11TH GABRIEL NETWORK MEETING

NOVEMBER 7-10, 2021
LES PENSIÈRES CENTER FOR GLOBAL HEALTH
VEYRIER-DU-LAC (FRANCE)

Organized by the Mérieux Foundation
with the support of bioMérieux

SPECIAL REPORT

From November 7 to 10, 2021, over 100 delegates, representing researchers, physicians, specialists in laboratory diagnostics, academics, and scientists, coming from both public and private sectors and over 15 countries, convened in person and online at the Les Pensières Center for Global Health in Veyrier-du-Lac, France, for the 11th GABRIEL network meeting. This report provides highlights of this symposium, including summaries of speeches and the main points of discussion.

The GABRIEL network meetings have become a forum for members to exchange experiences, present new collaborative opportunities, and review and examine the most recent scientific research in infectious diseases. For the first time, this year's program included presentations on the COVID-19 pandemic, as well as those on infectious diseases that have been regularly addressed in the past, such as tuberculosis, acute respiratory infections, and antimicrobial resistance. The full day session devoted to COVID-19 facilitaed discussions on advances in epidemiology, genomics, and surveillance of the infection in various parts of the world.

As in previous years, the program included the “GABRIEL Young Scientist Award”, created to enable promising young scientists from low- and lower-middle-income countries to advance their careers by allowing them to speak about their research at international scientific meetings. Two winners were selected for this year's best presentations.

Welcome Addresses

Alain Mérieux, the President of the Mérieux Foundation, presented his vision for the future of the foundation.

“For over 45 years, we have been working to ensure that every man, woman, and child has fair access to essential healthcare. We collaborate closely with local partners as part of multidisciplinary, complementary, and, most importantly, concerted approaches with a long-term perspective. We must immediately react to conditions as they emerge, as was the case when a Rodolphe Mérieux Laboratory was set up in Goma, RDC, in record time. We project to have a total 17 Rodolphe Mérieux Laboratories by the end of next year, thus expanding our network to Morocco, Burkina Faso, and Iraq. We have supported mother-child programs in Lebanon, a maternity ward in Ouagadougou, and a women’s foundation in Tunis. However, we must bear in mind that countries suffer hardships, such as violence, corruption, and poverty. It’s a never-ending struggle. According to Gramsci, it’s a battle between “the pessimism of intelligence and the optimism of willingness”. Our vision is to fight and prevail.”

Dr. Marc Bonneville, the Medical and Scientific Director of the Mérieux Foundation in France, welcomed the participants to the GABRIEL network meeting, recognizing that COVID-19 travel restrictions prevented some of the delegates from traveling and attending the conference in person. COVID-19, having shaken our lives, has become a priority in the GABRIEL network in terms of diagnosis and molecular characterization of the virus. There is also a need to focus on tuberculosis, acute respiratory infections and on antimicrobial resistance in the search for appropriate control measures and a better knowledge of microbial resistance to these diseases. GABRIEL offers the opportunity to strengthen synergies, explore ways to share expertise, and offer holistic scientific research initiatives. It is essential to build a critical mass of skills on technology platforms that must be structured by discipline as a priority.

Dr. Florence Komurian-Pradel, GABRIEL Network Manager, spoke about the Mérieux Foundation's main priority: its continuing support to scientific research on infectious diseases in developing and emerging countries. The specific mission of the GABRIEL network is to strengthen the research capacity of the 20 member laboratories located in sixteen countries and encourage researchers, physicians, and academics from around the world to join hands in promoting scientific training and conducting joint research projects.

The Rodolphe Mérieux Laboratory of the National Institute of Biomedical Research in Goma in the Democratic Republic of the Congo recently joined the GABRIEL network in 2021. Its Director, Dr. Daniel Mukadi, spoke about the laboratory's testing and research activities that cover the surveillance and response to outbreaks of Ebola, cholera, and COVID-19, as well as biological diagnostics and the training of laboratory staff. One current project aims at measuring the impact of mass cholera vaccination in the eastern part of the country. The laboratory shares its expertise with other laboratories in neighboring Rwanda and Burundi.

As a step to further structure the GABRIEL network, two research projects were recently selected to promote South-South collaboration: a multicenter evaluation of the impact of the COVID-19 pandemic on the spread of antimicrobial and biocide resistance from wastewater to the environment in Cameroon, Brazil, and Madagascar, and the phenotypic and genotypic characterization of isoniazid-resistant strains of M. tuberculosis from clinical isolates in Bangladesh, Cameroon, and Lebanon. The GABRIEL network's past capacity-building efforts to transfer molecular biology techniques to recipient laboratories have yielded excellent results.

Human capacity building within the GABRIEL network is being conducted through practical and theoretical academic training, the provision of PhD grants, and the transmission of webinars on COVID-19 variants. Collaborative research is also coordinated through interaction with other global networks, such as Pasteur International, the Global Virus Network, and the European Virus Archive Global.

The GABRIEL network provides support to a host of other South-South research projects dealing with acute respiratory infections, tuberculosis, COVID-19, and antimicrobial resistance, the topics of which range from vaccine impact studies to nosocomial transmissions.

SESSION I: COVID-19 (1)

Chaired by Dr. Jean William Pape, Haiti, and Dr. Marianne Abi Fadel, Lebanon

Keynote address: SARS-CoV-2: endgame or new start?

Prof. Bruno Lina, Head of the Virology Laboratory of the Hospices Civils de Lyon spoke about coronaviruses, which are transmitted through animal vectors and cause intermittent infections in humans. They are also responsible for having triggered past seasonal pandemics. The WHO is presently deciphering the events that led up to the appearance of the SARS-Cov-2 virus. Mutations in the F protein at the furin cleavage site of the virus lie at the origin of human-to-human transmission and animal-to-human passage. The sequencing of the SARS-CoV-2 genome has led to the discovery of several distinct variants. Many of them possess mutations that alter the spike protein's amino acid sequence.

Prof. Lina compared the outbreaks of the 1st, 2nd, 3rd, and 4th Covid-19 waves in various countries through October 2021 and displayed the COVID-19 case rate per 100,000 population that was plotted on a map of the European Union. According to sequencing data, the delta variant has largely overtaken all other variants as the pandemic progresses, accounting for 93% to 100% of the variants detected globally. Its higher transmissibility can be explained by the delta variant's F protein being more stable in the up position, increasing its likelihood to bind to the receptor.

The antigenic cartography of the major SARS-CoV-2 variant shows that exposure to one variant of the virus protects against exposure to others. Vaccination thus confers protections against all strains currently in circulation. However, when comparing Wuhan, alpha, gamma, and beta antibody titers in individuals, the quality of the immunity induced by a viral infection is not as effective as that acquired from a vaccination, which confers greater protection in all cases. Current vaccines offer the best solution currently available.

The question is whether the pandemic is nearing its end or whether new waves of infection are inevitable. Caution could be used in predicting an outcome based on prior experience.

Characteristics of the COVID-19 pandemic in Haiti

Prof. Jean William Pape, Director of GHESKIO Centers, began by discussing the COVID-19 pandemic in Haiti, which has been exacerbated by the country's complex situation in terms of political unrest, natural and environmental disasters, the population's low socio-economic status, and the country's public health challenges.

Confirmed COVID-19 cases in Haiti do not reflect the true scale of the pandemic, because most mildly symptomatic cases are not tested. Even though the pandemic has not been as severe in Haiti as in other Caribbean countries, the country has undergone four pandemic waves, the two most recent waves due respectively to the Gamma and Delta variants, both associated with the highest mortality, particularly among the elderly. Slum-dwellers living in crowded areas are being infected but are not clinically sick enough to require hospitalizations. The reasons why they have been spared are currently being investigated. The bulk of the population has been resistant to wearing masks and taking vaccinations due to people's lack of firsthand contact with COVID-19 cases and people dying from it as well and exposure to conspiracy theories through social media concerning the pandemic's validity and the danger of taking vaccines shots. Early measures to control the pandemic had negative impact on other health programs.

GHIESKO, in its leadership role, has been collaborating with Haiti's Ministry of Health to promote vaccinations, scale-up testing capacity, and develop a national operating strategy for behavioral change and community mobilization. Crisis management efforts include the setting-up of COVID-19 care centers and epidemiological surveillance, especially along the border with the Dominican Republic where viral transmissions have been very important.

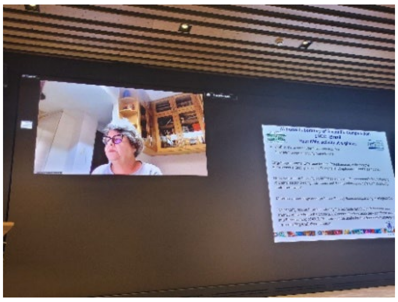
Wastewater-based epidemiology as a useful tool to track SARS-CoV-2

Dr. Paola Resende, a public health researcher at Fiocruz, Rio de Janeiro, Brazil, explained that wastewater-based epidemiology (WBE) can serve as a useful technique for SARS-CoV-2 surveillance. WBE is a low-cost, rapid, and near real-time method of monitoring disease prevalence in a large population, and can also serve as a predictive technique, because wastewater data may precede individual clinical testing. WBE was used in the past to track the spread of infectious diseases, such as polio, influenza, and hepatitis.

The Brazilian Ministry of Health and FIOCRUZ have jointly launched a pilot project for SARS-CoV-2 surveillance in residual water with the goal of improving the environmental surveillance of diseases that are a public health priority for the Brazilian Public Health Laboratory Network. One specific study, based on the detection of SARS CoV-2 dispersed in the sewage of the Niteroi municipality, was undertaken recently. Following the collection of wastewaters at designated points throughout the community, samples were processed to extract viral RNA for sequencing. Maps showing the dispersion of SARS-CoV-2 were thus obtained.

When data from whole-genome sequencing of clinical samples is combined with epidemiological data, it becomes possible for public health authorities to target its interventions more accurately, such as the application of restrictions to the population or the ordering of clinical testing.

Genomics surveillance in the state of Acre: crossing Amazonian borders



Dr. Ana Tereza Ribeiro de Vasconcelos from the National Bioinformatics Laboratory, Brazil, spoke about genomic surveillance of SARS CoV-2 in Acre, which is located along the western border of the country.

For this current study, samples were randomly collected from COVID-19 participants across various municipalities in the Acre. Genomic sequencing was performed on these samples to identify SARS-CoV-2, examine its evolutionary patterns, and monitor how it has evolved into gamma (P.1), lambda (C.37), and delta variants. This information should lead to a better understanding of the transmission routes of the infection from Manaus, in Brazil, to Peru and neighboring countries. However, little information is available currently regarding the impact of cross-border migration.

SESSION I – COVID-19 (2)

Chaired by Dr. Daniel Mukadi, Democratic Republic of the Congo, and Dr. Graciela Russomando, Paraguay

GABRIEL network initiative for the surveillance of emerging COVID-19 variants

Jean-Luc Berland from the Mérieux Foundation, Lyon, presented the current GABRIEL network initiative on COVID-19 variants screening. A variant is referred to as a VOC (variant of concern) when it acquires a clinical or public health significance due to its enhanced transmissibility, its failure to be detected by diagnostic assays, its higher virulence, or its more severe clinical outcome.

The 2021 GABRIEL network initiative goal is to set up a surveillance program of SARS-CoV-2 variants in countries that lack a national surveillance program, or if the member is not yet embedded in this program. A survey allowed the mapping out of the available skills in member countries to carry out the response. Five members benefit from the GABRIEL network initiative: over the short term, the GABRIEL network initiative should be able to target known variants, either by targeted rt-PCR, Sanger sequencing or next generation sequencing. As one of the outputs, it is expected health authorities will be notified about the results so that they can plan an informed response.

The GABRIEL network study in Lebanon

Dr. Marianne Abi Fadel from the Saint Joseph University (USJ) of Beirut, presented an overview of the COVID-19 epidemic in Lebanon. The Rodolphe Mérieux Laboratory (LRM) in Beirut was the first private university laboratory? to have responded to the call of the Ministry of Public Health, by providing tests for SARS-CoV-2 since the beginning of March 2020, in addition to the laboratory of the Rafic Hariri University (governmental) Hospital. The LRM is working on variant surveillance and deciphering, with the Laboratory of Biochemistry and Molecular Therapeutics at the School of Pharmacy at USJ. The findings are important to raise public awareness of the emergence and the rapid spread of new variants in Lebanon, as well as enforcing stronger prevention measures. The results will also be shared and compared with those obtained in other countries involved in the GABRIEL network study.

The GABRIEL network study in Madagascar

Dr Tahinamandranto Rasamoelina from the Charles Mérieux Center for Infectious Disease, Antananarivo, described the COVID-19 situation in Madagascar and the low number of cases that have been recorded. The GABRIEL network variant surveillance study is designed to monitor the frequency and spread of VOCs. Alpha and beta coronavirus variant has been found circulating in Madagascar. The steps that now remain to be completed involve variant screening, data analysis, characterization by Sanger and/or NGS, and redaction of the final report. The results will advise collaborating clinicians and the Ministry of Health about the identification of variants circulating in Madagascar.

The GABRIEL network study in Cameroon

Dr. Richard Njouom from the Centre Pasteur of Cameroon (CDC) described the three COVID-19 epidemic waves that have occurred in Cameroon since March 2020. A CPC network of 20 COVID-19 PCR laboratories is operating in Cameroon's ten regions. A nationwide strategy of SARS-CoV-2 genomic surveillance in Cameroon has been developed and should soon be validated by the Ministry of Health. This genomic surveillance in Cameroon will be maintained in conjunction with WHO/CDC Africa and Afroscreen networks.

The CDC is also involved in the Pathogen Genomics Initiative with the WHO/CDC Africa, in REPAIR with Pasteur Institutes International, and Afroscreen with the Agence Française de Développement. CPC has also developed PlaCARD, a nationwide database that analyzes and reports COVID-19 data.

The GABRIEL network variant survey will offer an overview of circulating SARS-CoV-2 VOC in Cameroon, identify the SARS-CoV-2 lineages, and correlate the viral variants with disease severity and disease rate. These results will be shared with Cameroonian health authorities and the scientific community.

The GABRIEL network study in Bangladesh

Dr. Shakeel Ahmed from the Bangladesh Institute of Tropical and Infectious Diseases signaled that the numbers of COVID-19 cases in Bangladesh have escalated to the point where they are creating a severe socio-economic burden in the country. The third wave of infections, which occurred from May to August 2021, was mainly caused by the delta variant and devasted the population. The overall seroprevalence rate in both urban and rural areas is over 60%.

From the GABRIEL network SARS-CoV-2 study, the team expect to learn about the country's circulating lineages in the country, as well as the prevalence and reinfection rates of each variant responsible for symptomatic and asymptomatic infections. This data will help in the development of health policies by health authorities. Sequenced data and appropriate metadata will be shared on public databases and with the scientific community. However, there is still a need for further sustainable funding for training and supervision, as well as support in bioinformatics.

Dr. Firdausi Qadri from ideSHi, Bangladesh, presented an overview of the COVID-19 pandemic in Bangladesh emphasizing that the third wave, which occurred between May and August 2021, was the most devastating. The overall seroprevalence rates in Bangladesh in both urban and rural areas is over 60%. IdeSHi carries out testing by RT-PCR and rapid antigen testing on individuals presenting COVID-like symptoms among Bangladeshis and the Rohingya population in Cox's Bazar, and the results are transmitted to the Directorate General of Health Services. IdeSHi is also evaluating antibody responses to COVID-19 vaccines in collaboration with other institutions.

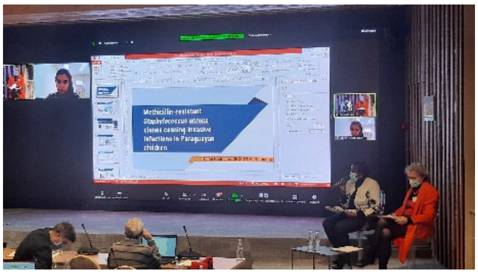
The team has set up the MinION sequencing platform and have sequenced more than 500 SARS-CoV-2 genomes. IdeSHi has formed a Genomic Consortium and through a Nationwide Systematic Surveillance is informing the DGHS of the circulating variants of SARS-CoV-2, with support of the GABRIEL network.

From the ongoing Variant PCR Project, the expectation is to understand the circulating lineages across the country and correlate the variants with diseases severity. Another objective is to determine the prevalence and reinfection rates by each variant responsible for symptomatic and asymptomatic infections so that a baseline can be established for further surveillance studies. The results are intended to help the health authorities of participating countries to make decisions and inform the scientific community.

SESSION III – YOUNG SCIENTIST AWARD PRESENTATION

Headed by the Scientific Award Committee and chaired by Dr. Sara Eyangoh, Cameroon, and Dr. Nathalie de Rekeneire, France

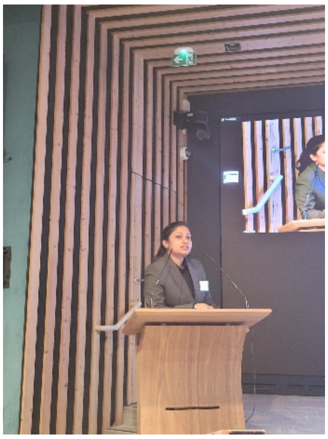
Methicillin-resistant Staphylococcus aureus clones causing invasive infections in Paraguayan children



Fátima Rodriguez from IICS-UNA, Paraguay, described her cross-sectional study that is aimed at molecularly identifying the main Methicillin-resistant *S. aureus* clones that cause invasive infections in Paraguayan children. The study was carried out using patient clinical data, antibody susceptibility tests, molecular characterization (virulence factors and methicillin-resistant genes detection and, multi-locus variable analysis) and clonal identification by cassette SCCmec, spa and, multi-locus sequence typing.

The results indicate that the most severe infectious S. *aureus* pathogens in Paraguayan children are predominantly associated with the Southwest Pacific clone CC30-ST30-IV. All the isolates analyzed carried at least one virulence factor that may be responsible for aggravating the infection.

Evaluation of saliva as a non-invasive alternative specimen for the detection of SARS-Co-V-2



Dr. Zannat Kawser from the Institute for Developing Science and Health initiatives (ideSHi), Bangladesh, pointed out that a recent Yale study found that the viral load from a saliva test is a stronger predictor of COVID-19 severity than one from an NP test. In addition, Saliva can be self-collected, does not cause coughing and sneezing unlike NPS and thus minimizes the risk of infection to healthcare workers in Bangladesh.

The study aimed at showing that saliva maintains over 90% sensitivity in clinical SARS-CoV-2 tests when compared to NPS samples. Sensitivity and specificity of saliva sample regarding NPS RT-PCR as the reference gold standard were determined. RBD-specific IgM and IgG antibody concentrations were measured and will be correlated with the concentration of secretory IgA in saliva (still under optimization). SARS-CoV-2-specific antibodies in the serum of patients who had previously tested positive on saliva and/or NP swab RT-PCR analysis were detected and measured.

It was found that saliva samples maintained clinical performance of over 90% sensitivity when compared to the nasopharyngeal swab samples as the reference method in adult patients. As a result, saliva is as effective as NPS for SARS-CoV-2 identification in infected symptomatic and asymptomatic individuals. Saliva sample collection is faster, and detection of SARS-CoV-2 can be run at a lower cost. Also, the self-collection of a saliva sample is possible, whereas one of an NPS is not. The use of self-collected saliva can improve patient compliance and satisfaction, particularly in the case of surveillance testing.

Microbiological profile of facial cellulitis in Mali

Dr. Elisabeth Sogodogo from the Charles Mérieux Center for Infectious Disease explained that a better understanding of the specific microbiological profile associated with cervico-facial cellulitis in patients in Mali can improve patient care. The research was aimed at identifying the microorganisms (bacteria and archaea) that cause cervico-facial cellulitis and attempted to determine their resistance profile.

Samples were obtained from infected patients by swabbing or by syringe puncture. The specific aerobic and anaerobic bacteria that were isolated from the samples were diverse, with *S. epidermidis* predominating. A similar study was conducted to detect methanogens. A genomic analysis and a study of the resistance profile of the pathogens are underway.

Multi-country evaluation of RISK6, a 6-gene blood transcriptomic signature for tuberculosis diagnosis and treatment monitoring

Rim Bayaa from the Microbiology, Health and Environment Laboratory (LMSE) in Lebanon stated that non-sputum diagnostics and techniques for monitoring tuberculosis treatment response are urgently needed. The drawbacks of sputum-based TB tests are because they are slow to culture and lack the sensitivity and specificity of smear microscopy. Furthermore, for certain patients, collecting sputum samples can be problematic.

The study aimed at evaluating how well RISK6, a PCR-based six-gene blood transcriptomic signature, performed in terms of TB diagnosis and treatment monitoring. The RISK6 scores were calculated from patient blood samples and were found to be directly correlated to TB detection in sputum samples. These results suggest that RISK6 signature meets the minimal requirement set by the WHO for non-sputum-based screening TB tests that discriminates patients with active TB in both healthy donors and latent TB infected groups.

RISK6 signature, as a TB biomarker, could also be potentially utilized to identify patients with high transmission risk and monitor the response to anti-TB treatment.

Current work in progress includes high-dimensional spectral flow cytometry analysis, in-depth characterization of the specific immune response against M. tb, as well as a study of the phenotypic and functional profiles of immune cell subsets and of immunological markers for monitoring the TB treatment response.

Evaluation and monitoring of the prevalence of extended spectrum beta-lactamase-producing Escherichia coli in Madagascar: The Tricycle project, year 2

Tiavina Rasolofoarison from the Charles Mérieux Center for Infectious Disease in Madagascar described the Tricycle project which is part of WHO's Global Antimicrobial Resistance Surveillance System (GLASS). This system provides a standardized approach to the collection, analysis, interpretation, and sharing of data, and monitors the status of existing and new national surveillance systems, with a focus on representativeness and quality of data collection.

The main objective of this project is to implement a shared, streamlined, and integrated multisectoral surveillance system to assess the prevalence of ESBL-producing E. coli.

The study followed the One Health concept, in that it was carried out in the environmental, animal, and human sectors. Samples were collected from four water sources in the Antananarivo area, from chickens sold in various local markets and from public laboratories belonging to a national network called RESAMAD.

The findings of the study will be used for the AMR surveillance system in collaboration with Madagascar's Ministry of Public Health. Further molecular characterization and sequencing are planned. Following on from this concept, another project called TRIuMPH will be carried out in various countries to identify carbapenemase-producing Enterobacteriaceae with the use of genomics and whole-genome sequencing as part of One Health Surveillance.

SESSION II – Acute respiratory infections

Chaired by Dr. Firdausi Qadri, Bangladesh, and Dr. Philippe Vanhems, France

Introduction: Roadmap and strategy on ARI-Pneumonia

Dr. Valentina Picot, head of Acute Respiratory Research and Interventions from the Mérieux Foundation, presented the Acute Respiratory Infections/Pneumonia roadmap with the three pillars, research, capacity building and advocacy, for the activities and interventions that will be deployed in the fight against ARIs, particularly childhood pneumonia in LMICs. A stepwise approach will be implemented to adapt an appropriate response that contributes to the prevention and improved care of pneumonia through the three pillars. The overall objective is to establish an integrated approach for better assessing and delivering care to pneumonia cases in crisis settings and reducing the frequency of severe cases. This can be achieved by addressing public health issues and promoting real practical solutions.

The main target populations are children under five years of age, persons living in low- to middle-income countries, and those in crisis settings, such as refugee camps.

The ARI work at the Mérieux Foundation started in 2010, a period over which about twelve projects have been conducted on this thematic in collaboration with numerous global stakeholders. The COVID-19 pandemic has given a new dimension to pneumonia globally and to our ongoing research projects. Through the NOSO-COR project, suspected cases of nosocomial SARS-CoV-2 infections have been characterized and a serological study performed on the immune status of health workers who have been infected or exposed to COVID-19. Through the FDP- COVID-19 project, epidemiological patterns have been investigated, as well as viral transmission, and the serological response of Forcibly Displaced Populations.

The findings contributed to unveiling information on pneumonia etiology in countries where little or very scarce information was then available, in guiding public health authorities for the introduction of PCV vaccines, in assessing diagnostics to better drive therapeutics, and globally aiming at the improvement of pneumonia case management in numerous LMICs.

Every Breath Counts Coalition

Leith Greenslade, Coordinator of the Every Breath Counts Coalition, outlined the efforts of Coalition members, including UN and global health agencies, businesses, donors, academic institutions and NGOs, to support national governments to reduce pneumonia deaths by 2030, including from COVID-19 and presented the findings of the Coalition's new report,

"The Missing Piece: why the global pandemic is an inflection point for pneumonia control".

Pneumonia is the world's biggest infectious killer of adults and children, claiming the lives of 2.5 million people in 2019 alone, including 672,000 children, according to the Global Burden of Disease. COVID-19 is forecast to more than double deaths from respiratory infections to 6.1 million in 2021. And the secondary impact of disruptions in vaccination services and rising rates of wasting could result in more child pneumonia deaths from other causes, further increasing mortality.

To reduce all-cause pneumonia deaths, Every Breath Counts promotes the development of reinforcement of pneumonia surveillance through national pneumonia management strategies and the Global Action Plan for Pneumonia. It also encourages financial support for pneumonia surveillance and investment in cutting-edge technology. National Pneumonia Control Networks, in partnership with industry and NGOs, can work jointly to achieve pneumonia preparedness.

NOSO-COR: A prospective, observational, hospital-basedmulticentre study on nosocomial SARS-CoV 2

Dr. Mitra Saadatian-Elahi from the Hospices Civils de Lyon, spoke about the NOSO-COR project, a prospective, observational, hospital-based multicenter study on nosocomial SARS-CoV-2 transmission carried out in 13 French hospitals and seven international centers. The international centers were coordinated by the Mérieux Foundation. The main objective was to describe the clinical characteristics at admission of community (CA) and hospital-acquired (HA) SARS-CoV-2 infections and to compare the outcomes, such as mortality, between the two groups.

In French hospitals, a total of 1,380 adult patients were enrolled during the first wave (Feb-June 2020) and followed-up until discharge or death. Based on the ECDC definition, 232 patients were classified as definite HA-COVID19 (symptom onset after 14 days from admission).

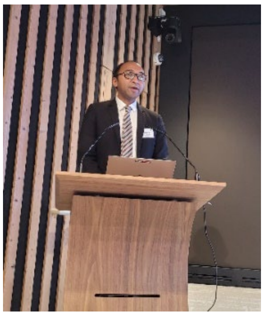
Comparison of the characteristics of CA and COVID-19 patients at admission showed that HA-COVID patients were older than CA-COVID patients and had more underlying chronic conditions, such as cancer and cardiovascular disease. The risk of in-hospital death was not different between HA and CA-COVID19 patients.

The sub-analysis of CA-COVID-19 patients included in Lyon University affiliated hospitals showed that time between the symptom onset and hospital admission was shorter for older patients. Older age and shorter delay between symptom onset and hospital admission were associated with decreased risk of hospitalization in ICU. The lower number of older adults treated in an ICU could be due to:

- A declining immune system in older adults compensated by more prudent behaviors, leading to earlier presentation at hospital and therefore more rapid management of these patients
- Widespread information about the low COVID-19 morbidity and mortality that may dissuade young people from seeking care.

In the investigation on the impact of patients' smoking habits on the severity of CA-COVID-19 patients, differences were observed in outcomes between active and ex-smokers. This confirms the relevance of distinguishing these two populations regarding the impact of smoking on the severity of COVID-19.

Further analyses are ongoing to identity the determinants associated with nosocomial onset. An ancillary study was also carried out with the aim of describing the serological response in CA-COVID-19 patients several months after the infectious episode.



Dr. Tahinamandrato Rasamoelina, from the Charles Mérieux Center for Infectious Disease, Antananarivo, spoke about the NOSO-COR project in Madagascar. The objective was to describe, and document suspected and confirmed cases of nosocomial SARS-CoV2 infections, as well as their clinical spectrum and determinants (risk factors) in three hospitals in Antananarivo. Cases of CA SARS-CoV2 likely to be a source of HA SARS-CoV2 infection were included. Finally, he described the procedures used to monitor SARS-CoV2 infections in a hospital setting.

From this study, the results should determine the proportion of medical staff and patients with suspected or confirmed nosocomial SARS-CoV-2 infection among individuals with a respiratory infectious syndrome suggestive of SARS-CoV-2 infection.

He also presented the NOSO-COR Immuno study, whose primary objective is to determine the seroprevalence rate among healthcare workers in three university hospitals who have been exposed to COVID-19. This immunological component to the NOSO-COR study will include a 6-month follow-up of health workers who have contracted COVID-19 or who have a history of potential exposure.

Dr. Daouda Coulibaly of the Côte d'Ivoire's National Institute of Public Hygiene noted that the COVID-19 epidemic has had a minimal impact on the country, with only 696 deaths and just over 60,000 cases reported so far.

Once completed, the NOSO-COR multicenter study on nosocomial transmission of SARS-CoV-2 virus in the country is expected to confirm the nosocomial risk of SARS-CoV-2 in healthcare facilities, raise awareness of the role of health care personnel in preventing the spread of the SARS-CoV-2 virus in hospitals, supplement existing recommendations by acquiring additional data concerning the transmission of the virus, and provide data that will support vaccination coverage among health workers.

According to available data, COVID-19 has a high risk of nosocomial transmission. The results showed a high prevalence of the disease at hospital admission, as well as the fact that the detection of COVID-19 takes a long time (for 63% of patients, time between onset of symptoms and confirmation of COVID-19 is greater than 7 days). Nosocomial transmission can be avoided by adopting control measures in hospitals and isolating COVID-19-positive health care workers.

FDP-COVID-19–SARS-CoV-2 detection and serological response cohort study in the FDMN (Forcibly Displaced Rohingya Myanmar National) population in Cox's Bazar: a retrospective and prospective study

Dr. Nabid Anjum Tanvir from the International Centre for Diarrheal Disease Research, Bangladesh, stated that among the one million Forcibly Displaced Myanmar Nationals (FDMNs) in Bangladesh, 60% are women and children living in refugee camps with inadequate sanitation and hygiene, public health facilities, and access to clean water. ARIs are responsible for the greatest rates of mortality and morbidity. In the under-five-year age group, the morbidity rate reaches 36%, and, overall, 32 % of people suffer from ARIs. The death rates due to ARIs are 30% (<5 children) and 36% (for all age groups). As a result of the COVID-19 pandemic, the situation has become increasingly desperate.

This study assessed the COVID-19 risk factors in two health facilities in Cox's Bazar since March 2021 as part of the SARS-CoV-2 detection and serological response investigation.

This study aims to:

- improve the understanding of viral transmission and epidemiological patterns of SARS-CoV-2 circulation in refugee camps, before and during the outbreak period,
- evaluate the serological response to SARS-CoV-2 from identified COVID-19 cases and their households at point zero and given endpoints of longitudinal intervals,
- assess the risk factors that heighten transmission, particularly in refugee camps settings, and
- improve best practices for the implementation of Infection Prevention and Control (IPC) measures in high crisis settings.

So far, the results have shown that the seropositivity among Covid-19 cases and household contacts is very high at the time of testing. Further testing will determine whether antibody levels persist. The investigation is still ongoing, and the study cases and households will be assessed for exposure and seroprevalence.

LARI-RMNs – Lower Acute Respiratory Infections – Etiology Research with a nested randomized controlled trial of POC diagnostics in the displaced Rohingya population settled in humanitarian refugee camps in Bangladesh

Dr. Abu Bakar Siddik from the Institute for Developing Science and Health Initiatives (ideSHi), Bangladesh, presented an etiological study that examines the disease burden among FDMN's in Bangladesh. According to prior data, ARIs account for 42% of reported cases in the under-five-year age group. Bacterial pathogens were isolated from blood and nasopharyngeal swab (NPS) samples and confirmed by RT-PCR as well as microbiological culture techniques. Antibiotic resistance patterns and serotype distributions were also measured in the obtained specimens. Moreover, viral pathogens isolated from NPS samples were tested by molecular techniques.

The results of the study suggest that in such crisis settings point-of-care diagnostics can lead to more assertive case management and treatment alternatives. In addition, the differentiation of S. pneumoniae serogroups should lead to improved PCV vaccine deployment. Pathogen identification should become more precise when more sensitive and specialized techniques are developed and implemented.

SESSION III – Antimicrobial resistance

Chaired by Prof. Abdoul Salam Ouedraogo, Burkina Faso and Prof. Luc Hervé Samison, Madagascar

Introduction: Roadmap and strategy on AMR

Dr. Florence Pradel from the Mérieux Foundation described the global challenge of anti-microbial resistance (AMR), which has been linked to antimicrobial overuse and misuse in humans, animals, and agricultural practices, as well as the spread of AMR genes in the environment, and AMR transmission from animals to humans through direct contact and the environment.

The World Health Global Action Plans aims to:

- raise awareness and understanding of AMR through effective communication, education, and training,

- strengthen knowledge through surveillance and research,
- reduce the incidence of infection through effective sanitation, hygiene, and infection prevention measures,
- optimize the use of AMR medicines in human and animal health, and
- make sustainable investments in the combat against AMR (new medicines, diagnostic tools, vaccines).

LMICs have difficulty in implementing AMR actions plans due to the inherent weaknesses of their laboratory systems, epidemiological surveillance, and research capacity.

The Mérieux Foundation seeks solutions to these problems by promoting surveillance programs and capacity-building for laboratory diagnostics through networks such as RESAMAD, RESAOLAB, and Fleming Funds Country Grants. It also contributes to the launch of initiatives aimed at the management and prevention of infections, better antibiotic prescriptions, and real-time monitoring of bacterial ecology through a One Health approach. The partnership with ANSES, the French agency for food, environmental, and occupational health and safety, strives to attain these objectives through shared training activities on AMR genomics and bioinformatics.

Multicenter evaluation of the impact of COVID-19 pandemic on the spread of antimicrobial and biocide resistance from wastewater to the environment

Dr. Valerie Donkeng from the Centre Pasteur du Cameroun gave a progress report on the COV-AMR project in Cameroon. The objective of the agreement between the CPC and the Mérieux Foundation is to examine the impact of the COVID-19 pandemic on the spread of antimicrobial and biocide resistance in the environment arising from the sewage of healthcare facilities treating COVID-19 patients. It also aims to compare the prevalence of antibiotic- and biocide-resistant bacteria and associated resistance factors and genes from different sampling sites, compare the level of antibiotic residues, and identify phylogenetic links between strains that have been isolated.

The team are currently working on sample analysis that includes the identification of antimicrobial- and biocide-resistant bacteria, DNA extraction, and the detection and quantification of SARS-CoV-2 in surface water bodies. Metagenomic analyses and quantification of antimicrobial residues are the ongoing activities to be conducted through July 2022 in Brazil.

Dr. Nazareno Scaccia from the Institute of Tropical Medicine, Brazil, spoke about the Brazilian component of the AMR project. The experimental work will rely on water samples that will be collected from three different locations in Brazil and the objectives will be to:

- detect and quantify SARS-CoV-2,
- compare the abundance of antibiotic-resistant bacteria and resistant genes,
- compare the level of antibiotic residues and biocides and analysis of the bacterial communities.

The protocols for water collection, antibiotic measurements, DNA extraction and so on were defined and shared with the participants from Cameroon and Madagascar. This work is based on a pilot study where antibiotic resistance in water bodies is being studied.

Pr. Luc Hervé Samison from the Charles Mérieux Infectious Disease Center, Madagascar, described the AMR project being conducted in Antananarivo. Water samples must be collected at two locations: one in a hospital receiving COVID-19 patients and one in a hospital with no COVID-19 patients and there must be comparison between water in rivers running through the city and a lake. The purpose of the genomic analyses on the isolated microorganisms is to determine whether the data obtained on AMR before the COVID-19 epidemic is the same as that found during the epidemic, and to measure the degree of correlation between changes in AMR and the onset of the COVID-19 epidemic.

Prevalence, risk factors and genetic characterization of ESBL E. coli isolated from healthy pregnant women in Madagascar

Milen Milenkov from the Mérieux Foundation spoke about Tricycle, the One Health project proposed by WHO, which is aimed at quantifying ESBL-*E. coli* prevalence in humans, the food chain, and the environment. Experiments include the phenotypic and molecular characterization of bacterial isolates, antibiotic susceptibility testing, identification of phylogenetic groups, search for resistance and virulence genes, point mutations, plasmid content, and the study of the genetic links between isolates of different origins.

The human component of the study comprised a cohort of asymptomatic ESBL-*E. coli* carriers in a group of pregnant women in Madagascar. Bacterial isolates from these participants were examined for their resistance profiles, phylogeny, strain-type diversity, geographical dissemination, number of virulence genes, and plasmid content.

The results show that community ESBL-*E. coli* has a high prevalence (34%) in Madagascar, the wet season being the sole risk factor linked to carriage. The bacterial isolates show great genetic diversity and mainly exhibit commensalism associated with phylogenetic group A. Few virulence genes were detected. However, a carbapenem-resistant strain was discovered in the absence of strong selective pressure.

Future research will focus on the analysis of resistance transmission mechanisms of isolates from animals and the environment. The impact of global warming is an unknown factor.

Identifying convergences and cooperation on AMR with the GABRIEL network

Dr. Jean-Yves Madec presented ANSES, the National Agency for Food, Environmental, Occupational Health and Safety, a public establishment based in Lyon that intervenes in the domains of food, the environment, health, and animal welfare, under the supervision of the French Ministries of Health, Agriculture, Environment, Labor and Consumer Affairs. ANSES assesses health risks to provide information to public decision-making bodies. It is made up of a network of nine French reference and research laboratories that work in the fields of animal health, food safety, and plant health. By incorporating the socio-economic components of health risks, it executes independent and pluralistic scientific expertise among groups of specialists.

ANSES coordinates the surveillance of AMR in animals at the national level with the National Monitoring Network called Resapath, at the EU level with EARS-Vet, and at the global level, with the FAO. ANSES has concentrated its efforts on the detection of ESBL-*E. coli*. The Agency has described these pathogenic strains and provided a quantitative assessment of the risks in various foods and has also assessed the impact of prevention and control measures utilized throughout the food production, distribution, and consumption chain (hygiene regulations, self-monitoring, consumer practices) on reducing the risk of ESLB-*E. coli.* Research is also being conducted on the role of AMR plasmids in food and food-producing animals.

ANSES shares its objectives with those of the GABRIEL network. It stimulates critical thinking and disseminates data on epidemiology through the One Health approach to all stakeholders. At the global level, it collaborates with partners in Africa, Brazil, and the Middle East to improve local applied research, improve laboratory-based characterization of AMR, and solve pertinent scientific issues through training and capacity-building.

Mapping of the AMR activities within the GABRIEL network

Speakers from various GABRIEL network member countries reported on the latest developments of AMR research in their laboratories.

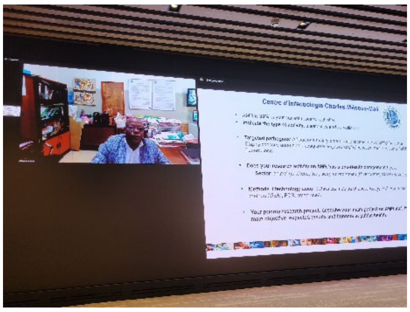
Dr. Sara Eyangoh from the Centre Pasteur of Cameroon presented the priority research project, “Metagenomics analysis of hospital effluents as a tool for monitoring antimicrobial consumption”, which describes the monitoring of AMR genes and antimicrobial residues in hospital wastewater by measuring the discharge of antibiotics by Cameroonian health facilities during the COVID-19 outbreak. Antimicrobial determinants and residues released into water bodies are measured for potential environmental and human health hazards. This information can be used to develop management control techniques based on microbial sensitivity.

Dr. Firdausi Quadri from ideSHi, Bangladesh, outlined the current AMR research in progress that includes studies on:

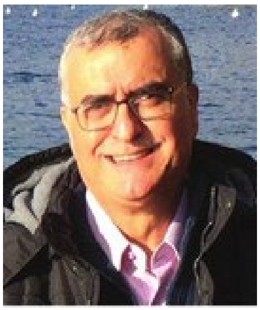
- lower ARIs among the Rohingya immigrant population in Cox’s Bazar,
- the correlation between COVID-19 and enteric pathogens,
- invasive and non-invasive K. pneumoniae infections in hospitalized patients and healthy controls.

Conventional microbiological methods and genome sequencing with bioinformatic techniques (ARIBA) are being utilized.

Dr. Shakeel Ahmed from BITID, Bangladesh, stated that the surveillance and diagnostic research on AMR represents 15% of BITID’s activities. The Institute of Epidemiology Disease Control and Research centralizes AMR surveillance, and its data is shared with the GLASS network. The main pathogens under study are E. coli, Klebsiella, Enterococcus, Pseudomonas for urine, and Salmonella, Shigella, and Vibrio for stool. Microscopic examination, culturing, and disk diffusion antibiotic susceptibility testing are some of the techniques used.

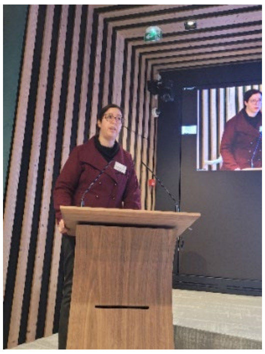


Prof. Bourema Kouriba from the Charles Mérieux Infectious Disease Center, Mali, reviewed the current research on hospital-acquired bacterial infections in the ICU and operating room of the Gabriel Touré Hospital. The incidence of bacteria responsible for nosocomial infections, as well as the antibiotic sensitivity of the isolated bacteria, have been determined. Multidrug-resistant bacteria and their molecular markers of antibiotic resistance have been molecularly characterized. These findings should help improve the quality of care in the ICU and reduce patient morbidity and mortality. A further project consists of identifying the genes responsible for antibiotic resistance and their mechanism of dissemination between the environment, animals, and humans. The results will be integrated into an AMR prevention and control initiative and should have an impact on the public understanding of AMR and the rational use of antibiotics in Mali.



Prof. Monzer Hamze from the Faculty of Public Health (FPH) and Doctoral School in Science and Technology (DSST) of the Lebanese University spoke about the priority research project involving the assessment of environmental pollution by antibiotics, antibiotic residues, and AMR bacteria that contribute to the spread of bacterial resistance. The results should serve to develop strategies for minimizing environmental contamination. Research is focused on M. tuberculosis, carbapenem-resistant Enterobacteriaceae (CPE), plasmid-mediated colistin resistance, and non-fermenting gram-negative bacilli (*Pseudomonas spp, B. cepacia, S. maltophilia*).

Prof. Luc Hervé Samison from the Centre d'Infectiologie Charles Mérieux outlined the TRIuMPH project, which involves a consortium of three countries from the South. Its goal is to upscale the monitoring and detection of ESBL *E. Coli*, CPE and the creation of a pipeline of WGS for One Health Surveillance. The results should generate data on ESBL *E. Coli* and CPE prevalence in humans, animals, and the environment. WGS analysis should reveal the diversity of CPEs circulating in various sectors and help determine whether animal and environmental CPEs are also found in humans.



Dr. Rosa Guillen Fretes from the Instituto de Investigaciones en Ciencias de la Salud, Paraguay, described the AMR research project underway that involves using *E. coli* that are resistant to beta-lactam antibiotics as indicator organisms to track the spread of AMR in humans, animals, and the environment. The results of AMR serving as baseline data for Paraguay will be compared with those from other local regions and countries around the world and should serve as a model for subsequent epidemiological surveillance systems.

Dr. Nazareno Scaccia from the Institute of Tropical Medicine, Brazil, explained that AMR accounts for 80% of the laboratory's research activity. The current project involves using the loop-mediated isothermal amplification (LAMP) technique to detect resistance genes in pathogens causing community urinary tract infections. LAMP is a rapid, economical, portable, and simple-to-use test for detecting antibiotic resistance in different environments, with results that can potentially improve health outcomes, especially in resource-poor countries, where other detection methods are too complicated or expensive. LAMP technology can also be used as a diagnostic platform for other targeted genes.

Dr. Ana Tereza Ribiero de Vasconcelos from the National Laboratory of Scientific Computation, Brazil, presented the AMR project that aims to determine AMR distribution in humans and animals resulting from bacterial transmission between animal hosts and the spread of AMR. The data obtained will be utilized to develop strategies for controlling the spread of AMR.

Enterobacteriaceae, Pseudomonas, Acinetobacter, Enterococcus, Staphylococcus, Salmonella, Streptococcus, and Haemophilus are the targeted microorganisms.

Overview of funding opportunities

Laurence Mazuranok from the Mérieux Foundation introduce the available funding opportunities to support AMR research either as ongoing or as future expected ones, with the eligible countries: EU4H-2021-PJ-14. This action offers grants earmarked for improved infection prevention and control (IPC) measures and antimicrobial stewardship (AMS) in hospitals and long-term care facilities. It complies with the EU One Health Action Plan against AMR to ensure patient safety in hospitals. This support includes funding for training in IPC and AMS, incentive schemes, and investments into healthcare infrastructure, but limited only to 2 members among the GABRIEL network (Georgia, Ukraine).

EDCTP3 (European & Developing Countries Clinical Trials Partnership): EU-Africa Global Health supports international research partnerships aimed at accelerating the clinical evaluation of drugs, vaccines, and diagnostics for sub-Saharan Africa's most dangerous infectious diseases. It seeks to provide people with greater access to breakthrough medical interventions, while also contributing to regional and global health security. This can be achieved through EU-Africa and South-South collaboration and capacity-development in clinical trials and implementation research and health interventions to tackle poverty-related infectious diseases and their interaction with non-communicable diseases.

HORIZON-HLTH-2022-DISEASE-07 has the aim of supporting actions that use effective disease management and international collaboration to combat diseases and reduce disease burden in the EU and around the world. It fosters expertise in research and innovation, as well as capacity building in the fight against communicable and non-communicable diseases. This fund provides grants to strengthen knowledge-based disease management strategies and policies, as well as steps to mitigate the threat of epidemics and the spread of AMR infections.

SESSION IV - TUBERCULOSIS

Chaired by Dr. Nestani Tukvadze, Georgia, and Dr. Delia Goletti, Italy

Introduction: Roadmap and strategy on TB

Dr. Jonathan Hoffmann of the Mérieux Foundation discussed the challenges of improving tuberculosis diagnosis and management through laboratory capacity-building and operational research.

The HINTT (HBHA immuno-monitoring for tuberculosis treatment) project is GABRIEL network's multi-country study (Madagascar, Lebanon, Bangladesh, Georgia, Paraguay) that evaluates TB diagnostic techniques for better TB treatment monitoring. The overall objective is to evaluate new diagnostic approaches that can distinguish patients who respond well to treatment compared to those who do not. The specific methods being studied are white blood cell counts, the QuantiFERON-TB Gold Plus and the heparin-binding hemagglutinin IFN-γ release assays, high dimensional cytometry, and blood-based RISK6 signature, all of which are powerful techniques used to detect subjects with active tuberculosis.

DEDICATE is a hospital-based study in Bangladesh that seeks to enhance TB patient management by using a non-sputum-based test that should improve TB diagnostics. By these means, molecular TB diagnostics can be decentralized at the community level.

The APRECIT project is designed to evaluate different screening strategies and improve the overall program management of latent TB infections in Madagascar and Cameroon. It entails assessing a community intervention strategy for TB identification and management of household members and individuals in high-risk groups (children under the age of five and HIV-positive individuals) in a quantitative and cost-effective manner. Diagnostics are performed with two interferon-gamma-release assays (IGRA) and GeneXpert MTB/RIF to predict the progression from LTBI to active TB infection from a sensitivity/specificity and a cost/efficacy perspective.

The Mérieux Foundation also works with the French National Research Institute for Sustainable Development (IRD) on operational TB research programs with the aim of developing innovative and efficient TB diagnostics for effective TB management in high-risk groups (children and PLHIV) at the community level.

The Lyon TB study group is dedicated to molecular epidemiology and basic TB research. It contributes to the study of contact tracing and TB transmission. Rapid anti-microbial susceptibility testing based on whole-genome sequencing, as well as the impact of microbial factors on the various clinical presentations and outcomes of tuberculosis are also topics of investigation.

Keynote address on childhood TB

Dr. Maryline Bonnet from the IRD, France, stated that of the estimated 1 million worldwide cases of TB in children in 2020, only 63% were notified to WHO most likely due to underdiagnosis for several reasons:

- diagnosis is complex in the context difficulty to collect respiratory specimen in young children and paucibacillary nature of childhood tuberculosis,
- the physiopathology of TB is not yet fully understood,
- childhood TB is a low priority in research,
- diagnostic techniques successfully developed for adults cannot be replicated on children,
- the lack of a reference standard for child TB complicates the development of new diagnostic techniques, and
- in health care systems of resource-limited countries with high TB incidence, child TB services are overcentralized,
- lack of systematic TB screening of children contacts of adult index cases.

The WHO has recommended initial diagnostic tests for children with presumptive pulmonary TB:

Xpert MTB/RIF and Xpert Ultra (or TB-LAMP and Truenat MTB using adults' data extrapolated to children) for pulmonary and extra-pulmonary tuberculosis,

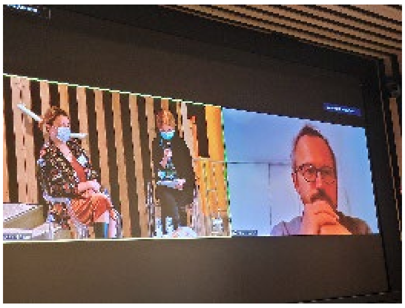
Urine LAM antigen point of care test used in HIV-positive children with presumptive TB or AIDS or who are seriously ill.

Although majority of children are initiated on treatment empirically, minimal attention is given to clinical diagnosis, especially at healthcare facilities. There is a lack of properly trained health staff and access to high-quality chest X-rays remains limited in limited resource settings. Special attention must be given to the TB screening of household child contacts, children with HIV infection or children with severe pneumonia.

Regarding treatment, the SHINE trial has shown that treatment can be shortened to 4 months in children with non-severe disease.

In the future, finding missing cases of child TB must become a top priority. With highly sensitive non-sputum-based assays, new strategies for screening and diagnosing tuberculosis in children must be developed. Biomarker research on MTB proteins, as well as host genomic and proteomic biomarkers, require more funding. In high-TB-incidence and resource-limited areas, there is a need to improve access to chest radiography and existing laboratory procedures. Health care workers must receive greater training in clinical diagnosis.

TB Speed



Dr. Olivier Marcy from the IRD, France, spoke about TB Speed, a research effort designed to improve pediatric tuberculosis services and the early detection of TB. Its purpose is to reduce TB childhood mortality in Cambodia, Cameroon, Côte d'Ivoire, Mozambique, Sierra Leone, Uganda, and Zambia, by devising a practical, cost-effective, and decentralized approach to diagnosis, and improving case-finding and access to treatment.

Up to 23% of children with pneumonia are diagnosed with TB. However, cases are frequently ignored or delayed. The SPEED-Pneumonia study aims to assess the extent to which 12-week mortality is impacted by implementing systematic TB detection with Xpert MTB/RIF Ultra on nasopharyngeal aspirates (NPA) and stools collected from children under five years of age with severe pneumonia.

Xpert MTB/RIF Ultra detection technique for TB was performed on one NPA and one stool sample from children with severe pneumonia at the time they were hospitalized. Most of the children, both in this group and in the control group, received standard care which included antibiotics and oxygen therapy. Twelve weeks later at the end of the study, the outcome of the children, whether dead, alive, withdrawn, or lost, was compared between the two groups.

It was found that screening with Xpert Ultra at the time of admission did not lead to reduced mortality in children with severe pneumonia. Death happened relatively early in the follow-up period after hospital admission. Even if Xpert Ultra had been effective, it may not have prevented early fatalities. Greater use of Ultra in this vulnerable group is nevertheless warranted by the high percentage of cases requiring TB treatment.

Deeplex Myc-TB: A new M.tb drug resistance prediction assay

Dr. Philip Supply from the CNRS, France, pointed out that the limitations of existing TB diagnostic and molecular testing are a primary factor that cripples the global fight against drug-resistant TB. This diagnostic difficulty has been further compounded by the updated definition of XDR-TB, which now includes resistance to fluoroquinolone, bedaquiline, and/or linezolid.

Whole-genome sequencing (WGS) is a promising candidate for comprehensive genotypic testing of drug susceptibility and resistance. WGS of more than 3,600 phenotypically drug-resistant or drug-susceptible *M. tuberculosis* isolates TB was done. In a subset of 2,099 genomes, 120 resistance mutations were algorithmically found, starting from a set of candidates associated with phenotypic resistance to first-and second-line drugs. This was used to predict either resistance or susceptibility in an independent set of genomes. Predictions were found to correlate with phenotypic results with 92.3% sensitivity and 98.4% specificity.

Then, starting with newly positive primary cultures, WGS was compared with standard clinical diagnostics, including phenotypic susceptibility testing, in a prospective study. WGS had a 93 percent accuracy rate for predicting drug resistance, was faster, and was even marginally cheaper. However, for routine clinical use, WGS still requires primary cultures, which take one or two weeks to grow, thus delaying diagnosis. However, in the WGS study with 3,600 *M. tuberculosis* strains, the major resistance determinants were concentrated in 14 candidate genes. This high concentration of genetic signal allowed to create a new tailored targeted next-generation sequencing (NGS)-based assay, called Deeplex Myc-TB.

The Deeplex-MycTB test is based on deep amplion sequencing instead of sequencing the complete genome. This is an all-in-one approach that amplifies 18 major resistance gene targets, as well as a mycobacterial identification target and M. tuberculosis genotyping targets, in a single 24-plex PCR reaction. The assay is directly applicable on a clinical sample, which is followed by preparation of amplicon libraries, and sequencing on a NGS platform.

The Deeplex-MycTB assay was compared to phenotypic drug susceptibility testing and WGS, using data from over 4,000 isolates and 1,600 sputum specimens from TB patients. This showed that Deeplex predictions had 95.3% sensitivity and 97.4% specificity versus phenotypic testing. The assay captured between 97 and 99 percent of the resistance phenotypes predicted by WGS performed on isolates.

Deeplex Myc-TB testing has recently been shown to be applicable to stool specimens. A Deeplex drug resistance report showed a high degree of concordance with phenotypic testing done on sputum when stool specimens of the same patients were *M. tb*-positive by qPCR were tested. In another study, Deeplex Myc-TB also revealed resistance to all first-line drugs in isolates that were only identified as isoniazid monoresistant by the standard diagnostic algorithm in place in South Africa. Furthermore, most of thses isolates that were reclassified as multi-drug resistant shared same SNP patterns and an S-type spoligotype, indicating potential outbreak clone. This was confirmed by WGS, which revealed that this clone acquired resistance to all first-line treatments before spreading longitudinally.

APRECIT study

Mame Diarra Bousso Ndiaye from the Institut Pasteur, Madagascar, presented the ongoing APRECIT project entitled, “Development and evaluation of a multiplex assay for detection of SARS-CoV-2 IgM and IgG antibodies in Madagascar”. In the wake of the COVID-19 pandemic, highly performant multiplex anti-SARS-CoV-2 serology tests are needed to precisely describe and date the onset of infections.

A multiplex assay based on the Xmap technology of Luminex, was developed to target specific IgM and IgG antibodies against the Spike 1, Spike 2, Receptor Binding Domain and the nucleocapside protein of SARS-CoV-2. Blood samples over a 12-month period from 43 COVID-19 cases in Madagascar were tested. Receiver operating characteristics curves were generated to determine the cut-off limits and the sensitivity and specificity of the multiplex assay.

In terms of results, the test performed well in detecting anti-IgG and anti-IgM antibodies at day 14 after enrollment. The sensitivity and the specificity for IgGs were equal to 100% for S1, RBD, and NP. This multiplex assay was compared with two commercialized ELISA tests (IDVet IgGNP and Wantai Ig-RBD). The results showed that the in-house multiplex assay had higher sensitivity. Principal Component Analysis was performed using these eight parameters (IgGs and IgMs against four targets) and successfully differentiated the patients based on both time of sampling and clinical presentations.

This high-performance multiplex assay that quantifies the IgG-IgM response to SARS-CoV2 allows for the approximate timing of the infection event. This technique could be valuable for global surveillance and timing of SARS-CoV-2. This assay will be used to monitor the COVID-19 status of TB patients and their household contacts.

Phenotypic and genotypic characterization of Isoniazid-resistant strains of M. tuberculosis from clinical isolates in Bangladesh, Cameroon, and Lebanon

Dr. Valerie Donkeng from the Centre Pasteur of Cameroon explained that isoniazid (INH), an antibiotic used in the treatment of tuberculosis, exhibits mycobactericidal activity by inhibiting mycolic acid biosynthesis. INH-resistance commonly occurs due to mutations in the katG gene or the inhA regulatory regions. From 20 to 30% of INH-resistant strains of M. tuberculosis lack mutations at the katG and inhA genes.

This project aims to screen alternative mechanisms associated with INH-resistant strains of M. tuberculosis complex in clinical isolates from Cameroon, Bangladesh, and Lebanon.

The present study hypothesizes that reduced expression of catalase- peroxidase (enzyme necessary to activate the prodrug INH) activity and the enhanced expression of efflux pumps have an impact on the mechanisms by which M. tuberculosis complex clinical isolates develop resistance against INH. The study design includes a total of 850 M.tb (832 INH-resistant and 18 INH-susceptible strains) clinical isolates. Phenotypic and genotypic DST will be carried out on these isolates.

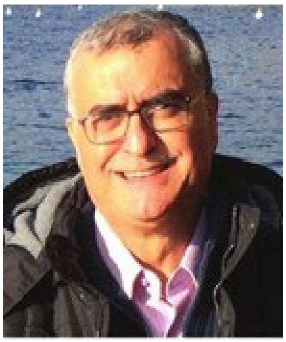
From the results obtained, isolates that are resistant phenotypically but genotypically susceptible will be of interest for this study. MIC will be carried out on these isolates of interest, followed by DNA extraction for katG gene sequencing. Proteins will be extracted from these isolates and used for the quantification of KatG levels using Western blot technique. RNA will be extracted and used for the quantification of the expression levels of the MDR efflux pumps (efpA, pstB, mmpL7, Rv1258c, Rv2136c) using the RT-qPCR.

This study could aid in the development of an assay that can rapidly diagnose all INH-resistant cases and, as such, reduce the spread of MDR-TB and improve effective TB management.

Dr. Mohammad Khaja Mafij Uddin from icddr.b, Bangladesh, explained that 20-30% of phenotypically INH-resistant *M. tb* isolates lack a detectable mutation in the katG or inhA genes linked to INH resistance. The absence of mutations in these target genes suggests that additional or alternative mechanisms of resistance to INH are at work.

The study aims at estimating the prevalence of INH-resistant M. tb isolates without these mutations and to determine the reduced or lack of expression of the katG gene responsible for the INH-activating enzyme catalase-peroxidase. Another goal is to examine the expression of efflux pumps in *M. tb* isolates that are INH-resistant and those that are INH-sensitive.

Research is currently underway despite the delays due to the COVID-19 pandemic and the countrywide lockdown.



Prof. Monzer Hamze from the Microbiology, Health and Environment Laboratory (FPH-DSST), Lebanon described the ongoing work to determine the prevalence of M. tb isolates resistant to INH by culture-based phenotypic tests but lack mutations? revealed by molecular assays. Two types of isolates found in Lebanon are under study.

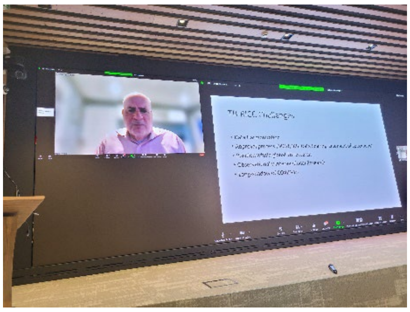
The first type is composed of INH-isolates molecularly tested for resistance mechanisms. In this pool, four isolates are phenotypically INH- resistant, but show no molecular resistance mechanism detected by Deeplex. Most of the isolates showed no drug-resistance-associated mutations despite strong coverage in katG, inhA, fabG1, or ahpC. by Deeplex. A single case of INH-resistant phenotype had an unpredictable INH result using Deeplex-MycTB, due to limited coverage of corresponding gene targets.

The second type of isolates is composed of 150 isolates that have not been tested for drug susceptibility. These isolates were collected from December 2017 to June 2018 throughout the Lebanese Governorates. The team have been facing delays due to the economic crisis in Lebanon and the COVID-19 pandemic.

TB Management and laboratory capacity-building in emergency setting: accomplishments and lessons learned from the Lebanese crisis

Dr. Josette Najjar-Pellet from the Mérieux Foundation, Lebanon, described the 2017-2021 Lebanese National Strategic Plan for the elimination of tuberculosis, which calls for the designation of a National Referral Laboratory for Tuberculosis. The plan provides for laboratory capacity-building through sustainable partnerships with local and international actors, infrastructure upgrades, personnel training, initiation of nationwide studies on TB, implementation of SOPs, and other initiatives. However, the country has gone through considerable unrest in recent years, and the priorities of health facilities have shifted from TB to COVID-19 as consequence of the ongoing epidemic. Health personnel testing for COVID-19 now includes latent TB screening. The August 2020 blast that damaged the national TB center, as well as the impact of the Lebanese financial crisis, have made it difficult to meet financial commitments, procure reagents and equipment, retain staff, and obtain funding. However, we are rehabilitating our facilities and taking action to ensure the continuity of diagnosis and treatment. Looking ahead, we expect to overcome the crisis all the while bearing in mind the value of long-term partnerships, the need for investments during peacetime, the targeting of expertise, and the maintenance of quality standards.

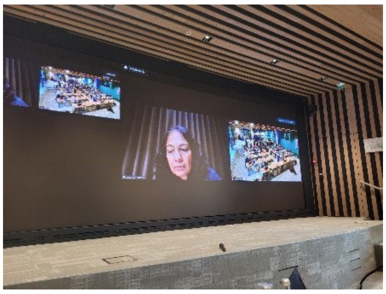
RePORT network: overview and focus on biomarker activities



Dr. Jerrold Ellner from Rutgers Global Health Institute, USA, presented RePORT International that aims to foster the development of TB biomarkers, vaccines, drugs, and diagnostics. The primary purpose of the RePORT International project is to provide a platform for coordinated TB research by establishing a common set of standards and definitions that can be used in the context of observational clinical TB research. This will enable research studies to use pooled data and well-curated biological specimens for future analysis.

The RePORT International Common Protocol encourages TB programs and HIV/AIDS programs to collaborate, for example, to share point-of-care diagnostic laboratory testing, human resources, blood sampling circuits and other resources that are already in place. The work is done within the framework of national TB programs to ensure that activities continue to be viable after the project has ended to make a lasting impact on TB incidence. It should ultimately strengthen health systems overall and help deliver better care to patients.

The project will contribute for at least 6 more years and will help reach the third target of the Strategy to End the Global TB Epidemic by 2035 - namely, to ensure that no family must shoulder the financial burden of TB health care.



Dr. Padmini Salgame from Rutgers-New Jersey Medical School described the RePORT International Coordinating Center (RICC) Biomarker Protocol. Its goal is to find biomarkers that can predict recurrence-free cure and can be used to develop point-of-care (POC) diagnostics.

The aim is to:

- test the efficacy of defined qRT-PCR signatures in predicting cure vs. unfavorable treatment outcomes,
- establish NanoString as an alternative transcriptomic platform,
- test RNA treatment response signatures in MDR-TB,
- test plasma cytokine treatment response signatures,

- test T cell activation markers, and
- compare RNA and protein signatures to treatment response prediction based on clinical variables.

Young Scientist award ceremony

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

After careful deliberation, the GABRIEL network's jury, composed of Dr. Nathalie de Rekeneire, Dr. Delia Goletti, Dr. Sara Eyangoh, Pr. Abdoul Salam Ouedraogo, and Pr. Philippe Vanhems, decided to award the two prizes to:

- Rim Bayaa for her work on the multi-evaluation of RISK6 for TB treatment and monitoring, and
- Tiavina Rasolofoarison for her research on the evaluation and monitoring of beta-lactamase-producing *E. coli*.

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

Close of the Gabriel Network Meeting

Dr. Marc Bonneville concluded this year's edition of the GABRIEL network meeting by thanking all the participants for their contribution. He conveyed his heartful gratitude for the achievements of the Rodolphe Mérieux Laboratories that have earned them international recognition. The Mérieux Foundation's work continues to focus on serving public health and setting up educational programs in all member countries. Today's context is difficult and complex in view of the COVID-19 pandemic. In addition, some countries are not as secure as they once were. But we should never lose sight of the mission: to share knowledge, to keep the laboratories at the heart of the efforts, and to make a real impact on public health, scientific innovation, relief to populations, and scientific career opportunities for the youth of the developing world.