. The study was also set up to determine whether viral or bacterial co-infections are associated with the risk of severe pneumonia. Another objective was to identify *Streptococcus pneumoniae* serotypes in nasopharyngeal and blood samples, and to characterize new infectious agents or variants in pneumonia of unknown etiology.



This was a prospective case-control study with the cases being children hospitalized for pneumonia, and the controls being children hospitalized for other diseases without any symptoms suggestive of respiratory illness.

The procedures included clinical examinations, sampling from blood, pleural effusion, and nasopharynx fluids, a chest X-ray, and a clinical history of antibiotic use.

In terms of results, this non-PCV population of children displayed pneumonia attributable to *S. pneumoniae*, RSV, hMPV, and influenza A virus. A dose response relationship was apparent between the number of microorganisms found in nasal swabs and the risk of being a case for pneumonia. In conclusion, increased pneumococcal conjugate vaccine coverage in children could significantly reduce the burden of pneumonia in the sub-Saharan.

The LACORIS Project in Laos

Dr. Valentina Picot from Fondation Mérieux, France, presented LaCoRIS, an ongoing prospective community-based cohort study on respiratory disease surveillance among cohorts in Vientiane, Laos. This project is part of a larger international study, run in collaboration with NAMRU in Cambodia that should eventually lead to cross-country comparisons of burden and epidemiology. Its objectives are to measure the incidence rates of acute respiratory diseases, and identify the pathogens responsible for acute respiratory diseases in urban and peri-urban areas within the established catchment area. The promotion of capacity building for disease surveillance in Laos is also an objective to develop and sustain the activity of participating institutions along with the Ministry of Health to implement public health interventions and training in an area with a well-defined epidemiology for the purpose of education and competency testing.

The catchment area of the study population included 998 households from 22 villages surrounding Vientiane whose 4,885 household members consented to having nasal and throat swabs and sputum samples taken. Pathogens were then identified on all the samples with preliminary data showing that *S. pneumoniae*, *Chlamydia pneumoniae*, and the influenza A virus are the most prevalent.

When complete (extension of the project expected through 2017), 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.

Improving Case Management and Public Health Interventions of Patients with Community-Acquired Pneumonia (CAP) in Humanitarian Crisis Settings

Dr. Thomas Kesteman from Fondation Mérieux, Lebanon, stated that, in Lebanon, respiratory tract infections are a major health problem among refugees. There is a need for adapted treatment guidelines for CAP among Syrian refugees. The feasibility of studying this problem during a humanitarian crisis needs to be evaluated. To address this, the project PEARL (Pneumonia Etiology Among Refugees and Lebanese population) aims to 1) estimate the proportion of CAP attributable to specific viral and bacterial pathogens in a population of refugees, 2) assess the feasibility and performance of nuclear acid-based diagnostic tests conducted during a humanitarian crisis, 3) identify the serotypes of *Streptococcus pneumoniae*, 4) identify antimicrobial susceptibility profiles, 5) provide improved treatment and care protocols for local healthcare staff and public health decision-makers,



6) identify modifiable risk factors for CAP in this population, and 7) refine current data on the incidence of CAP in vulnerable populations in Lebanon.

The study will be run by identifying cases of CAP in a population of refugees, analyzing the blood, nasopharyngeal, and urine samples collected to identify the pathogens present, and by calculating the proportion of CAPs caused by each pathogen. The results of the study will make it possible to determine the fractional distribution of each causative microorganism according to time period, age of patient, and site. The study will also show the incidence of ALRI and other diseases, the agent-specific hospitalization rate and fatality rate as a function of the socio-demographic characteristics of the patients, the clinical signs observed, and operational characteristics of diagnostic tests.

In the long run, the study should contribute to lower morbidity rates due to CAP, improve know-how and methodology, provide evidence of dominant etiologies of CAP, guide population-based health interventions, such as immunization campaigns, and ultimately strengthen the capacity and preparedness of local health systems.

Status of Childhood Pneumonia in India

Dr. Shally Awasthi from King George's Medical University, India, described India as a country where one child out of 21 dies before the age of 5 years. About 15% of these deaths are attributable to pneumonia, and 9% to diarrhea. Mortality and severity requiring hospitalization varies inversely with age, and the case fatality rate (higher in boys than in girls) ranges from 2.5% to 11.8%. However, seeking qualified care is often delayed, because it is considered to be a last resort measure once traditional healers and local doctors have been consulted. Traditional village-based caregivers and practitioners, usually the villager's first choice for health advice, are unclear about breathing patterns in pneumonia and may not recognize that fast breathing is a sign of pneumonia. Even Community Health Workers fail to report signs of severe pneumonia, thus delaying proper treatment.

A study underway is assessing community perceptions toward pneumonia. Among the different local populations, their description and taxonomy (local dialect names) given to the symptoms of pneumonia can vary considerably. Care-seeking behavior, attitude toward risk, and perception of constraints also differ widely from one population group to the next. Therefore, changing care-seeking behavior represents a challenge, because the decision-making process within families is complex.

A specific website in India has been created, fightpneumonia.org, as part of a public awareness campaign, and as a step forward in building faith among the people in the public health system. This has set off a behavior change communication project in rural India. Immunization campaigns and awareness campaigns describing risk factors (e.g. lack of handwashing, moderate to severe malnutrition, overcrowding, indoor air pollution, etc.) are part of this approach. The goal (one of the Sustainable Development Goals) is set to reduce the under-5 mortality rate to 25 deaths or fewer per 1,000 live births. The fight against pneumonia can help achieve this.

Enterovirus D68 and Beyond

Dr. Jianwei Wang from the Chinese Academy of Medical Sciences first reviewed the complicated infection cycle of the enteroviruses (EV), and EV-associated diseases or conditions. Specific genotypes including D68 that cause upper and lower tract respiratory diseases have been identified. The EV is



appearing in new epidemics across the world, but its origin remains uncertain. Since 2005, the epidemics of EV-68 have been increasing and the virus has been identified in more than 22 other countries. The largest outbreak of EV-D68 infections associated with severe respiratory illness and neurological complications emerged from the United States in 2014. It has attracted global public health concerns. The circulation of EV-D68 in China has been identified since 2006, but cases were sporadic and did not display neurological symptoms reported in China. The infection of EV-D68 has appeared in a broad age range (1 month to 80+ years of age) with no sex predilection, affecting mostly school-aged children, many of whom have had a history of asthma. The disease can go from mild to severe, requiring care and ventilation. Anti-EV drugs, pleconaril, pocapavir and vapendavir, appear to be ineffective. EV-68 outbreaks are also characterized by seasonality and a biennial periodicity.

Three clades of EV-D68 have been identified based on phylogenetic analysis. The six coding mutations (M291T, V341A, T860N, D927N, S1108G, and R2005K) associated with neurovirulence reported in American strains were not found in Chinese strains. Moreover, 2014 Chinese strains had a unique R220A mutation in the puff region of VP2, while a R220E mutation occurred in other strains. Like other enteroviruses, the loop sequences of the domain X and Y in the 3' -UTR of the Chinese strains are complementary. However, the X loop sequences of the 2014 American strains were not complementary but identical to Y loop sequences. These results indicate that different EV-D68 strains circulated in China and America and the mutations might be responsible for different prevalence. Our findings also provide new evidence for the sequence diversity of EV-D68.

Future research will seek to determine whether mutations are a source of different clinical manifestations. To further evaluate the emerging EV-D68 epidemic, we isolated the circulating viral strain and investigated the seroprevalence of neutralizing antibodies (NAbs) in Beijing between 2004 and 2011. The titres of EV-D68 NAbs were generally low in all age groups in sampled populations in 2004, but significantly higher in 2009. From 2007 to 2011, the NAbs against EV-D68 significantly increased over time. These findings indicate that EV-D68 has spread widely in the Chinese population in recent years, although only a limited number of cases have been reported.

Session II: NEW RESEARCH INITIATIVES WITHIN GABRIEL

Chaired by Robert Heyderman, United Kingdom and Luc Hervé Samison, Madagascar

Tuberculosis Prevalence in the Slums of Port-au-Prince, Haiti

Dr. Jean William Pape, Director of the GHESKIO Centres, Haiti, described the GHESKIO-TB Reach project, the purpose of which is to 1) detect undiagnosed tuberculosis in an urban slum of Port-au-Prince more quickly, 2) reach a greater number of urban slum-dwellers for TB screening and testing, 3) recruit more patients for TB treatment and improve treatment outcomes, and 4) evaluate the use of GeneXpert® tests in Haiti. Overall, TB prevalence is decreasing in the world. However, Haiti has the highest prevalence in the Americas. In 2014, WHO reported that TB incidence in Haiti was 200 per 100,000 individuals. Haiti's HIV/TB co-infection rate is 2.0 times higher than the global average. 19% of TB cases were also HIV-positive in 2015, and the HIV/TB co-infection rate was 35 per 100,000. In January 2010, Haiti sustained a devastating earthquake displacing over 1.5 million Haitians into crowded slums that have become a breeding ground for ongoing TB transmission.



The GHESKIO-TB Reach project ran from August 1, 2014 to May 19, 2016. It consisted of TB Active Case Finding (ACF) through systematic, community-based screening for chronic cough. Community health workers (CHWs) screened households home by home in eight slum communities for cough sustained for over two weeks. All TB suspects were referred to GHESKIO for HIV and TB screening and were evaluated for TB with CXR and sputum acid-fast bacilli (AFB) smear and GeneXpert tests. Patients diagnosed with TB were immediately started on treatment on the same day.

Through the REACH program, 80 CHWs screened 140,697 individuals for cough in eight Port-au-Prince slums through active case reports. Of these, nearly 12,000 (12%) TB suspects were identified and referred for TB evaluation at GHESKIO. Out of this group, 2,027 (17%) patients evaluated for TB were diagnosed with TB, of which 1,766 (87%) were bacteriologically confirmed TB cases, and 15% of this last group were co-infected with HIV.

In conclusion, household-level screening of slum dwellers for chronic cough by CHWs under the TB Reach study was highly effective. It also led to the detection of a high rate of co-infection with HIV. There was also the added benefit of GeneXpert MTB/RIF testing. Further studies are needed to determine the cost-effectiveness of these strategies.

Cryptococcal Meningitis in HIV-Infected Patients in Madagascar: High Prevalence, Lethality and Therapeutic Challenges

Dr. Mala Rakoto-Andrianarivelo from the Charles Mérieux Infectiology Center (CICM), Madagascar, reported that there is little information on the epidemiology of *Cryptococcus neoformans* in Madagascar. The objective of the study, expected to run until December 2016, was to estimate the prevalence of cryptococcal meningoencephalitis (CM) in Madagascar and to describe the cases presenting this pathology. HIV-infected patients (n=118) were selected according to their immunological CD4 status. Blood samples and cerebral spinal fluid were tested for cryptococcal antigen by the newly developed method Lateral Flow Assay, IMMY®. Overall prevalence of cryptococcal antigenemia in Madagascar was high (16.4%) compared to that observed in some sub-Saharan African countries (5 to 13%) and the prevalence of CM was 15.1%. Fever, headache, neck pain and night sweats were the most common signs observed among CM cases. The Case Fatality Rate was unacceptably high and the major factors of death include high antigen titre at baseline and high CSF pressure.

Fluconazole is the only therapeutic option in Madagascar. Challenges remain to facilitate the access to more effective molecules to treat patients with CM due to hindrance from heavy administrative formalities linked to drug importation and weakness of the national control program.

Ultimately this study is expected to: make laboratory-based diagnosis for early detection of cryptococcosis available to the HIV/AIDS National Control Programme; help establish adequate cryptococcosis treatment; understand the epidemiology of the *C. neoformans* complex in Madagascar; and serve as a model for similar studies to enhance the understanding of other opportunistic infections in HIV-infected patients.



Laocol-VP: Cervical Cancer Screening among Women Living with HIV in Lao PDR

Dr. Phimpha Paboriboune of Christophe Mérieux Infectiology Center, Laos, spoke about the LaoCol-VP project that is designed to compare the efficacy and cost-effectiveness of careHPV™, a biological test for molecular screening of oncogenic human papillomavirus (HPV) for cervical cancer in women living with HIV in Laos, with the Pap smear, an alternative technique that is the current strategy of reference.

A multicenter cross-sectional study was conducted in four care centers for HIV in the hospitals of Setthathirath, Mahosot, Luang Prabang and Savannakhet in Laos. From February 2014 to May 2015, 644 women living with HIV, aged 25 to 65, after having granted their informed consent, received a careHPV test, a Pap smear, and a systematic colposcopy combined with a biopsy for a histological examination. HPV genotyping was also systematically carried out.

Severe abnormalities were diagnosed in 41 women, of whom 36 had pre-cancerous lesions and 5 had cancerous lesions. Follow-up care by cone biopsy or hysterectomy was provided free of charge. More than 200 women were identified as oncogenic HPV carriers and thus needed to be closely monitored.

Preliminary results have been sent to project partners in Laos. A final analysis is underway to comparatively evaluate the performance of the different screening strategies.

Antibiotic Resistance of Acute Respiratory Pathogens in 4 Referral Hospitals in Cambodia

Dr. Monidarin Chou from the Rodolphe Mérieux Laboratory at the University of Health Sciences, Cambodia, described the objective of this ongoing study, which is mainly to determine the etiological agents in adult patients with ARI symptoms, investigate prevalence of viral infections in the four referral hospitals, to investigate prevalence of pathogenic bacteria and characterize their anti-microbial drug resistance.

The case group consisted of 657 individuals presenting a fever and respiratory symptoms. The control group had 89 individuals with no respiratory symptoms. Nasal wash samples were tested by real-time PCR and bacteria culture subjected to antibiotic sensitivity testing.

The results showed that 25 bacterial and viral pathogens were identified in the samples, that rhinovirus, enterovirus, and influenza viruses were the viruses most frequently detected, and that *H. influenza* and *S. pneumoniae* were the most common bacterial pathogens detected by PCR. *S. aureus* and *K. pneumoniae* were the bacterial agents the most frequently identified by culture. Bacterial resistance per antibiotic varied according to the type of antibiotic and bacterial species. More than 60% of isolated *Staphylococcus aureus* were proven to be MRSA-positive.

Evaluation and Validation of a New Molecular Assay for the Diagnosis of Typhoid Fever

Stéphane Pouzol from the Emerging Pathogens Laboratory, Fondation Mérieux, France, reviewed the current status of typhoid fever. Its global incidence, mainly in developing countries, is estimated at 21 million cases with 216,000 deaths per year. Current diagnostic techniques are inadequate.



The current study is designed to develop a molecular tool to monitor the burden of typhoid in developing countries. The methodology consists of identifying by real-time PCR *S. Typhi*, *S. Paratyphi A*, and *S. enterica* directly from 1ml of whole blood after a pre-enrichment step.

In the first phase of the study, we developed the multiplex molecular assay in terms of sensitivity and specificity on characterized bacterial strains, as well as in vitro samples and assessed performances on clinical samples. In a second phase, the assay was evaluated in Bangladesh on larger volumes of blood samples and compared to blood culture. Presently, the third phase of the project consists of validating the assay in African context and improving the knowledge of the burden of typhoid fever in that region.

In conclusion, we have developed and validated a method with a turnaround time of one day to identify pathogens responsible for enteric fever from whole blood using a limited volume of blood. Work is in progress to render this assay suitable for clinical diagnostics. The next step is to develop a strategy to disseminate this novel technique.

Session III: YOUNG SCIENTIST PRESENTATIONS

Chaired by Glaucia Paranhos-Baccalà, Brazil and Florence Komurian-Pradel, France

Molecular Characterization of Multidrug-resistant *Salmonella* Isolated from Humans and Chickens in Cameroon

Ariane Nzouankeu from Centre Pasteur, Cameroon, explained the problems in poultry farming and human health due to food-borne diseases caused by *Salmonella*. Chickens are the main reservoir of this bacterium, and the irrational and unmonitored use of antimicrobials has led to the emergence of AMR. The objectives of the current study are 1) to evaluate AMR and perform molecular characterization of *Salmonella* isolated from poultry and humans, 2) to estimate *Salmonella* contamination rates in chickens sold in Yaoundé markets and analyze *Salmonella* isolates from patients who arrived at the Centre Pasteur between January 2006 and February 2007, 3) to study the phenotypic and genotypic AMR factors in *Salmonella* isolates from humans and animals, and 4) to evaluate the genetic relationships between isolates from both sources.

Sampling, antimicrobial susceptibility testing, and identification of *Salmonella* serotypes were carried out. The genetic relationships of the different serotypes were determined and resistance patterns of the isolates were analyzed.

In conclusion, there is a great diversity of *Salmonella* serotypes in Yaoundé, but only four serotypes are shared by both humans and chickens. There is susceptibility of isolates to third-generation cephalosporins and fluoroquinolones, and emerging resistance to certain other antibiotics in human isolates. Forty percent of the *Salmonella* isolated from humans and chickens in Yaoundé are multidrug resistant. This study highlights the need to raise awareness about the abusive use of antimicrobials, especially in human and veterinary medicine, in Cameroon. An antimicrobial resistance surveillance system for *Salmonella* and other enteric bacteria in Cameroon is recommended.

Targeting the Chemokine Receptor CXCR2 in Murine Models of Primary and Secondary Lung Infections: Therapeutic Potential?



Cristiana Garcia from Fiocruz, Brazil, explained the role of the chemokine receptor, CXCR2, in the inflammatory process. It is a key regulator of the recruitment, the infiltration, and activation of blood leukocytes, such as neutrophils, in the case of influenza and pneumonia infections, and post-pneumonia pathogenesis. Receptor antagonists block the receptor from producing a biological response, as it has been observed in the case of a CXCR2 antagonist, DF2162, protecting mice during an influenza infection, and preventing mouse mortality during pneumococcal meningitis. DF2162 reduces lung injury, but does not necessarily diminish the burden of infectious bacteria. The question for the present study has to do with whether the control of neutrophil recruitment with DF2162 would represent an effective therapeutic strategy in three models: influenza, pneumococcal pneumonia, and secondary pneumococcal pneumonia.

The CXCR2 antagonist prevented lethality or weight loss caused by influenza, *S. pneumoniae* or secondary infection. It was associated with decreased inflammation (neutrophil numbers, proinflammatory cytokines) and lung damage. Viral loads were reduced but bacteria counts were not affected by DF2162 treatment in single or secondary pneumococcal infection.

However, the CXCR2 antagonist, DF2162, reduced systemic pathogen dissemination in secondary pneumococcal infection. In conclusion, it appears to be a potential therapeutic agent for the treatment of pulmonary infections, by reducing inflammation, lung damage and lethality. Further pre-clinical investigations are underway.

Surveillance of Acute Respiratory and Enteric Infections in Children Under-five in Two Dhaka Hospitals

Asifuzzaman Rahat from ideSHi, Bangladesh, pointed to the dire situation in Bangladesh regarding the mortality and morbidity rates of children under five, which are much higher than in developed countries and mainly attributable to preventable diseases, such as pneumonia, diarrhea, and enteric fever. The objective of the current study is to determine the incidence and the antimicrobial susceptibility patterns of the bacterial/viral isolates obtained from patients with acute respiratory infections (ARI), acute watery diarrhea, and enteric fever.

Samples were collected from patients in two hospitals with ARI (nasal swabs), diarrheal disease (rectal swabs), and enteric fever (blood). Bacterial pathogens were isolated and tested for antibiotic susceptibility. In the case of ARI and enteric fever, these isolated pathogens were identified and antimicrobial susceptibility patterns determined. ESBL detection was carried out in multidrug-resistant strains. Viral pathogens were identified by PCR and information on co-infections with bacterial pathogens was obtained. In the case of diarrheal disease, the study is still in progress.

Future studies would entail carrying out a more comprehensive analysis with involvement of more hospitals and a larger enrollment of patients. Nationwide surveillance studies are required to better understand the full impact of human activities on antibiotic resistance ecology in Bangladesh.



Session IV: TUBERCULOSIS

Chaired by Delia Goletti, Italy, and Jabin Akhter, Bangladesh

Tuberculosis Working Group Update

Jean-Luc Berland, from LPE, Fondation Mérieux, France described the developments from the most recent workshop of the GABRIEL TB working group that was held in January 2016. The objective of this workshop was to brainstorm proposals, to reinforce South-South initiatives, trigger and develop innovative ideas and solutions, design new collaborative research projects, and find ways of securing external funding. Three main topics were addressed at this recent meeting. First, surrogate biomarkers used as a measure of disease rate or change are being evaluated as indicators contributing to the diagnosis of TB and to the monitoring of active TB cases. They can also measure latent TB infections that are triggered to become active cases. The second topic was the diagnostic algorithm and the use of GeneXpert for the evaluation of Whole Genome Sequencing (WGS) in the algorithm for drug resistance testing. To what extent is WGS an alternative to drug resistance testing? Finally, the transmission dynamics of TB are to be further explored so as to design interventions for infection control.

Molecular Epidemiology of *Mycobacterium tuberculosis* and Antibiotic Resistance in Lao PDR

Silaphet Somphavong from the Christophe Mérieux Infectiology Center, Laos, presented the preliminary results of an ongoing study on “Molecular epidemiology of *Mycobacterium tuberculosis* and antibiotic resistance in Lao PDR”. The aim is to study, for the first time, the *M. tuberculosis* population circulating in Laos and to explore the level of drug resistance. LPA assays (Hain tests) were used to identify *M. tuberculosis* and to detect isoniazide (INH) and rifampicin (RIF) resistance. Spoligotyping and 24 loci MIRU-VNTR were performed to genetically characterize the *M. tuberculosis* population.

Among the Lao population-based sampling (National prevalence survey, 2010-2011), 201 isolates were confirmed as belonging to *M. tuberculosis* complex, only one case of multi-drug resistance (MDR, isolates resistant at least to INH and RIF resistant) and 9 mono INH resistant isolates were detected. Five *M. tuberculosis* families were described: EAI, Beijing, T, CAS, LAM. EAI was the predominant, followed by Beijing (63.2% and 12.4% respectively). The proportion of EAI family is similar to Cambodia (60%) and Myanmar (48.4%), but is quite different from North Vietnam (38.5%) and Taiwan (21.85%). It is worth noting that this family was present in all the regions of Laos, while Beijing is mostly observed in the north, decreasing in the center and absent in the south. The Beijing family in Laos is present at a lower rate compared to Thailand (58%), Vietnam (38.5%), Cambodia (30%) and Myanmar (31.9%). Nevertheless, this family displays a higher clustering rate (18.5%) compared to EAI (12.2%), based on spoligotyping and MIRU-VNTR data, suggesting a higher involvement of the Beijing family in recent transmission.

Among the hospital sampling collected between 2010 and 2014, 145 positive cultures from MDR-TB suspected patients in routine were analyzed by Hain tests. 131 (90.3%) were confirmed as belonging to *M. tuberculosis* complex and 14 (9.7%) were non-MTBC. 17 out of the 131 *M. tuberculosis* isolates were resistant to at least rifampicin and isoniazid (MDR-TB) (12.9%). This proportion is lower than those observed in Cambodia (28%) and in China (25.6%). Nevertheless, 59% of the MDR strains were identified as belonging to the Beijing family and 22% to EAI. In parallel, 23 out of the 131 isolates were



selected for a whole genome sequencing (WGS) analysis. The concordance was of 100% for INH resistance and of 97% for RIF resistance between LPA and WGS.

This preliminary study shows that the Beijing family is present in low percentage, but is more likely related to recent transmission and MDR-TB cases in Laos in agreement with the data worldwide. Furthermore, LPA appears to be a valuable diagnostic tool to detect MDR-TB cases in Laos. The next step is to further analyze the first line and second line drug resistances and their link with the genetic background of the isolates to determine the spread and the evolution of drug resistance in Laos. The final goal is to provide data for TB infection control strategy and actions.

Review of Biomarkers for Diagnosis and Treatment Efficacy in Tuberculosis

Dr. Jonathan Hoffmann, from the Emerging Pathogens Laboratory, Fondation Mérieux, France, spoke about the need for proper diagnostic tools that can monitor the effectiveness of treatment for TB and identify patients with LTBI who are at risk for developing active tuberculosis (ATB). Tuberculosis drugs are subject to limitations related to their toxicity and the risk of having resistant microbial strains develop. Surrogate biomarkers appear to be the answer to overcome these problems by their potential capacity to shorten anti-TB therapy, to increase the TB cure rate, and to limit the emergence of resistant bacterial strains. They are also useful to monitor the effectiveness of treatment, identify the risk of developing ATB, and distinguish ATB from a latent infection. It appears that HBHA, a surface protein of *M. tb* acting as an antigen, may serve as a biomarker for these purposes. An experimental test is being designed to measure the in vitro immune response to HBHA and QFT-P in whole blood in a study population of HIV-infected patients.

Surrogate biomarkers have distinct advantages over classic anti-TB medication in the management of TB treatment. Their use can potentially help decrease the proportion of non-compliant and defaulted patients (which also impacts TB transmission) and monitor the effectiveness of a preventive treatment of children in contact with an ATB index case. Surrogate biomarkers could qualify as a research project in a multicenter study in the GABRIEL Network.

Tuberculosis Transmission in Households

Dr. Tsira Chakhaia from the National Center for Tuberculosis and Lung Diseases (NCTLD), Georgia, mentioned that early case detection is one of the most important steps for reducing the risk of TB transmission among household (HH) members in Georgia. Investigating the contacts of patients with TB has been a priority public health measure in high-income countries to detect new TB cases, and to identify the close contacts, who may benefit from LTBI treatment to prevent progression to active TB. TB Contact Investigation has typically been a low public health priority in most high TB-incidence LMIC countries. In the past, in Georgia, TB contacts did not come in for follow-up visits, nor were they visited by healthcare workers. After a roll out of a nationwide contact tracing in 2012, Georgia Public Health now does home contact tracing with a team of epidemiologists who work in conjunction with NCTLD and other TB facilities.

The aim of the study 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 identified household TB 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 a delivery of proposals for improvement through an infection control plan. Training for capacity building in laboratory techniques in molecular biology was also carried out.

A future project is envisioned, called “Capacity Building for Diagnostic and Clinical Management of MDR and XDR TB in Civil and Penitentiary Sector in Georgia”, which will be run in the context of new anti-TB drug implementation and surveillance of X/MDR-TB transmission in Georgia.

Effect of Active Case Findings on Pulmonary TB Transmission among Inmates of Dhaka Central Jail

Mazidur Rahman from icddr,b Bangladesh, described the TB control situation in the Dhaka Central Jail. In the passive approach that has been taken for case findings, sputum samples are collected from inmates at least once a week and/or when requested. Microscopy is carried out in the nearby DOT Center in the absence of culture or DST. The objectives of the current study are to understand the transmission dynamics in a high-risk setting such as a prison and how interventions affect TB transmission and drug resistance patterns in prisons. It also seeks to evaluate the use of Next-Generation Sequencing (NGS) for clinical diagnosis of drug resistance.

12,464 inmates were screened. From the active cases, 285 isolates were collected and drug susceptibility testing, spoligo- and MIRU-VNTR typing, and NGS sequencing (Miseq, Nextseq) were performed. The findings reveal that the longer the incarceration, the higher risk there is for a TB infection. There is no difference in smear score classes, and smear status at onset does not match the dynamics of transmission. Resistance patterns in a prison environment are similar to those found outside prison walls. TB management of inmates can be potentially as efficient as on the outside.

In conclusion, active screening and interventions have the potential of draining the reservoir of tuberculosis inside the prison. After three years of active screening, the number of clustered isolates dropped three-fold, indicating a considerable reduction of transmission inside the prison. Compared to the general population, no new or specific resistance profile was detected.

Drug Resistance in the Zaporozhye Region

Associate Prof. Olga Konakova, from the Zaporozhye State Medical University, Ukraine, stated that despite the decline in the incidence of TB observed in Ukraine since 2004, two negative trends have appeared: the increasing rate of MDR-TB and XDR-TB in the population, and the increasing rate of TB - HIV co-infections. Among newly diagnosed TB patients, the number of new smear-positive cases increased from 30% to 47.8%, and the percentage of TB-drug resistant cases was 23% (among relapsed cases, 53.5%). Among patients whose TB treatment failed, 41% displayed TB-drug resistance.

The project currently underway between Fondation Mérieux and Zaporozhye State Medical University involves a GenoType MTBDR plus assay for rapid MDR-TB detection in the Zaporozhye region. This project uses molecular-based technologies for timely diagnosis of MDR-TB in the Zaporozhye region,



Ukraine. This project will help develop an infrastructure that will augment the capacity to carry out high quality and internationally significant TB-related research.

The techniques required for this project, comprising a biobank with MTB isolates and isolates for molecular testing, typing, and sequencing will be applicable to future research projects in this field. In Ukraine there is an increasing need to develop the capacities of biological laboratories for the early detection of MDR-TB and monitoring of inter-patient transmission. 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.

MDR-TB in Mongolia

Nyamdavaa Naranbat, from GYALS Medical Center, Mongolia, spoke about the dramatic rise of MDR-TB prevalence in Mongolia since 2005, as well as that of the number of deaths attributable to MDR-TB.

A recent nationwide survey of anti-TB drug resistance was carried out to investigate drug resistance levels among new and re-treated TB cases. Specifically, the prevalence of resistance to rifampin, streptomycin, isoniazid and ethambutol among TB patients with and without prior anti-tuberculosis treatment was determined. The findings revealed the relatively low prevalence of multidrug resistance among patients without a history of prior treatment, as compared to a very high prevalence among previously treated patients. This suggests that retreatment is deficient and poses a threat to continued transmission, which has not yet manifested itself among new patients. Drug resistant patterns were determined according to mono- or multiple resistances to INH and RIF, and classified by age and sex distribution of the individuals tested. This was achieved by using symptom and chest X-ray screening combined with diagnosis using smear microscopy/culture, as well as GeneXpert MTB/RIF, as recommended by WHO.

Session V: ASSURANCE QUALITY INITIATIVE

Chaired by **Graciela Russomando, Paraguay, and Bourema Kouriba, Mali**

Overview of the GABRIEL Network Quality Evaluation

Anh-Thu Ngo from Christophe Mérieux Infectiology Center Laos, presented a summary of the responses received from the self-evaluation questionnaire sent to 14 of GABRIEL's member laboratories. The purpose of this questionnaire is to evaluate the overall performance of laboratory practices and to identify the quality assurance priorities that must be taken into account. The evaluation is broken down into 12 modules: Facilities and Safety, Organization, Personnel, Equipment, Purchasing and Inventory, Process Management, Information Management, Documents and Records, Customer Focus, Assessment, Nonconformity Management, and Continual Improvement. Most laboratories are in the process of fulfilling the recommendations needed to qualify for ISO 15189:2012 accreditation (requirements for quality and competence of medical laboratories). None of the modules has yet been fully completed to meet ISO accreditation in any of the laboratories. However, the first eight modules obtained a score of 67% to 80%, fulfilling the required guidelines. Accreditation can also be obtained for ISO 15190:2003 (requirements for safety in medical laboratories) and ISO 17025:2005 (general requirements for calibration and testing in laboratories).



The Quality Initiative: Results and Perspectives

Dr. Nicolas Steenkeste from Fondation Mérieux, France, shared the significant benefits of adopting a Laboratory Quality Management System (LQMS): reducing costs, better responding to the needs of patients, providing accurate and timely diagnoses, meeting regulatory safety requirements, reducing waste, improving turnaround time, and helping the decision-making process of clinicians. ISO 15189, strongly recommended by WHO, 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 then progressively phase in the requirements of the internationally-recognized ISO standards. The management process includes human resource management, communication, regularly scheduled meetings, internal audits, and SOP management. The operational processes include sample preparation, media management, biological validation, and transmission of results. And the support processes cover biosecurity, equipment maintenance, archiving, and inventory management. At Fondation Mérieux, a working group on quality has come up with a flowchart designed to lead 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.

The Young Scientist Award Ceremony

Hubert Endtz, Florence Komurian-Pradel, and Delia Goletti 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 the 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 the quality of their presentations.

After careful deliberation, GABRIEL's 6-member jury decided to award the two prizes to:

- **Ariane Nzouankeu** for her work on the molecular characterization of MDR Salmonella isolated from humans and chickens,
- **Cristiana Garcia** for her research on the feasibility of using the chemokine receptor, CXCR2, in the therapy of primary and secondary lung infections.



CLOSING WORDS

Prof. Hubert Endtz brought this year's edition of the GABRIEL meeting to a close with warm thanks extended to all the participants for their contribution. Our challenge is to maintain our drive to obtain financial support from foundations, and industrial and financial partners. As government administrations are often reluctant to fund long-term projects in their country, they need to understand the true impact of GABRIEL's work in terms of health benefits, scientific innovation, relief to populations, and scientific career opportunities for the youth of the developing world. There is hunger for greater interaction and more profound intellectual maturity. Both are prerequisites for sustainability.

"This year's GABRIEL meeting was all about new ideas, testable hypotheses, and project designs. We should be proud of our achievements and we should look forward to our next phase of accomplishments," he concluded.

