AHRI ANNUAL REPORT 2017



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Armauer Hansen Research Institute
Since 1970

Contents

Foreword

2017 was another great year at the Armauer Hansen Research Institute (AHRI). The Government of Ethiopia has approved the construction of a cutting edge laboratory complex for the Institute. This project is exclusively financed by the government and is expected to be completed within 3-4 years. Illumina NextSeq 500 Sequencer is now up at AHRI and is expected to be running within the next few weeks. We have substantially increased the number of research workforce through hiring new staff. Further, we have expanded our partnership base and won new competitive grants. Ethiopia has joined the European and Developing Countries Clinical Trial Partnership (EDCTP) Association and Coalition for Epidemic Preparedness Innovations (CEPI) and AHRI is a focal Institute representing the country inboth partnerships.

AHRI has continued generating high-quality evidence that could shape health policy and implementation in Ethiopia and beyond. In 2017, we implemented more than 60 research projects and our researchers have contributed 38 peer reviewed articles to the global scientific community. As biomedical research arm of the Federal Ministry of Health of Ethiopia, the focus of our research and innovations is guided by the priorities of the Ministry and health Sustainable Development Goals (SDGs). For instance, we have consolidated the mapping of national burden of viral hepatitis; we have continued the surveillance of antibiotic resistance in the country; and we have supported a dozen of health innovations that could potentially improve the much desired maternal and child health in Ethiopia. Balancing Ministry's priorities with AHRI's core competency, we have improved the depth and breadth of our research in biomedical sciences particularly in tuberculosis and leprosy. Cognizant of the growing importance of the One Health approach, we have increased investment in research in infections that co-affect humans and animals. Our capabilities in conducting clinical trials have grown. As non-communicable diseases are considered new frontiers of public health, our research follows these evolving priorities of the health sector. In all research we have conducted, we have ensured ethical transparency and scientific integrity, and balanced the value and volume of our work.

AHRI has continued its partnership with universities in the country through supporting young researchers advance their career with exposure to the best laboratory facilities and fine supervision. In this regard, 13 PhD students are supported by AHRI through Sida-funded Biomedical Sciences Post-graduate Programme and 34 others are funded through other sources; 4 have graduated in 2017. Relatedly, 52 Masters level students were supported by AHRI, of these 28 defended in 2017. Furthermore, number of researchers from universities all across the country has received short-term trainings in immunology, molecular biology, research ethics and other research courses.

The results we delivered in research and research training could be attributed to the generous support of our essential partners, the strategic guidance by AHRI Scientific Advisory Board (SAB), commitment of the staff and voluntary participation of men, women and children in our research. We are privileged to have Sida and Norad, as well as Federal Ministry of Health of Ethiopia as the main source of funding. We are deeply grateful to Sida, Norad and Federal Ministry of Health. We have immense respect and gratitude to AHRI SAB's continued support and guidance. As we continue to diversify our research themes, we have started to increasingly partner with study participants who unreservedly share their information and biological samples with us. Our highest appreciation goes to our study participants. We rarely recognize our team but they have tremendously contributed to the impressive progress seen at AHRI.

We are still at a learning phase in driving policy, implementation and investment in health in Ethiopia. We should continue our long journey, with impact in sight. I am confident that this journey will continue providing cutting edge solutions to the defining public health challenges of Ethiopians.

Sincerely,

Taye Tolera Balcha, MD, MPH, PhD

Director General, Armauer Hansen Research Institute

Executive Summary

The research conducted, trainings provided and innovations supported by AHRI in 2017 emanated from the mission of the Institute. They were also responsive to pressing public health challenges of the country. Additionally, adequate reference was made to global health SDGs. In 2017, the Institute implemented more than 60 research projects and reported 46 peer-reviewed publications, supported 14 health innovations and provided research support to 99 postgraduate students from partner universities across the country. The design work of AHRI laboratory complex was completed and construction is expected to begin in the beginning of 2018.

Mycobacterial diseases have continued to be front and centre of the studies conducted at AHRI. In line with global End TB Strategy, our research projects have been focused both on latent infection and active disease. A broad range of areas including pathogenesis, prediction of disease progression, biomarkers and vaccine discovery science and disease mapping tailored to programmatic needs have been studied. Few notable ongoing research projects in TB include *Molecular Epidemiology of tuberculosis and role of M. bovis in settings with high dairy development in Ethiopia, Evaluation of host biomarker-based point-of-care tests for targeted screening of active TB, and Evaluation of a novel microbiological test for latent tuberculosis infection in Ethiopia.* The mycobacterial diseases research directorate also coordinates TB Research Advisory Committee (TRAC) which was established by the MoH and its stakeholders atthe Institute 17 years ago. TRAC is positionedto enhance TB research-programme nexus and propel Ethiopia to be a pathfinder in TB elimination.

Ethiopia is on the brink of eradicating leprosy. New leprosy cases are currently concentrated in few districts of the country. To address this, the Institute has been mapping new leprosy cases and their household contacts in hot spot geographies. In close partnership with the national and sub-national programme managers, AHRI works with Health Extension Workers to detect leprosy cases early and minimize the currently high rate of disability. The evidence generated from this study is robust enough and it will likely influence national policy in the path and pace towards leprosy eradication. Other studies included the evaluation of the diagnostic potential of Auramine O staining for leprosy which showed its superior diagnostic performance to the other routine diagnostic tests. Further, planning is underway to conduct more studies in coherence with national leprosy eradication efforts.

The Institute has been systematically generating evidence in bacterial and viral infections. Guided by the national burden of diseases and public health needs, our bacterial research hasbeen focused on typhoid fever, bacterial pneumonia, sepsis, meningitis and antibiotic resistance. We have ongoing surveillance of severe typhoid fever in Oromia and Southern Nationals, Nationalities and People's regions. Based on the request of the Ministry of Health, national surveillance of antibiotic resistance has continued. A few research projects including *Etiology, disease severity and diagnostic challenges of bacterial meningitis during non-epidemic seasons in Ethiopia, Assessing bacterial etiologic agents that cause neonatal sepsis and their antimicrobial susceptibility pattern, Phenotypic and Genotypic Characterizations of Streptococcus pneumoniae Strains Isolated from Pediatric Patients in Addis Ababa, Ethiopia and*

Characterization of the interaction of microbiota and pathogenic enteric bacteria in an Ethiopian traditional fermented food could be highlighted here. Applying the evidence obtained from those and other ongoing studies could help the Ministry design and implement improvedpublic health approaches againstcommon bacterial diseases.

Although the Institute still lacks a virology laboratory, several studies have been conducted on viruses of public health importance in Ethiopia. We have been mapping arboviral infections and the burden of viral hepatitis in Ethiopia. Given the paucity of data and limited national guidance in these areas, our studies will potentially be precursor for strong health system measures responsive to viral infection including viral hepatitis and arboviral infections. Few of the studies currently underway include *Estimating the vertical transmission of hepatitis B infection, Molecular Epidemiology of Hepatitis B Virus Genotypes and pattern of mutations among HIV co-infected and HBV mono-infected adults, Characterizing the unmet HIV prevention needs and HIV risk vulnerabilities of adolescent girls and young women in Ethiopia*. As adolescent girls and young women are 'left behind', the Ministry could apply tailored interventions based on the evidence reported by the Institute.

An area of top-tier priority for the Government of Ethiopia, and hence is under establishment at AHRI is health biotechnology. The major pillars of national health biotechnology include development of vaccines, diagnostics, medicines and medical devices. In this regard, there are startup activities particularly in preparation and evaluation of in-house monoclonal antibodies, development of lateral flow assay for the diagnosis of TB, vaccine development for hemorrhagic septicemia in cattle and plasma fractionation. Further, in-house production of diagnostic kits for visceral leishmaniasis and immune-phenotyping for diagnosis of hematologic malignancies are at an advanced stage of development. The Institute is also currently establishing a platform for genomic research.

Surveillance and research on infections that co-affect both human and animals have received prominent attention. In this regard, AHRI's One Health Initiative has substantially expanded in recent years. The major areas of focus include zoonotic TB, brucellosis, Middle East Respiratory Distress Syndrome (MERS) and several other infections that impact on humans and livestock. Nationally significant research projects include *Jigjiga One Health Initiative, Brucellosis surveillance in livestock and pastoralists in Afar and Somali Region and Vaccine development for hemorrhagic septicemia in cattle and Tuberculosis in non-human primates.*

As malaria and most neglected tropical diseases (NTDs) are candidates for global and national elimination, our research in these conditions follow the elimination strategy. In malaria, for instance, *the dynamics and implications of asymptomatic malaria as a source of malaria infection, clustering of asymptomatic malaria infections, the epidemiology of the drug resistance alleles in Plasmodium falciparum, the population genetics of Plasmodium vivax and its prevalence, and genotype of glucose-6 phosphatase dehydrogenase deficiency and in vitro drug resistance assessment techniques for different stages of Plasmodium species*have been notable areas of research. Further, several studies investigating important aspects of cutaneous and visceral leishmaniasis are currently underway. The findings will provide the Ministry with data on operational realities, challenges and solutions as it transitions its

malaria and NTDs strategy from control to elimination.

Clinical Trials Directorate is working to position itself as a platform that could conduct high-quality research. It has also successfully completed few clinical trials that have led to impactful policy decisions. Currently, there is a multi-country trial which aims at evaluating the non-inferiority of shorter treatment regimen for multi-drug resistant TB compared with the current World Health Organization standard of treatment. The directorate has completed preparation for locally-driven clinical trials including *Efficacy and safety of locally manufactured external fixator versus conventional external fixator for treatment of long bone fractures in Ethiopia: a randomized controlled trial and the safety and efficacy of Ethiopian highland herb in the treatment of psoriasis- a randomized controlled clinical trial.*

Grounded in national priorities and community needs, the Institute has widened its scope to include research non-communicable diseases. We have established human leukocyte antigen (HLA) laboratory to support Ministry's new renal transplant surgery initiative. As the tests are otherwise run abroad, the new HLA laboratory will save considerable amount of time and cost related for patients who undergo renal transplant surgery. Further, several studies are underway in common cancers including hematologic malignancies and breast cancer. The overwhelming purpose of these research activities is to enhance early detection and improve treatment outcomes of common malignancies in Ethiopia. Completed or ongoing research projects worth mentioning include *Diagnostic utility of immunophenotyping by flow cytometry and comparison with morphology and cytochemistry for diagnosis and classification of acute leukemia, Leukemia characterization by flowcytometry, cytogenetic and molecular markers at TikurAnbessa Specialized Hospital, Addis Ababa, Ethiopia, BCR-ABL kinase domain mutations to Tyrosine Kinase Inhibitor drugs in Ethiopian CML patients attending tertiary hospitals, Lymphoma phenotyping by flow cytometry and Fluorescence In-Situ Hybridization, Immunologic, virologic and genetic correlates of Hodgkin's lymphoma in Ethiopia, Molecular characterization and assessment of viral tumorigenesis in breast cancer among women in Addis Ababa, Ethiopia* and several others.

Grand Challenges Ethiopia initiative is an innovation arm of the Institute aiming at stimulating and supporting health innovations to catalyze much-needed improvement, particularly in maternal and child health. In this regard, the Institute has supported 14 health innovations and the second round of call for proposal is out. The overarching plan is to support the Ministry transition promising health innovations into scale and spur the progress towards achieving SDGs and universal health coverage.

Medical research training is embedded within our research and innovations. The Institute has supported several short-term trainings related to conduct of research including clinical trials, data analysis and reporting of research findings and research bioethics mainly for researchers based at partner institutions. It has also supported the biomedical research training of 99 postgraduate students. Internally, 9 and 5 researchers are pursuing their studies at doctoral and masters levels, respectively. Of a total of 14 AHRI postgraduate students, 42.8% are female.

The Institute has received core fund from Norad, Sida and the Government of Ethiopia. It has also won significant amount of funding through applying for competitive grants. We have also forged robust research partnership with several domestic or international organizations. In 2017, Ethiopia joined

European and Developing Countries Clinical Trial Partnership (EDCTP) Association and Coalition for Epidemic Preparedness Innovations (CEPI) and delegated AHRI as a focal Institute representing the country at both partnerships.

The progress in 2017 testifies to the strong resilience of the Institute in responding to challenges of transition to a national role as a Ministry of Health clinical research arm consolidating areas of prior strength in biomedical sciences while expanding into new mandates of non-communicable disease research and health biotechnology, expanding its workforce base underconstraints of space (albeit transient) andsmoothly harmonizing operational procedures with government guidelinesmaintaining best practice standards in the process.

Highlights of 2017

The Future Building of AHRI

The FMOH has allocated a budget to build a modern laboratory and office complex to AHRI and a total of 5401.17 m^2 land within the ALERT compound is allocated for this purpose. The total floor area of the complex will be 23,828.48m^2.

In 2017, the engineering team from FMOH and a committee established at AHRI has been facilitating the selection of a consultant company. The team critically evaluated the submitted documents including the architectural designs in consultation with the AHRI management and as a result Yohannes Abbay Consulting Architects and Engineers won the bid.

The consultant group had 5 meetings including the kickoff meeting with the team from FMOH and the team at AHRI including senior researchers where the architectural design was presented and commented. As a result of the discussion, some major changes like having additional floors for the lab arm and functional reorganizations were made. Structural, electrical and sanitary designs were also presented by the group. The design is finalized and now approved by the FMOH and a bid document for selecting a contractor is ready.

Architectural Design for the future AHRI's Laboratory and Office Complex

AHRI Leads H3Africa Tuberculosis Project

AHRI is leading a multi-country project on host-pathogen genomics oftuberculosis that is awarded by the H3Africa program funded by Wellcome Trust and coordinated by the African Academy of Sciences. AHRI will coordinating this project which will be conducted in four African countries (Eritrea, Sudan, Cameroon and Ethiopia). A Bioinformatics Research Training center will be established at AHRI to serve all project partners. The project will be officially launched in January 2018.

Illumina NextSeq 500 Sequencer installed at AHRI

As part of the new initiative to strengthen the biotechnology and bioinformatics capacity of AHRI, IlluminaNextSeq 500 Sequencer has been purchased and installed at AHRI. The installation was made by engineers from Illumina.

Following the installation, test run and training was given for three days starting from November 6 - 8, 2017 for 7 AHRI staff members by FarazShaheed. The training includes: Basic concepts on NGS technology, sequencing test run using φX library on NextSeq 500, create sample information, data transfer protocols, routine machine washing steps, issue reporting for remote support, general safety and data quality checks.

The instrument is now ready for function and is expected to give service (whole genome, exome, RNA sequencing) as well as bioinformatics support not only to AHRI but also to all interested researchers/ institutions in the country based on specific agreement.

Training on IlluminaNextSeq 500 Sequencer

HLA Laboratory Established

Establishing an HLA lab has been one of the priorities of AHRI in the last 2 years. The main purpose of establishing the lab is to support the national kidney transplantation program and along with it to support research activities in both communicable and non-communicable diseases.

In 2017, four researchers were trained on HLA fusion software which is required for the analysis of HLA data and one engineer was trained on Luminex machine installation and maintenance. A Luminex machine was donated by the Lagitre International Srl. and a one week-long training was provided on anti-HLA antibody detection tests (Lab screen mix and Single antigen beads), Luminex application and data analysis to researchers and lab technologists at AHRI.

The lab will be able to perform all tests including flow cross-match, LAB screen and LAB type in 2018.

Co-hosting the second Health, Science and Higher education Conference

AHRI has a long standing and fruitful partnership with the Norwegian institutions and people at large. In addition to this strong partnership, AHRI's track record in the field of biomedical research has made it a choice for co-hosting the second Health Science and Higher education Conference with Oslo University Hospital. More than 250 researchers, academicians and practitioners attended the conference. Moreover, Norway's Crown Prince Haakon Magnus and Princess Mette-Marit opened the conference and paid a visit to AHRI showing their acknowledgment of AHRI's scientific contribution.

The Crown Prince and Princess of Norway visiting AHRI and ALERT

AHRI Tore Godal Award Ceremony

In 2017 we had two AHRI Tore Godal Award Ceremonies. Congratuations to the16th AHRI Tore Godal winners: Metasebia Tegegn, Dr. Beyene Moges, Brook Tesfaye and Taddesse Alemu.

We also congratulate the winners of the 17th AHRI-Tore Godal award winners; Samuel Ayele and Eshetu Nigusie. This award was special due to the presence of Dr. Tore Godal who presented the award to the winners.

Left to right: Dr. Beyene Moges, Metasebia Tegegn, H.E. Dr. Kebede Worku, Brook Tesfaye and Tadesse Alemu

Left to right: Dr. Taye Tolera, Eshetu Geleta, Samuel Ayele, Dr. Tore Godal

Research and Innovation

Mycobacterial Diseases Research

Summary

As in the past years, this year was also productive for the mycobacterial diseases research directorate (MDRD) in terms of number of research activities conducted, postgraduate student training, publication outputs, grants written and initiation of new projects. This report briefly summarizes the overall activities conducted in 2017 within the directorate: a brief summary of research activities, publication outputs, grant applications and new initiatives as well as other networking activities such as training and conference participation.

Two team members (Dr.Liya and Dr. Markos) continued to actively participate in the Tuberculosis Research Advisory Committee (TRAC) document preparation and identification of gaps and setting national TB research priorities. Following up on the recommendations of TRAC, the Directorate has sorted out priority research questions to be addressed by researchers/students at AHRI. In close consultation with our Deputy Director General for Research and Innovation (Dr. Abraham Aseffa), the Directorate has identified what research programs it would focus on in both TB and leprosy.

Based on key observations from studies conducted by PhD students, a one-day workshop was held on the situation of TB in prisons of the Southern Nations, Nationalities and Peoples Region and on active case detection of leprosy in Kokosa area of Oromia Region. Different health practitioners (nurses, laboratory heads and health officers) working in various institution in SNNP and Oromia Regions have participated in the workshops. The main purpose of the workshops was to produce at least two policy documents for the active TB and leprosy control programs. Similarly, we had a half day workshop with Clinicians at ALERT Center working on leprosy. Challenges in leprosy management at ALERT Center and at national level were discussed. The team also drafted one policy document on bleach microscopy for possible use of improved microscopy for diagnosis of tuberculosis.

I. Completed research

Molecular Epidemiology and Drug Resistance of Tuberculosis in Southern Ethiopia

Yared Merid[1,2,3], Yimtubezinash Woldeamanuel[2], Markos Abebe[1], Daniel Gemechu[4], Tsegaye Hailu[1],Getnet Habtamu[1], Gebeyehu Assefa[1], Russell R. Kempker[5], Henry M. Blumberg[5], Abraham Aseffa[1].

[1]Armauer Hansen Research Institute; [2]Addis Ababa University; [3]Hawassa University; [4]REACH Ethiopia, lSTM, [5]Emory University School of Medicine, Atlanta, GA, USA

Tuberculosis (TB) is a major public health problem in the Horn of Africa with Ethiopia being the most affected. The development and spread of drug-resistant *M. tuberculosis* also threatens national TB control

programs in several countries in the region. Molecular typing of strains helps in predicting transmission rates and identifying dominant strains associated with outbreak, disease severity and drug resistance. However, in most African countries including Ethiopia, there is limited molecular epidemiological data on tuberculosis and this has hampered our understanding of the disease dynamics. In this PhD study, the aim wasto describe the molecular epidemiology and drug resistance pattern of TB to better understand the transmission dynamics of TB and the development and spread of drug resistance in southern region of Ethiopia.

A cross-sectional study design was carried out in 9 health facilities in Shashemene town. Culture was carried out on 250 sputum samples. Drug susceptibility testing, deletion typing and spoligotyping assays were performed. Whole genome sequencing is being done on selected samples.

Of the 174 isolates tested for drug susceptibility test for the first anti-TB drugs, 22(12%) showed resistance to one and more drugs and among them one isolate was MDR-TB. RD9 deletion typing and spoligotyping were also done for 200 of the isolates, confirming all the isolates were M. tuberculosis by DR9 deletion typing comprising a few spoligotype patterns. Byspoligotyping, 60 patterns were identified; 159 of the 200 isolates (79.5%) belong to Lineage 4 (Euro-American), 23 (11.5%) to Lineage 3 (India, East Africa), 7 (3.5%) to Lineage 7 (previously reported from Ethiopian highlanda), and there were 11 (5.5%) new or orphanspoligotype patterns. The majority of them were SIT 53 (48, 24%) and SIT 149 (44, 22%) strains. The majority of the drug resistance strains belonged to the SIT 149 strain.

A high diversity of strains are circulating in the study areas. SIT 53 and SIT 149 strain types were the major strains involved in current transmission of TB in the study areas.

Mycobacterium tuberculosis Infection: Effect on Immunological Synapse formation and T cell activation

Meseret Habtamu[1,2],Greger Abrahamsen[1], Emawayish Andarge[2], Abraham Aseffa[2], Markos Abebe[2], Anne Spurkland[1]

[1]Department of Molecular Medicine, Institute of Basic Medical Sciences, University of Oslo, Norway, [2]Armauer Hansen Research Institute, Addis Ababa, Ethiopia

The immunological factors of *Mycobacterium tuberculosis* (*Mtb*) infection leading to disease progression, latency or clearance are poorly understood. While protective immunity against *Mtb* is the result of complex interaction between innate and adaptive immune response, most studies have focused on cytokine secretion profiles of T cells. The aim of this PhD study is to assess the immunological synapse between T cells and monocytes during TB infection.

We assessed T cell-monocyte interaction and NF-κB translocation within conjugated T cells from Ethiopian subject samples. Fluorchrome stained cells were analyzed by imaging flow cytometry (IFC) after 6 hours stimulation and results were compared among active pulmonary tuberculosis (PTB) cases, latent tuberculosis infected (LTBI) and endemic controls (EC). Ds-red expressing BCG (BCG) and ESAT-6 (ES6) were used as stimulating antigens.

A similar proportion of T cell-monocyte conjugates was observed between unstimulated (UNS) and BCG stimulated samples, whereas ES6 stimulation resulted in a significantly higher conjugates. Among the study cohorts, active PTB cases exhibited lower T cell-monocyte frequencies than LTBI and EC subjects, with the highest proportion observed amongthe LTBI group. On the other hand, active PTB cases showed the highest frequency of T cell conjugates displaying NF-κB translocation. No significant differences in any of the parameterswere observed between LTBI and EC groups.

Our results showed an impaired communication between T cells and monocytes without affecting the response in T cells as measured by NF-κB translocation, highlighting the need to consider multiple approaches in the search for immune protection surrogates specific for tuberculosis.

Evaluation of innate immune markers in latently infected and non-infected school children and adolescents

Birhan Alemnew[1,2], Tamrat Abebe[1], TBTEA consortium, Liya Wassie[2]

[1]Addis Ababa University, Department of Microbiology, Parasitology, and Immunology; [2]Armauer Hansen Research Institute

Latent TB infection, a phase where non-persistent replication of the *M. tuberculosis* bacilli and continuous activation of the immune system persist in equilibrium, is a critical stage of *M. tuberculosis* infection and has recently been considered as a target for an effective TB control strategy. Little attention has been given to understanding the role of innate immunity in protection against TB. Toll-like receptors (TLRs) are innate immunity markers expressed on leukocytes; including macrophages that play a significant role in TB pathogenesis via recognition of pathogen associated molecular patterns. In this MSc study, we assessed the differential expression of selected TLRs (TLR-1, TLR-2, TLR-4, TLR-6 and TLR-9) in latent TB.

The objective of this study was to investigate the expression levels of TLRs in whole blood of latently infected and non-infected children and adolescents as screened by tuberculin skin test (TST) using quantitative real-time PCR (*qRT-PCR*).

This study was part of an on-going project supported by EDCTP (TB-TEA and ADO-TB). A total of 64 cDNA samples (32 TST positive and 32 TST negative) and their linked archived data were retrieved from AHRI biorepository using convenient sampling (availability of samples), to analyze the mRNA expressions of TLRs (TLR-1, TLR-2, TLR-4, TLR-6 and TLR-9) using *qRT-PCR*. A total of 1 µg of cDNA was used in a total of 12.5 µl reaction volume, run in duplicates on the Rotor-Gene RG-3000 thermocycler. Specific primers and florescent-labelled probes were used to span exon-intron junctions to prevent amplification of genomic DNA. We used human ribosomal protein (HuPO) as a housekeeping gene and internal control throughout the experiment and the comparative CT method (also known as $2^{(-\Delta\Delta CT)}$) to describe the mean fold change in the relative expression of TLR genes. Data were later exported to GraphPad Prism 7.03 for Windows for further statistical analyses. This study was approved by the

AHRI/ALERT Ethics Review Committee (AAERC), AAU-CHS IRB and the National Research Ethics Review Committee (NRERC).

Overall, an increased mean fold change in the mRNA expression of TLRs was observed in latently infected individuals relative to non-infected ones. Significant fold change increase was observed for TLR-2 and TLR-6 genes in latently infected individuals relative to the non-infected; whereas a slight fold decrease was observed for TLR-1 gene. Similarly, the relative expression of TLR mRNAs was compared between children and adolescents and between sexes. However, no apparent difference was observed in the fold change expression of TLRs across age and between sexes.

The up regulation of these genes during latency possibly suggests the role of TLRs during TB infection. The fact that no apparent difference was observed across age contrasts with our previous observation suggesting the need for further studies with inclusion of younger age children. In addition, investigation of other innate markers such as pulmonary surfactant proteins in a wider cohort including TB patients is warranted.

Funding Source: EDCTP and AHRI core budget

Comparison of immune cells subsets and their activation status between Lymph Node Cells and corresponding Peripheral Blood Mononuclear Cells of Calves Exposed to Natural Mycobacterium Bovis Infection: Flow-cytometry and Immunohistochemistry

Fekadu Desta[1, 2], Gobena Ameni[2], Javier Salguero Bodes[3], Rawleigh Howe1

[1]Armauer Hansen Research Institute; [2] Aklilu Lema Institute of Pathobiology, Addis Ababa University; [3]Department of Pathology and Infectious Diseases, School of Veterinary Medicine, University of Surrey,Guildford, UK

Cell mediated immunity and development of necrotic granulomas in *Mycobacterium bovis* (*M. bovis*) infected lymph node (LN) is pathognomonic for bovine tuberculosis (BTB). This delayed hypersensitive host response involves a complex interaction of cellular and immune mediators within systemic circulation and LN. Hence, tuberculosis immunological response in tuberculosis should ideally be independently investigated at the peripheral blood and LN tissue level. The objective of this study was, therefore, to compare the cell surface and cytokine expression between immune cell from peripheral blood and LN and their differential expression across different stages of granuloma in (Bacillus Calmette–Guerin) BCG vaccinated and non-vaccinated calves. Twenty pairs of peripheral blood mononuclear cells (PBMC) and lymph node cells (LNC) from *M. bovis* naturally infected calves during BCG vaccine experiment trial were isolated and investigated by flow-cytometry. Immunohistochemistry (IHC) staining was done on a total of 45 lymph node tissue blocks, from which a total of 122 (29 stage-I, 27 stage-II, 30 stage-III and 36 stage-IV) granulomas were considered for analysis. Byflow cytometry the proportion of CD25+ expressing fresh cells was significantly higher ($P<0.05$) in CD4+ and CD8+ T cells isolated from lymph node than that of peripheral blood. However, such difference in CD25+ expression was not observed in γδ (marker WC1)T cells. Contrary to CD25 *in-vivo* expression, IFN-γ and TNF-α

producing cells was greater (P<0.05) in T cells of the peripheral blood than T cells of lymph node after PMA + ionomycin stimulation. This difference in IFNγ and TNFα responses was also statistically significant between vaccinated and non-vaccinated group. IL-4 producing cell were not evident in PBMC and LNC. Further flow cytometry phenotyping evaluated CD2, CD14, CD21, CD28, CD45RO, CD44, CD62L, CD80, CD86, CD205, CD335, HLA-DR and CD1w2 as markers for subtypes of T memory and *in vivo* activation and other subsets of of PBMC and LNC (data analysis is on processes). Immunohistochemistry revealed that all l markers except CD3 was found to be significantly higher in granuloma compared non-granulomatous regions within a given specimen. IHC staining for cell surface markers CD68+ (macrophage lineage cells) and CD3+ (T cells) were lower across all granuloma stages of BCG-vaccinated calves compared to non-vaccinated group while iNOS, IFN-γ and TNF-α staining were higher in the BCG vaccinated group (P<0.05). Collectively, these results illustrate differences in the phenotypic and functional properties of cells between lymph node and peripheral blood. Moreover, by IHC of lymph node tissue, while T cells and macrophage numbers were lower in BCG vaccinated calves, the proinflammatory markers were higher, consistent with a more potent response.

Evaluation of Auramine O Staining and conventional PCR for Leprosy diagnosis: a comparative cross-sectional study at Armauer Hansen Research Institute, Addis Ababa, Ethiopia

Selfu Girma[1], Kidist Bobosha[1], Kassu Desta[2], Munir Idris[1], Yohannes Tsegaye[1], Shimelis Nigusse[3], Tsegaye Hailu[1], Phillipe Busso[4], Charlotte Avanzi[4], Stewart Cole[4], Abraham Aseffa[1]

[1]Armauer Hansen Research Institute; [2]Addis Ababa University, School of Medical Laboratory Technology; [3]ALERT Hospital; [4]École PolytechniqueFédérale de Lausanne

Diagnosis of leprosy mainly relies on clinical examination due to the inconsistent sensitivity and non-reproducible performance of the current laboratory diagnosis test. Utilization of alternative methods to the standard Ziehl Nelsen (ZN), Fite-Faraco (FF) and Haematoxylin and Eosin (H&E) staining may eventually improve leprosy diagnosis. In this comparative study, the performance of Auramine O (AO) staining and polymerase chain reaction (PCR) was assessed in different skin samples using a combination of references test which incorporates ZN, FF and H&E staining as gold standard. Sensitivity was 87.6%, 59.3% and 77% for H&E, ZN and FF. The sensitivity of AO in SSS, AO in tissue section, and PCR was 65.5%, 77.9% and 91.1%, respectively. Auramine O staining on both SSS and tissue section was superior over with the routine ZN and FF diagnostic tests. The sensitivity of PCR was considerably higher than all methods evaluated in this study. Therefore, we recommended AO staining for the diagnosis of leprosy in lower health institutions and on the other hand PCR diagnosis at least at referral labs level.

Funding: AHRI Core budget and École PolytechniqueFédérale de Lausanne

Molecular detection of Mycobacterium leprae in Slit Skin Smear from leprosy patients and the correlation of molecular and histopathological findings with clinical Data

Azeb Bereket[1,2], Selfu Girma[1], Kidist Bobosha[1], Tufa Gemechu[2]

[1]Armauer Hansen Research Institut; [2]Department of Pathology, College of Health Sciences, Addis Ababa University

Mycobacterium leprae is a causative agent of leprosy which is a chronic infectious disease. The disease mainly involves the skin and peripheral nerves. Clinical examination, ZeilNeelson (ZN) staining and although not routine H&E staining are the available diagnostic tools of leprosy and have their own limitations. Introducing advanced techniques like polymerase chain reaction (PCR) as diagnostic tool for leprosy may fill the limitations of the common diagnostic tools.

The objective of this study was to evaluate the diagnostic performance of PCR on archival Slit Skin Smear samples collected from clinically confirmed leprosy cases.

A total of60 Slit Skin Smear (SSS) samples from 43 multibacillary (MB) and 17 paucibacillary (PB) cases stored at histopathology lab of AHRI were used for this study. DNA was extracted from each SSS slides and PCRwas done.H&E, ZN and clinical reports of the patients were reviewed and compared with the PCR result. Data entry and analysis was performed using SPSS version 20. Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were calculated and a p-value less than 0.05 were considered statistically significant.

The PCR on SSS was positive in 5 (29.41%) PB patients where 5(13.89%) were from AFB negative slides. The PCR was also positive in 22 (51.16%) MB patients.The H&E result was positive for 36 (83.72%) MB and 9 (52.94%) PB patients.The sensitivity, specificity, PPV & NPV of PCR on SSS with the gold standard H&E was 58% (95% CI: 42% - 72%), 93% (95% CI: 68% - 100%), 96% (95% CI: 81%-100%) and 42% (CI: 25%-61%) respectively

Although the PCR showed low detection as compared to H&E and FF staining, by improving some technical procedures in sample collection and handling, it can be used for diagnostics at referral laboratories where PCR machines are available.

II. Ongoing research

Systematic review and meta-analysis of the performance of GeneXpert testing of stool specimens for the diagnosis of tuberculosis in children

Meseret Gebre[1], Lindsay Hatzenbuehler[2], Liya Wassie[3]

[1]ALERT Hospital; [2]Emory University; [3]Armauer Hansen Research Institute

Diagnosis of childhood TB has long been challenging due to low bacillary load, lack of tussive force to produce sputum, low sensitivity of smear and culture, limited access to mycobacterial laboratory culture facilities in resource poor-settings and long turn-around time for culture results. A test with reasonable sensitivity and specificity on samples which is easy to collect is needed to diagnose pediatric TB. This study is one of the Ethiopia-Emory Tuberculosis Research and Training Program (EETB-RTP) initiatives, aiming to conduct a systematic review and meta-analysis to evaluate the evidence supporting

the use of GeneXpert on stool specimens to diagnose TB in children.

We used a systematic literature review using Pubmed, Cochrane, EMBASE, and ClinicalTrials.gov as main source engine databases to retrieve both published and unpublished original articles and conference proceedings conducted or presented on pediatric TB using fecal samples for GeneXpert analysis. The last search date was on February 15, 2017. We used a standardized and Cochrane endorsed tool, the population, intervention, comparison and outcomes (PICOS) approach to define the inclusion and exclusion criteria and articles were evaluated for eligibility using the preferred reporting items for systematic reviews and meta-analysis (PRISMA) checklist. In addition, quality assessment was done using a tool for the quality assessment of diagnostic accuracy studies (QUADAS 2) tool. Finally using Revman 5.3 software (with the assumption of random effects model and DerSimonian analysis), results will be presented as weighted sensitivity/specificity of aggregate data, flow diagram (PRISMA), forest plot and funnel plot.

A total of 34 studies were identified with systematic literature search, where nine studies met the inclusion criteria. Risk of bias was assessed using QUADAS tool by two independent reviewers and any disagreement was resolved through discussion and involvement of a third reviewer. With the QUADAS tool, the majority of the included studies (8/9) had a bias assessment score ≥6. In addition, study level and outcome variables were also extracted from these studies and the result for meta-analysis is currently underway.

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Molecular Epidemiology of tuberculosis and role of M. bovis in settings with high dairy development in Ethiopia (ETHICOBOTS project)[1]

Hawult Taye[1], Adane Mihret[1], Eschcolewyene Fekadu[1], Shewit Haile[1], Bizuneh Belachew[1], Fantanesh Melesse[1], Nahom Getachew[1], Bamlak Tessema[1], Abraham Aseffa[1], Stefan Berg[2], James Wood[3]

[1]Armauer Hansen Research Institute, [2]Animal and Plant Health Agency, [3]Cambridge University

The main objective of this project is to gather evidence relevant for future strategies to minimize the zoonotic impact of TB on high risk populations. Such populations include dairy farm workers and their families and those at risk as the emergent dairy livestock system continues to expand.

The prevalence of bovine TB (bTB) is being investigated through isolation of mycobacteria from suspected patients. Two approaches have been used to recruit study participants: active case detection, where workers in high risk bTB settings are screened for pulmonary and extra-pulmonary TB, and passive case detection, where TB patients are recruited at health facilities upon diagnosis from sites in Addis Ababa and the surrounding, Hawassa, Gondar and Mekelle.

1 ETHICOBOTS is a 5 years research project (2014-2019) funded by the UK under the Zoonoses and Emerging Livestock Systems (ZELS) scheme and currently led by the University of Cambridge (UK) (Prof. James Wood) and the Animal and Plant Health Agency (UK) (Prof. Glyn Hewinson) in collaboration with researchers from University College London (UK), AHRI, the National Animal Health Diagnostic Investigation Center (NAHDIC, Ethiopia), Addis Ababa University (AAU), the Ethiopian Institute of Agricultural Research (EIAR), and the Swiss Tropical Public Health Institute (Switzerland).

So far, 1406 sputum and 392 FNA samples have been collected, of which 237 and 87 were culture positive, respectively. Deletion typing was also conducted on 324 of the isolates, with only 2 *M. bovis* isolates confirmed. Sample collection will continue until the target of collecting 600 isolates is reached.

Funding: Biotechnology and Biological Sciences Research Council (BBSRC), UK

Evaluation of host biomarker-based point-of-care tests for targeted screening of active TB (Screen TB)

Adane Mihret[1], Sosina Tadesse[1], Azeb Tarekegne[1], Bamlak Tessema[1], Gerhard Walzl[2], Annemieke Geluk[3], Paul Corstjens[3], Hazel Dockrell[4] Rawleigh Howe[1]

[1]Armauer Hansen Research Institute, [2]Stellenbosch University, South Africa, [3]Leiden University Medical Center, The Netherlands, [4]London School of Hygeine and Tropical Medicine, UK

Host serum protein biomarkers that indicate a high likelihood for active TB disease represent attractive targets for integration into screening tests. The EDCTP1-funded African European Tuberculosis Consortium (AE-TBC) has previously investigated host biosignatures in whole blood culture supernatants after overnight stimulation with *Mycobacterium tuberculosis* (MTB) specific proteins.

A promising serum host inflammatory signature was identified during this project after investigation of more than 70 serum host inflammation markers, including acute phase proteins, T helper cell 1, T helper cell 2 and regulatory cytokines, soluble cytokine receptors and growth factors. The original set of markers for screening were chosen according to the availability of multiplexed assays (Luminex platform) and their known roles in inflammation and represented a wide spectrum of markers with diagnostic potential. The most promising host serum protein signature was subsequently validated on 687 people from five African countries with suspected TB, regardless of HIV infection status or ethnicity, providing the basis for the follow-up work suggested herein. The six-analyte signature of C-reactive protein (CRP), Interferon gamma (IFN-γ), pre-albumin, complement factor H (CFH), apolipoprotein A1 and inducible protein 10 (IP-10) ascertained TB disease with a sensitivity of 89% (CI 78 – 95%) and specificity of 76% (CI 68 – 83%) (positive predictive value of 61%; negative predictive value of 94%). During the AE-TBC project, partner Leiden University Medical Centre (LUMC) developed and validated a user-friendly lateral flow assay (LFA) for simultaneous detection of multiple host TB biomarkers (cytokines and antibodies) in blood or other body fluids. These LFAs are likely to assist in rapid TB diagnosis in low-resource settings and were tested in the AE-TBC field sites. Importantly, our test formats utilize novel, nano-sized upconverting phosphor (UCP) reporter particles as read-out. The UCP-technology delivers flexibility with respect to sensitivity and robustness as the ultrasensitive fluorescent label is not hampered by common auto-fluorescence background. Moreover, the label does not fade, allowing UCP-test strips to be stored indefinitely for reanalysis. The LFA can also be modified for measurement of markers in finger prick blood, which will further enhance its POC utility. This is an EDCTP funded project aiming to incorporate the six-marker signature into an UCP-LFA format, the TransDot assay, enabling finger prick blood testing. The end point of the study is the accuracy (sensitivity and specificity) of the UCP-LFA TransDot test on finger prick blood for active TB and will be prospectively compared

against composite gold standard diagnostic criteria of GeneXpert, mycobacterial growth indicator tube (MGIT) culture, TB sputum smear, CXR, TB symptom screen, response to antibiotics and response to TB treatment.

Out of 200 targeted treatment naive presumptive TB cases (those presenting with cough of more than 2 weeks and at least one of the signs-symptoms complex such as fever, weight loss, hemoptysis and night sweats), about 93 pulmonary TB suspects have been recruited so far in Addis Ababa, of whom 17 were GeneXpert and culture positive; 3 GeneXpert and culture negative and 1 GeneXpert positive, culture negative. Participants will be recruited from primary health care clinics in Cape Town, South Africa, Windhoek in Namibia, Addis Ababa in Ethiopia, Banjul in The Gambia and Kampala in Uganda.

Funding: EDCTP

Molecular Epidemiology, drug resistance pattern of *M. tuberculosis* and clinical outcome evaluation in Woldiya region, Ethiopia

Elena Hailu[1], Tesfaye Sisay[2], Markos Abebe[1], Cheryl Day[3], Melanie Newport[4], Abraham Aseffa[1]

[1]Armauer Hansen Research Institute; [2]AAU Biotechnology Department; [3]Emory University; [4]Brighton and Sussex Medical School

The causative agent of human tuberculosis, *Mycobacterium tuberculosis* complex (MTBC), comprises seven phylogenetically distinct lineages associated with different geographical regions. Lineage 7 of *M. tuberculosis,* recently characterized at AHRI, is restricted to Ethiopia and it represents a phylogenetic branch intermediate between the ancient and modern lineages of *M. tuberculosis*. It was shown that modern lineages are generally more virulent and more globally successful, compared to other more geographically restricted lineages. Differences in immunogenicity, severity of disease, and transmission consistently indicated that Lineages 2 and 4 are more virulent than Lineages 1 and 6 but nothing is known about Lineage 7. This PhD study is aimed to investigate Lineage 7 of *M. tuberculosis* experimentally and in clinical settings. The experimental part includes infection of the THP-1 macrophage cell line and macrophages isolated from healthy donors with locally isolated *M. tuberculosis* from either Lineages 1, 3, 4 and 7. Culture supernatants will be analysed for cytokine content and cell responses to different lineages compared.

Clinical studies are being carried out in Woldia town, South wello zone, Amhara region where the highest prevalence of Lineage 7 was reported previously. Woldiya Hospital and five surrounding health centres were selected for this study. All new pulmonary TB patients (smear negative and smear positive) attending the health care facility in this region were included in this study. Sputum and blood samples were collected from the patients. *M. tuberculosis* strains are being isolated from sputum samples and molecular techniques are being used for strain typing. Plasma is separated from blood samples for cytokine analyses. Anti-coagulated blood (with EDTA) is being stored with RNA Later solution for further RNA isolation.

To date, a total of 245 blood and sputum samples have been collected. The culture yield from smear positive and negative patients was 93-95% and 0-33%, respectively, varying among health centres.

Lineage 4 is the most prevalent lineage following by Lineage 3. Two Lineage 7 strains have been found so far. One strain belongs to Lineage 1 orancient Lineage, previously reported only from Southern part of Ethiopia. Different lineages show difference in growth characteristics related to high or low smear bacterial load. Two rifampicin resistant strains have been isolated. The findings of this study will indicate the overall pattern of lineages distributions, drug resistance pattern and their correlation with cytokine profile among TB patients in Woldiya region. In addition, immune response patterns revealed from THP-1 cells and macrophages infected with different lineages will also contribute to further understanding of phenotypic consequences of genomic variation.

Funding: AHRI core budget

Epidemiologic determinants of TB disease, timeliness of obtaining proper diagnostic services and related expenditure

Ezra Shimelis[1, 2], Melaku Tilahun[2], Tsegaye Hailu[2], Gebeyehu Assefa[2], Fikre Enkusilassie[1], Abraham Aseffa[2]

[1]Addis Ababa University School of Public Health and College of Health Sciences; [2]Armauer Hansen Research Institute

This PhD study aims to determine factors that contribute to occurrence of TB disease, including both drug sensitive and MDR-TB, and assess the duration spent by TB patients before obtaining proper diagnosis at health services and their associated expenditure.

The study is primarily a health institution based matched case control study. Cases will be new smear-positive pulmonary TB patients and the controls include two different groups. The first control will be age and sex matched health center attendees, while the second will be community controls. Household contacts will also be assessed for TB. It is being conducted in Addis Ababa city with a total sample size of 230 cases. A similar number of controls are also planned to be included in the study. The study will also involve confirmed MDR-TB patients to explore the risk factors for MDR-TB, and the calculated sample size for this part is 167. Both quantitative as well as qualitative study methods will be applied. The major data collection techniques will include individual interview, review of medical charts, focus group discussion, key informant interview, mycobacterial culture, Region of Difference 9 (RD9) deletion typing, and spoligotyping. Data entry and analysis will be conducted using SPSS for windows statistical software. Analysis of qualitative data will be done through the thematic analysis technique by categorizing, coding and summarizing the common 'themes'. Important measures will be taken to ensure data quality. The study has been approved by the ethical review committee at the School of Public Health, and College of Health Sciences, Addis Ababa University, AHRI/ALERT Ethics Review Committee and Addis Ababa Health Bureau Research Unit.

Currently, data from 195 cases, 195 controls and a proportional number of household and community controls have been collected. Sputum samples from 195 TB cases were collected and cultured. Data entry has been completed for all collected cases and controls. RD9 deletion typing was conducted for 88 cultures positive isolates and all were confirmed to be *M. tuberculosis*. Data collection and entry were completed from 167 MDR-TB cases from ALERT Hospital MDR-TB treatment center. Preliminary analysis for MDR-TB data is currently underway.

Funding: AHRI Core budget

Monocyte Function in TB, HIV and TBHIV Co-Infected Patients

Wegene Tamene[1,2,3], Ulrich Sack[2], Desta Kassa[3], Amha Kebede[3], Meseret Abebe[1], Liya Wassie[1], Vincent Markoni[4], Rawleigh Howe[1]

[1]Armauer Hansen Research Institute; [2]University of Leipzig, Germany; [3]Ethiopian Public Health Institute; [4]Emory University

People living with HIV are 20-30 times more at risk to develop active TB than HIV negative people. Cells of the monocyte-macrophage lineage are crucial cells in the pathogenesis and protection of TB disease, as well as to pathogenesis of HIV disease. Studies have suggested that HIV infected patients have reduced monocyte function and abnormal distribution of subsets of peripheral blood monocytes even after ART, which may contribute to increased susceptibility and/or progression to TB. Thus, this project aimed to investigate the role of TB, HIV and TB/HIV co-infection on phenotypic and functional properties of subsets of peripheral blood monocytes in Ethiopian patients. This study focuses on characterization of monocyte subsets defined by the expression of CD14 and CD16 on cell surface. We have evaluated toll like receptors (TLR2, 4 and 9), chemokine receptors (CCR 2, 4, 5, 7 and CX3CR1) and activation/death markers (HLADR, CD54, PD-L1 and PD-1) by flow cytometry. Characterization of cytokines and molecules of the Vitamin D axis at the protein and mRNA level will be performed. Finally, we will correlate the expression of these molecules and *in vivo* generated molecules including plasma cytokines and mycobacterial products such as LAM.

Funding: AHRI core budget

Evaluation of antigen specific T-cell response and serum biomarkers for the diagnosis of smear negative TB patients, Ethiopia

Ahmed Esmael[1,2], Adane Mihret[1], Tamrat Abebe[2], Rawleigh Howe[1], Jyothi Rengarajan[3],Liya Wassie[1]

[1]Armauer Hansen Research Institute; [2]Addis Ababa University, College of Health Sciences; [3]Division of Infectious Diseases and Emory Vaccine Center, Emory University School of Medicine

The continual public health burden of TB is mostly attributed to the limitation of currently available diagnostic tools. This is even more complex and challenging for smear negative TB cases. This PhD study is an Emory initiated project aiming to look at selected antigen specific T cell responses and plasma screening of cytokines/chemokines in smear negative presumptive TB cases to ultimately identify potential candidate biomarkers for diagnosis of smear negative TB cases. This is a longitudinal study, recruiting newly diagnosed smear negative HIV negative adult TB patients compared with selected control groups in selected health facilities of Addis Ababa. The study has been ethically approved by the AHRI/ALERT and the National Research Ethics Review Committees and is currently awaiting procurement of reagents to conduct the study.

Funding: AHRI core budget

Active case detection of new leprosy cases and tracing of household contacts in KokosaWoreda, West Arsi zone, Oromia region, Ethiopia

Tsehaynesh Lema[1,2,3]; Kidist Bobosha[2]; Saba Lambert [3]; Tsegaye Hailu [2]; Samuel Ayele[2] , Girmay Medhin[7]; Annemieke Geluk[5]; Sven Britton [6] ; Christa Kasang4; YimtubezenashWoldeamanuel[1]; Abraham Aseffa[2]

[1] College of Health Science, Addis Ababa University; [2]Armauer Hansen Research Institute; [3]All African Leprosy, Tuberculosis, Rehabilitation and Training Center; [4]German Leprosy and TB Relief Association; [5]Leiden University Medical College; [6]Karolinska Institutet; [7]Aklilu Lema Institute of Pathobiology

Leprosy is a chronic mycobacterial disease caused by *M. leprae* which has a big impact physically, socially, economically and psychologically on the affected population. In 2014, 3758 new cases were reported from Ethiopia where 482 were children and, 384 patients had grade 2 disabilities (G2D) indicating an alarming continued presence of ongoing transmission. The annual incidence of leprosy has remained the same in Ethiopia for over a decade with more than 76% of the reported new cases coming from Oromia and Amhara Regional States. All cases reported are from passive case detection which is known to underestimate true prevalence. In Ethiopia from a recent mapping study, 93 Woredas were identified as leprosy high burden areas. Hence a survey with active case detection will better approximate the true burden of leprosy in the country.

In this study, our main objective has been to investigate the factors responsible for the high burden of leprosy in one "hotspot area" in order to develop guidelines for elimination of leprosy in similar clusters across the country. The specific objectives include the identification of new leprosy cases and tracing household contacts at risk of developing leprosy by active case detection and map the distribution of leprosy cases to understand factors for clustering. Objectives also include assessment of health care facility performance status in leprosy control and evaluation of leprosy treatment out comes at study sites. The investigations have been conducted in one of the high leprosy burdened Woredas in Ethiopia, Kokosa located in West Arsi Zone, Oromiya region.

A cross-sectional study was conducted in Kokosa Woreda, 359 kms from Addis Ababa via Hawassa to Kokosa. Household screening was made by the Health Extension Workers (HEWs) supervised by the TB/Leprosy Focal persons and 36,495 household members were screened. Leprosy suspects were examined for cardinal signs and symptoms by leprosy experts and dermatologists. Index cases were encouraged to bring their household contacts (HHCs)to the health facilities for screening.

Knowledge, attitude and practice (KAP) of the health professionals working in the Woreda was assessed and training was given before launching the study. A total of 72 new cases,55 multibacillary (MB) and 17 paucibacillary (PB) cases were brought to treatment by active case detection (ACD) within a year. Among the confirmed new cases, 17 were children below the age of 14. Eight previously treated cases (couple of years ago) came with new lesions and were put on treatment. The number of new cases detected in a single year (72) was much higher than the average new cases per year (45) detected in the past nine years in the area. A total of 38 HHCs with inconclusive signs and symptoms of leprosy are under follow up.

The active case detection initiated as part of this study is completed and the follow up study is in progress. All collected samples have been brought to AHRI and the whole blood stimulated in microtubes is shipped to the Netherlands for multicytokine assays. ELISA assays for IFN-γ and IL-10 were done at AHRI. Data analysis is underway and other lab assays are in progress.

Funding: AHRI BSPP (Sida), GLRA Germany, TLMiE, AAU, and ALERT

Tsehaynesh Lema in Kokosa with Health Extension workers

Tsehaynesh and Azeb performing ELISA at AHRI lab

The correlation of antimicrobial peptide LL37 expression in different forms of Leprosy

Mahlet Osman[1,2], Kidist Bobosha[1], Markos Abebe[1], Shimelis Nigussie[3], Adane Mihret[1]

[1]Armauer Hansen Research Institute; [2]School of Medicine, College of Health Sciences, Addis Ababa University;

[3]All Africa Leprosy and TB Rehabilitation and Training Center

Leprosy is a curable infectious disease caused by *M. leprae* which affects hundreds of thousands of people every year. It attacks mainly the skin and peripheral nerves where macrophages and Schwann cells are the target cells. There are different clinical forms of leprosy, including tuberculoid leprosy (TT/BT) and lepromatous (BL/LL) forms. The spectrum of these clinical manifestations is correlated with the level of cell mediated immunity (CMI). Other than the difference in clinical forms, the occurrence of leprosy reactions (type 1 and type 2) is the major challenge in the management of leprosy. LL37 is the only human cathelicidin antimicrobial peptide encoded by the gene CAMP. It referred to as LL37, since it has 37 amino acid sequences starting with two leucines. This antimicrobial peptide has a broad-spectrum activity against bacteria, fungi and viruses.

The objective of this study is to investigate the association of LL37 with different forms of leprosy.

A total of 100 HIV negative individuals who fulfil the inclusion criteria will be enrolled. This will comprise 25 subjects TT/BT, 25 with BL/LL, 25 household contacts, and 25 healthy endemic controls. RNA and protein expression of LL37 will be measured.

Funding: AHRI core budget (Sida and Norad)

The role of Neutrophil Fcγ receptors in the pathogenesis of Erythema Nodosum Leprosum

Dareskedar Tsehay[1,2], Kidist Bobosha[1], Aster Tsegaye[2], Edessa Negera, Kassu Desta[2], Stephen L Walker[3], Saba Lambert[3], Abraham Aseffa[1], Diana Lockwood[3]

[1]Armauer Hansen Research Institut; [2]Medical Laboratory Sciences, Addis Ababa University; [3] London School of Hygiene and Tropical medicine

Erythema NodosumLeprosum (ENL) or Type 2 leprosy reaction is a neutrophilic immune-complex-mediated complication of leprosy with significant morbidity and mortality. It is characterized by crops of painful, erythematous nodules with systemic manifestations such as fever, malaise and inflammation producing iritis, arthritis, lymphadenitis, orchitis and neuritis. This study will investigate the association between the neutrophil expression of human Fc γReceptors and ENL. FcγRIIIb and FcγRIIa on neutrophils can interact with immune complexes (IC). A low copy number in FcγRIIIBgene has been associated with susceptibility to several IC-associated conditions. We hypothesize that the development of ENL in lepromatous leprosy patients is associated to a low copy number of FcγRIIIBgene, which leads to decreased expression of FcγRIIIb on neutrophils.

The objective of this study is to investigate the association between neutrophil expression of human Fcγ Receptors and ENL compared to those leprosy patients without ENL.

We have collected samples from ENL patients and lepromatous leprosy patients with no history of ENL from ALERT Hospital Addis Ababa, Shashemene referral Hospital Kuyera and East Gojjam zone, Ethiopia.

Genetic association will be done using a Droplet Digital PCR machine (ddPCR). Copy number variation of FcγRIIIBgene, which leads to reduced expression of FcγRIIIb on neutrophils will be compared to cases and controls. Data will be analyzed using graph pad and STATA.

Funding: AHRI core budget (Sida, Norad) and Malta grants for leprosy research (MALTALEP)

Treatment outcomes and Associated factors among MDR-TB patients who were on treatment during 2011-2016 at ALERT Hospital

Netsanet Aragaw[1,3], Mekonnen Tefri [1] , Oumer Ali [1]and Emawayish Tesema [2] , Kifle Woldemichael [3], Habtamu Jarso[3]

[1]. Armauer Hansen Research Institute (AHRI) [2]. ALERT Hospital [3]. Jimma University

Background: Tuberculosis (TB) is a major public health problem throughout the world. About a third of the world's population is estimated to be infected with tubercle bacilli and hence at risk of developing active disease.

According to 2016 WHO report Ethiopia is one of the 30 high TB, TB/HIV and drug resistant TB (DR-TB) burden countries globally and stands third among African countries with annual estimates of 2100

DR-TB cases among annually notified TB cases.

MDR-TB treatment course takes a minimum of 20 months and at least 18 months after culture conversion. Being diagnosed with MDR-TB and undergoing this longer treatment period imposes significant psychological, social, and economic stress on patients this potentially affects the MDR-TB treatment outcomes. As WHO report in 2015, only 52% of the MDR/RR-TB patients who started treatment in 2013 were successfully treated, while 17% of patients died and in 9% of patients their treatment failed (22% were lost to follow up or not evaluated)

The aim of this study is to assess MDR-TB treatment outcomes and associated factors among MDR-TB patients in ALERT Hospital.

Objective: To assess MDR-TB treatment outcomes and associated factors among MDR-TB patients who were on WHO's longer treatment regimen during 2011-2016 at ALERT Hospital.

Methods: A retrospective cohort study will be conducted. Data will be collected by record review using structured data collection format (checklist). All Patients registered from 2011 to 2016 will be included in the study based on inclusion and exclusion criteria. Data will be double entered to RedCap and exported to R 3.4.0 for analysis. Descriptive statistics will be computed to get summary results and multinomial logistic regression analysis will be used to predict factors which affect dependent variables. The association will be estimated by the relative risk (RR) together with the 95% confidence interval.

Significance of the study: It will be important to evaluate the programmatic management of DR-TB and TB treatment control program to set strategies for increasing treatment success rate. It can also serve as important background for the upcoming newly recommended WHO shorter regimen for management of MDRTB since Ethiopia is on the way to implement soon.

Status: Data collection was finalized and data entry is on-going.

Source of Budget: AHRI Core Fund

AHRI - APOPO Project Activity

Nigussie Beyene

Summary

The AHRI-APOPO project has two wings: enhanced case finding among presumptive TB cases attending TB clinics in Addis Ababa (routine case finding) and mass screening inmates and prison staff in 35 prisons across Ethiopia so as to find active TB cases (active case finding).

The routine case finding project aims to contribute to the national tuberculosis control program of Ethiopia by increasing the number of identified TB patients by at least 35% in the short term, whilst building a local capacity of TB detection rats and quality personnel to create a long-term impact on reducing the TB problem in Ethiopia. Financial support is obtained from Skoll Foundation for the startup

(construction of the facility and equipment procurement) and 2 years operation.

The three years active case finding project aims to screen 52,500 prison inmates and staff in 35 prisons all over the country. It is anticipated to find about 930 active TB cases that serve as a TB reservoir in the general population in general and the prison community in particular. This prison project is supported by Elton John AIDS Foundation. It will be implemented in collaboration with the German Leprosy and Tuberculosis Relief Association in Ethiopian (GLRA-Ethiopia) and the Federal Prisons Administration Commission.

Achievements so far:

- Construction of the project building (landed on 192 square meters but with a total floor area of 288 square meters due to a half-basement structure) was completed in October 2017.
- Construction of a modern incinerator (double combustion chamber) with waste storage area was completed.
- Office and laboratory furniture, lab equipment, reagents and chemicals are imported/procured and installed.
- Five lab technologists, 5 animal handlers, 4 sample collectors, a health officer and a data clerk are hired. Support staff (a lab assistant, a janitor, a driver, security guards, and a gardener) are going to be availed by AHRI.
- The animal handlers and 2 lab technologists attended training in Tanzania.
- The 30 TB centers that are going to be part of the enhanced case finding project are selected.
- Baseline data collection at the 10 selected 10 prisons that are to be covered in the first year of the prison project is underway in collaboration with GLRA and the Federal Prison Administration.

New initiative

TBRU ASTRa (TB Research Unit - Role of antigen-specific T cells in the control of TB):

This is a new collaborative study on latent TB with Emory and New York Universities. The Protocol is finalized and will be submitted to IRBs. This is an NIH funded project aiming at identifying biosignatures for detection of progressors among LTBI individuals. Within the project, students will have the opportunity for PhD training as part of capacity building, which will also include exchange visits, high throughput data analyses and data management capacity building.

TBRU meeting at AHRI in Nov 2017

Bacterial and Viral Diseases Research

Summary

AHRI has a rich tradition in mycobacterial diseases and great strength in many disciplines of infectious diseases research of public health relevance. The institute also has a platform to study immunobiology, microbial pathogenesis, genetics and evolution, bioinformatics, and epidemiology of different bacterial, viral and parasitic infectious diseases. Bacteriology-Mycology and Virology are two teams of AHRI Bacterial and Viral Diseases Research Directorate (BVDRD). Detecting bacterial, fungal and viral infectious agents and characterizing these pathogens is a basic strategy to fight against the diseases they cause. In this regard, the BVDRD is engaged in research activities that contribute to control pathogens affecting the nation. Other important public health challenges that warrant intervention include drug resistant microbial pathogens and emerging/re-emerging diseases occurring in epidemics or sporadically. In addition to research undertaking, the BVDRD is engaged in capacity building through enrolment of PhD and MSc students who have registered at different local and foreign Universities. Access to training and opportunities to undertake actual bench work at AHRI as well as at collaborative labs abroad are arranged for students as well as AHRI research staff. For instance, a collaborative study with Norwegian scientists on meningococcal meningitis *("Etiology, disease severity and diagnostic challenges of bacterial meningitis during non-epidemic seasons in Ethiopia: 2012-2013"*) was implemented with a capacity building component leading to a PhD dissertation. Students attached to BVDRD are accomplishing or have recently completed their research activities in collaboration with laboratories in Belgium, Sweden and Norway. In 2017, 7 PhD and 11 MSc students have been working under BVDRD supervision and oversight. The different research activities under the directorate are presented as follows:

A. Bacterial-Fungal Diseases Research Team

I. Completed Research

Etiology, Disease Severity and Diagnostic Challenges of Bacterial Meningitis during Non-epidemic Seasons in Ethiopia

Wude Mihret[1], Beyene Petros[2], Abraham Aseffa[2], Gunnstein Norheim[3]

[1]Armauer Hansen Research Institute; [2]Addis Ababa University, [3]Norwegian Institute of Public Health

Bacterial meningitis (BM) is a severe infectious disease of the nervous system that needs urgent medical attention. Ethiopia, a country located at the eastern end of the "meningitis belt", is frequently affected by meningitis epidemics. Studies have rarely focused on non-epidemic season strains of BM and use of less sensitive diagnostic tools have impeded characterization of its causative organisms. A prospective case-based study was launched from 2012-2013 on 139 patients clinically diagnosed with BM. The objective of the study was to define etiologies of BM, diagnostic challenges and disease severity in Ethiopia during non-epidemic seasons. Cerebrospinal fluid (CSF) samples taken from the study participants were

subjected to bacterial culture, molecular and immunological lab analyses while sera were evaluated by immunological assays. Patient age were varied from 2 days to 78 years old and more than half the total study population were younger than 12 years of age. Younger age and male gender were associated with higher levels of disease severity (i.e. death or sequelae). Bacteria were isolated in 10.1% of the cultures, and etiologies defined by RT-PCR in a third of these (46 total). . Of these, *N. meningitidis* was identified in 27 of the 46, , comprising genogroups A (11), W-135 (7), C (1), X (1) and non-groupable (7). *S.pneumoniae* was identified in 18 of the 46 isolates and *H. influenzae* in 1. . Levels of inflammatory cytokines in the CSF were significantly elevated in BM caused by *S. pneumoniae* compared to that of *N.meningiditis*, consistent with a worse outcome by the former. Affordable, multivalent meningitis vaccines composed of serogroups A, C, W-135 and X are urgently needed for use in Ethiopia and possibly in all countries within the African meningitis belt.

Status: Completed and PhD dissertation defended in May 2017.

Fund: Grants from the Research Council of Norway (no. 192477 to E Rosenqvistand no. 220829 to DAC).

Clinical Laboratory Professional Competency assessment on Gram stain examination and interpretation in Addis Ababa Hospitals

Adugna Tsehay[1], Kassu Desta[2], Adane Mhiret[1], Hilmineh Sinishaw[2], Kirubel Eshetu[2], Addisu Gize[2]

[1]Armauer Hansen Research Institute; 2 School of medical laboratory Sciences,Addis Ababa University

Gram staining is one of the standard laboratory test methods actively used to differentiate bacteria. However, gram stain examination and interpretation is not always straightforward and requires significant experience. In Ethiopia, particularly in Addis Ababa, no studies have been performed to assess competency of medical laboratory professionals on gram stains technique. Such a study could help to identify gaps and offer suggestions for improvements to educators and policy makers. We evaluated 190 laboratory participants from hospitals in Addis Ababa.

Forty eight (25.3%), 78 (41%) and 64 (33.7%) received scored low, medium and high skill levels, respectively. Among all participates, 1140 observations were made and of these, 4% were major errors and 321 with very major errors. Most medical laboratory professionals worked without supervision and training on gram stain examination and interpretation. These results suggest that the knowledge level and skill level of many medical laboratory professionals are not satisfactory, and this could lead to errors in patient diagnosis and management.

Fund: AHRI core budget.

Bacterial Profile, Antibacterial Susceptibility Pattern and Associated Factors among Mothers and Children visiting hospitals for Bacterial Infectios in Ethiopia

Biruk Yeshitela[1], Ebba Abate[2], Abebaw Bitew[2], Berhanu Seyoum[3], Daniel Demissie[3], Tesfaye Kassa[4], Zeleke Gizachew[4], Tamrat Abebe[5], Zeleke Ayenew[5], Tsegaye Alemayehu[6], Anteneh Amsalu[6], Rawleigh Howe[1], Abraham Aseffa[1]

[1]Armauer Hansen Research Institute; [2]University of Gondar; [3]Haromaya University;[4]Jimma University;[5]Addis Ababa University, [6]Hawassa University

Bacterial infection is an important cause of maternal and child morbidity and mortality. Maternal sepsis, endometritis, urinary tract infection and surgical site infections are the most common infections in mothers. Most of the morbidity and mortality in children is caused by sepsis and diarrheal diseases. There is a difference in the causative organisms of maternal and child infection in different countries as well as different geographic settings of same country. The objective of this study was to determine the bacterial profile, antibacterial susceptibility pattern and associated factors among mothers and children who contracted infection.

A Cross-sectional study was conducted from March 2016 to April 2017 to isolate pathogenic bacteria from clinical specimens and test for antibiotic susceptibility. The studies were conducted among women and children attending hospitals in Gondar, Dire Dawa, Hawassa, Addis Ababa and Jimma among.

Results of the Individual Study sites

1. Bacterial profile, antibacterial resistance pattern and associated factors among women attending postnatal health service at the University of Gondar Teaching Hospital, Northwest Ethiopia

Out of 107 specimens collected bacterial strains isolated in 90 (84.1%) cases. The predominant isolates were *S. aureus* (41.6%), *E.coli* (19.8%), *K. pneumonia* (13.9%), *and Coagulase negative staphylococci* (12.9%). The majority of isolates were resistant to ampicillin (66.7%) and amoxicillin (66.7%) but susceptible to ceftriaxone (100%). Multidrug resistant bacteria, defined as resistance to antimicrobials of three distinct categories, were seen in 54.5% and 95.8% for gram negative and gram positive bacteria, respectively. Procedures such as cesarean section and episiotomy for delivery and premature rapture of membrane had strong association with bacterial infections.

2. Assessing bacterial etiologic agents that cause neonatal sepsis and their antimicrobial susceptibility pattern at University of Gondar Hospital, North -West Ethiopia

Of the 251 study participants suspected of neonatal sepsis, 117 (46.6%) showed bacterial growth and 120 strains were isolated. Gram positive bacteria were the most common 81 (67.5%). The most identified bacterial species was *S. aureus*, 49 cases, (40.8%) followed by coagulase negative staphylococci, 26 (21.6%) and *K. pneumoniae*, 19 (15.8%). Multidrug resistance was observed among 78 (65%) of the isolates, including 56 (69.1%) among Gram positive and 22 (56.4%) among Gram negative bacteria..

Independent risk factors for the occurrence of neonatal sepsis included Apgar score within 5 minutes of < 7, birth weight < 1.5 kg, birth weight between 1.5–2.5 kg , gestational week <37 weeks and caesarian section delivery .

3. Prevalence, Drug susceptibility pattern of bacterial isolates and associated factor of septicemia infection at Dil-chora referral hospital Dire Dawa, Eastern Ethiopia

The prevalence of septicemia among collected blood cultures was 12.9% and coagulase negative staphylococcus was found to be the most frequent isolate,= (28.1%) followed by *E.coli* (22.8%), *Pseudomonas aeruginosa* (10.5%) and *Proteus* spp. (3.5%). Antimicrobial sensitivity was highly variable, ranging from 0-94%.. Multiple vaginal examinations and multiple pregnancies were identified as significant risk factors associated with sepsis.

4. High burden of nosocomial infections caused by multi-drug resistant pathogen among pediatric patients at Hawassa University Comprehensive Specialized Hospital

Culture confirmed nosocomial infections were reported in 82 patients (21.4% of total patients evaluated by culture) with a total of 88 bacterial isolates. *Klebsiella spp.*, 21(23.9%), and *S.aureus*, 16(18.2%) were the most frequently isolated bacteria. Among the *S,aureus*, 62.5% were methicillin resistant (MRSA) while 88.9% of all bacterial pathogens were multidrug resistant (MDR). Among gram negative bacteria, all isolates except *E.coli* were multidrug resistant. Length of hospital stay and malnutrition were significantly associated with nosocomial infections.

5. Enteric Pathogen Profile and Antimicrobial Susceptibility Pattern among Pediatric Patients with Diarrhea in Selected Health Facilities, Addis Ababa, Ethiopia

Among 290 study patients examined for complaints of diarrhea and from whom stool samples were obtained, *E.histolytica/dispar,* 75 (25.8%), *G.lamblia,* 13 (4.5%) *and H.nana,* 4 (1.4%) were identified parasites. The majority of bacterial enteropahogens isolated in the study were *Shigella* Spp. 22 (7.6%), enterohemorrhagic *E.coli* O157:H7, 13(4.5%), and *Salmonella* Spp. 7(2.4%). Of the *Salmonella* Spp. evaluated; 42.9% and 14.3%, showed resistance to trimethoprim-sulphamethoxazole and chloramphenicol, respectively. 77.3% of *Shigella* Spp. was resistant to ampicillin, 68.2% to trimethoprim-sulphamethoxazole and 36.4% to augmentin. *E.coli* O157:H7 isolates were frequently resistant to ampicillin (69.2%), to trimethoprim-sulphamethoxazole (46.1%), Augmentin (38.5%), ciprfloxacin 23.1%), whereas resistance to Amikacin, ceftriaxone and gentamycin was 15.4% among such O157:H7 isolates.

6. Multi-drug and/extensive-drug resistance in bacteria from reproductive age women with Urinary Tract infections (UTI) visiting Jimma University Specialized Hospital, Southwest Ethiopia

The prevalence of urinary tract infections among symtomatic reproductive aged women was 22.9% and *E. coli* (56.7%) was the most frequent among isolated bacteria followed by *Klebsiella species* (24.7%). Over 90% of the isolates were multi-drug resistant. Resistance to Ampicillin was 100%, followed by Tetracycline (92.4%) and Colistin (86%); in contrast, minimal resistance was observed for Imipenem (13%). Multivariate analysis revealed that risk factors such as previous history of hospitalization, extended spectrum beta-lactamase production and strong biofilm production were significantly associated with multidrug resistance at p-value <0.05.

Funding: MoH

II. ONGOING

Epidemiology and molecular characteristics of extended spectrum beta lactamase producing Enterobacteriaceae at Jimma University Teaching Hospital, Ethiopia

Tsegaye Sewunet[1,2,3,4], Daniel Asrat[2], Yimtubezinash Woldeamanuel[2], Adane Mihiret[3], Christian Giske[4]

[1]Jimma University; [2] Addis Ababa University; [3]Armauer Hansen Research Institute; [4] Oreboro University, Sweden

Enterobacteriaceae are common causes of morbidity and mortality in health care settings. The genetic plasticity of these bacilli and a variety of virulent factors they code for help them to adapt to different environments. These organisms acquire resistance genotypes and phenotypes to many antimicrobials; resistance to newer generations' of β-lactam antibiotics are frequently encoded by extended spectrum beta lactamase (ESBL) genes. Currently, epidemiology of ESBL producing strains varies across different countries and world regions. The current study is designed to define and characterize such strains at among patients visiting Jimma University Teaching Hospital (JUTH).

A cross sectional study was conducted from June 2016 – Oct 2016. Clinical specimens were collected from patients in consultation with attending physicians, and processed at Jimma University medical microbiology laboratory. Species identification was performed by conventional biochemical tests. ESBL determination was initially based on drug sensitivity ephenotypes, and later validated by MALDI_ TOF at the clinical microbiology lab at Karolinska University hospital. Whole genome sequencing was performed on Illumina Hiseq 2500 NGS platform at Sci Life Laboratory.

A total of 1045 study participants were included in the study, and the overall prevalence of ESBL is 22.1% among Enterobacteriaceae isolated from study participants. While there was no gender specific differences, the prevalence was much higher among admitted patients (26.9%) than in outpatients (9.5%), highly significant statistically (P = 0.000). CTX-M, TEM-1B, OXA-1 were the most commonly identified ESBL subtypes in this collection. Most of the *E.coli* and *K.pneumoniae* strains were resistant to at least three classes of drugs.

Conclusion: The total prevalence of ESBL producing strains in this study, particularly among hospital

patients is high, and should warrant policy changes.

Funding: AHRI (BSPP/Sida)

Characterization of the interaction of microbiota and pathogenic enteric bacteria in an Ethiopian traditional fermented food

Yared Hailaye[1,2,3,4] , Solomon G/Selassie[2], Abraham Aseffa[3] , Per -Erik Olsson[4]

[1]Debereberhan University; [2] Addis Ababa University; [3]Armauer Hansen Research Institute; [4] Orebro University, Sweden

Based on the report from WHO and CDC, Ethiopia is the fifth among fifteen countries with the highest number of annual child deaths due to diarrhea, also the second leading cause of death among all ages. The main etiologies of diarrhea are enteric bacteria transmitted by the feco-oral route. Teff is the staple grain within Ethiopia and used to produce Injera, a principal source of nutrition. Although the macro and micronutrient benefits of Teff are well established, there is no knowledge of the presence of potentially harmful bacteria which survive the fermentation and baking process during injera preparation, and which could contribute to suboptimal health including diarrheal disease among consumers.

The current study aims to characterize the microbial diversity of Teff fermentations from high and low-temperature areas of Ethiopia and investigate the interactions between the different microbial groups. The microbial dynamics was followed during the entire fermentation process and evaluated by plating. To date, we have found that obligate and facultative heterofermentative lactic acid bacteria, yeast and *Bacillus spp* persisted throughout the fermentation process in low and high-temperature fermentation. Bacteria from orders Corynebacterialles and Micrococcales also persisted in most parts of the fermentation process. The presence of diverse groups of microorganisms at the end of the fermentations warrants additional studies to determine the antimicrobial activity of fermentation. The role of the different groups in the sensory and safety of Teff Injera is critical for household consumption and commercialization to the global market. The antimicrobial activity of the fermentation and some of microbiota identified from fermentation is currently under investigation.

Funding: AHRI (BSPP/Sida)

Identification of Akaki River Pollutants and their Biological Effect on Livestock and Humans in Addis Ababa Ethiopia

Meron Talu[1,2,3,4], Gezahegne Mamo[2], Adane Mihret[3], Abraham Aseffa[3], Per -Erik Olsson[4]

[1]Asossa University; [2] Addis Ababa University; [3]Armauer Hansen Research Institute; [4] Orebro University

Water pollution has become a global challenge threatening health. The lack of enforcement to implement unnecessary waste disposal is a major problem in developing countries. Identifying the source of pollution and taking the necessary measures to improve the water quality is important. The source of pollution can be domestic sewage, effluents from industries, pharmaceuticals, hospitals. Depending on the source of pollution, the level and type of contaminants also varied from microbial to highly dangerous chemicals and trace elements. Little and Big Akaki rivers are the two most polluted rivers in

Ethiopia receiving all the domestic and industrial contaminants or pollutants released from Addis Ababa. However, the pollution level of toxic chemicals (pesticides, herbicides, Azo dyes, disinfectants.) and the biological effect of these pollutants on different organisms (aquatic, terrestrial) are unclear. In this study, Akaki River river water will be evaluated by measuring chemical pollutants (organic and inorganic) and biological impact *in vitro* using organisms *Caenorhabditis elegans*, and Daphina *magna.*

Water samples from the first phase (rainy season) has been collected and other samples will be collected within the coming two months.

Funding: AHRI (BSPP/Sida)

Phenotypic and Molecular Characterization of Potential Pathogenic Bacteria in Akaki River and their Biological Effect on Model Organism, Ethiopia

Berhan Yitayew[1,2,3,4], Daniel Asrat[2], Yimtubezinash Woldeamanuel[2], Abraham Aseffa3, Adane Mihret [3], Per-Erik Olsson[4], and Jana Jass[4]

[1]Debre Birhan University; [2] Addis Ababa University; [3]Armauer Hansen Research Institute; [4] Orebro University

Despite modern techniques for disinfection and water purification, sanitation and water borne diseases still threaten human health. Water-borne diseases are caused by bacteria, viruses, parasite and fungi. However, the majority of the outbreaks are caused by bacteria. Akaki river is the major river running through the centre of Addis Ababa, Ethiopia. It is polluted by industrial and municipal solid and liquid waste. The polluted river water is used by downstream residents for domestic purposes and to grow vegetables which are sold and consumed by inhabitants of the city. Pathogenic bacteria have been detected in many rivers associated with large cities worldwide. Identification of these pathogenic agents in water resources is beneficial for controlling and prevention of infectious diseases.

The aim of the current study is to determine the phenotypic and molecular characteristics of potential pathogenic bacteria in the Akaki river and their biological effect on model organisms from July, 2017 to January, 2019.Samples will be collected from five upstream and downstream points during the dry and rainy seasons. A microbial nucleic acid quantitative detection assay will be used to determine the profile of clinically important bacteria and major genes conferring resistance to beta-lactam, fluoroquinolone, tetracycline, sulfonamide, macrolide, chloramphenicol and aminoglycoside antibiotics will be assessed. . Bacteria will be isolated from standard cultures and identified using MALDI-TOFMS, and subject to standard antimicrobial susceptibility assays. Drug resistant bacteria will be further subjected to whole genome sequencing to determine drug resistant and virulence genes. The expression of antimicrobial genes, oxidative, stress, developmental and heat shock genes will be determined in *Caenorhabditis elegans* exposed to pre-and post-filtered water. In addition, a *P. aeruginosa* model strain will be treated with Akaki river water and changes in expression of drug resistance, virulence and stress genes determined.

Funding: AHRI (BSPP/Sida)

Prevalence of Burkholderia pseudomallei and other bacterial pathogens in community acquired infections in Addis Ababa and Bahir Dar regions of Ethiopia

Emawayish Andarge[1], Kassu Desta[2], Mekonnen Teferi[1], Biruk Yeshitela[1], Abraham Aseffa[1], Ivo Steinmenz[3]

[1]Armauer Hansen Research Institute; [2]Addis Ababa University, [3]Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz, Graz, Austria.

The Gram-negative pathogen, *Burkholderia pseudomallei* is the causative agent of melioidosis, a serious, often fatal disease of both humans and animals. *B pseudomallei* is an environmental saprophyte found in wet soils. It mostly infects adults with an underlying predisposing condition, mainly diabetes mellitus. The disease is acquired through environmental contact. Melioidosis has enormous clinical diversity, spanning from asymptomatic infection, localized skin ulcers or abscesses, chronic pneumonia mimicking tuberculosis, to fulminate septic shock with abscesses in multiple internal organs. The prevalence of melioidosis in Ethiopia and the environmental distribution are not known.

The aim of this study is to assess the prevalence of *B. pseudomallei* and other bacterial pathogens in selected clinical specimens from community-acquired infections and to determine the corresponding susceptibility pattern and to identify environmental *B. pseudomallei* in soil and surface water. So far 75 samples have been collected.

Funding: Burkholderia project fund

Severe Typhoid in Africa (SETA) Program

Mekonnen Teferi[1], Biruk Yeshitela[1], Melese Yeshanbaw[1], Ashenafi Alemu[1], Melaku Yidenekachew[1], Marechign Yimer[1], Oumer Ali[1] and Abraham Aseffa[1] and SETA Investigators

[1]Armauer Hansen Research Institute

Typhoid fever (TF) and invasive non-typhoidal *Salmonella* (iNTS) annually causes approximately 21.7 and 3.4 million cases and 217,000 and 681,000 deaths, respectively. Disease burden appears to be highest in countries where access to safe water and appropriate sanitation is restricted. While data demonstrating the burden of TF and iNTS exists for Asia, in sub-Saharan Africa (sSA) this information is limited.The Typhoid Surveillance in Africa Program (TSAP) provided data on incidence rates; however, many issues remain undefined. The goals of Severe Typhoid in Africa Program (SETA) are to estimate the burden and severity of invasive salmonellosis; report the long-term sequelae and associated cost of illness; and assess the immune response to natural infection over 1 year. SETA will generate TF, para typhoid fever (PF) and iNTS disease burden and immunological data to drive vaccine development and inform evidence-based prevention and control policy (including vaccine policy).

The Severe Typhoid in Africa Program aims to implement a surveillance program that will allow for the collection of the information mentioned above through a comprehensive integrated design in six African countries (Ghana, Ethiopia, Burkina Faso, Nigeria, Democratic Republic of Congo, Madagascar). The program will employ a combination of passive surveillance activities for febrile diseases at tertiary, secondary, and primary healthcare facilities where clinical and microbiological data will be collected from suspected TF and iNTS febrile patients.

The project is currently enrolling eligible patients from three sites Welayita-Sodo, Adama-Wenji and Addis Ababa. Accordingly, currently eligible patients are enrolled from Wolayta Sodo University Hospital, Wolayta Health Center, Adama Hospital Medical College,Wonji Gefersa Health center , Wonji Kuriftu Health center , Alem Tena Health center, Black Lion Hospital and St. Paul Hospital. Since the start of the project, July 2017 to Oct 2017, a total of 125 eligible febrile patients were recruited and samples were processed. Patient recruitment and data entry will be on-going until July 2019.

Funding: Bill and Melinda Gates Fouundation through IVI

Systematic Study of Hospital Acquired Infections and Antibiotic Resistance: insight into the epidemiology and genetics of infection

Biruk Yeshitela[1], Lena AlHassen[2], Abraham Aseffa[1]

[1]Armauer Hansen Research Institute; [2]Sussex University, UK

Hospital-acquired infections (HAI) affect a considerable number of hospitalized patients worldwide, with numerous outbreaks leading to morbidity and mortality.

The aim of this study is to investigate the epidemiology of hospital acquired infections and to genetically characterize antimicrobial resistance mechanisms. The project is being undertaken at Tikur Anbessa Specialized University Hospital. Since project initiation, a total of 100 samples have been collected and processed, and patient data has been captured and under database entry.

Funding: AHRI core budget.

Phenotypic and Genotypic Characterizations of Streptococcus pneumoniae Strains Isolated from Pediatric Patients in Addis Ababa, Ethiopia

Abel Abera Negash[1,2], Abraham Aseffa[1], Daniel Asrat[2], and Mario Vaneechoutte[3]

[1]Armauer Hansen Research Institute; [2]Addis Ababa University; [3]Ghent University

Pneumococcal vaccine 10 (PCV10) has been introduced in Ethiopia in September 2011. Many studies performed in developed countries have indicated that the introduction of PCV often results in "serotype replacement disease" resulting in replacement of vaccine serotypes by non-vaccine serotypes. The continued study of pneumococcal disease and carriage isolates is therefore important for understanding selective effects of PCV 0 upon regional population structures of this species. In addition to PCV, trends in pneumococcal carriage and disease epidemiology is also influenced by the use of antimicrobial drugs.

The aims of this study are to determine the magnitude of pneumococcal diseases, the e serotype and genotype of invasive and non-invasive *S. pneumoniae* isolates, and the prevalence of antibiotic resistance, clonal spread and genetic variability among *S. pneumoniae* isolates with emphasis on macrolide and penicillin resistance. The target population is HIV+ and HIV- children who are clinically suspected with meningitis, pneumonia, bacteremia, septicemia, and acute otitits media and referred by physicians in four major government hospitals and one pediatric speciality center in Addis Ababa, Ethiopia.

Samples have been collected from 845 pediatric patients with suspected disease, including 649 children

with community acquired pneumonia, 95 with sepsis, 55 with acute otitis media and 46 with meningitis. Subculture has been performed on samples from 711 blood cultures and trans-isolate media (TIM) bottles, 459 nasopharyngeal swabs and 55 middle ear swabs. In addition, antibiotic sensitivity testing has been performed on 340 isolates. 810 isolates 322 whole blood, 491 blood culture broth aliquots and 480 nasophrayngeal swabs have been stored. Currently, DNA extraction and PCR on whole blood, blood culture broth aliquots and culture negative nasopharngeal swab samples are being performed at University of Ghent. Serotyping by sequetyping and real time multiplex PCR, genotyping with MLST and identification of clonal spread and genetic variability of macrolide resistant and pilus-encoding genes will also be performed.

Funding: A PhD Training Scholarship by VLIROUS, Belgium

Humoral Immune Response to Pneumococcal Vaccination in HIV-1 infected children on ART

Mahlet Lemma[1,2], Beyene Petros[1], Meseret Gebre[3], Raweligh Howe[2] , Francesca Chiodi[4]

[1]Addis Ababa University; [2]Armauer Hansen Research Institute; [3]ALERT Hospital; [4]Karolinska Institute

HIV is a global public health problem with heavy burden in Sub-Saharan Africa. Although antiretroviral treatment changes HIV infection from a progressive illness with fatal outcome into a chronic manageable disease, HIV infection is still characterized by progressive destruction of the immune system that could eventually lead to AIDS. One indicator for the detrimental effect of HIV on the immune system is the presence of a low number of pneumococcal antigen specific memory B cells in HIV infected individuals that received pneumococcal vaccination. Some studies have suggested that early initiation of ART may limit the detrimental effect of HIV on the immune system and result in adequate humoral immune response to pneumococcal vaccination. Therefore, the aim of this cross-sectional study is to investigate humoral immune responses to pneumococcal vaccination and natural infection in HIV-1 infected Ethiopian children receiving ART.

This study will measure the level of pneumococcal antigen specific IgG, IgM and IgA in children who have received a full dose of PCV-10 in the national immunization program using stored samples from vaccine naïve children. In addition, it will assess the frequency of memory B cell subsets and marginal zone memory B cells in HIV infected children on ART compared to age matched healthy controls. Pneumococcal antigen specific memory B cells will also be enumerated by using flow-cytometry. To date pneumococcal antigen specific IgG for 13 serotypes that are incorporated in PCV-13 have been measured using stored samples from vaccine naïve children. . In addition, measurement of the frequency of classical memory B cells subset and marginal zone memory B cells is ongoing at Karolinska Institute, Stockholm, Sweden using the same samples.

Funding: AHRI (BSPP/Sida)

B. Viral Diseases Research Team

A five-year viral diseases programmatic research plan has been prepared based on need assessment, facility assessment and Health Strategic Development Plan directions focusing mainly on development

of in-house molecular viral diagnostics including detection and monitoring of drug resistance, studies on efficacy of viral vaccines and immunity and molecular epidemiology of circulating viruses in Ethiopia. The following are identified thematic areas: HIV/AIDS, viral hepatitis, oncoviruses excluding (HBV and HCV), emerging and re-emerging viral diseases, viral gastroenteritis, and viral respiratory diseases. Four rooms are needed for molecular virology techniques which include master mix preparation room, nucleic acid extraction, amplification room and detection room. The existing AHRI complex is renovated as stated above to meet the minimum BL2 standard of both RNA and DNA handling. This BL2 laboratory enables us for handling genetic materials from patient samples with extraction, processing and molecularly amplification for qualitative and/or quantitative detection and sequencing for molecular characterization.

I. Completed research

Viral Hepatitis Projects

Adane Mihret[1], Ebba Abate[2], Getnet Ayalew[2], Mekuanint Geta[2], Berhanu Seyoum[3], Fitsum Abebe[3], Seifudin Usman [3], Tesfaye Kassa[4], Rebie Kedir[4], Belayneh Dima[4], Seifegebriel Teshome[5], Habtamu Biazin[5], Tamrat Abebe[5], Abraham Aseffa[1], Rawleigh Howe[1]

[1]Aramuer Hansen Research Institute; [2]University of Gondar; [3]Haromaya University; [4]Jimma University; [5]Addis Ababa University

This project has 3 components. The first component focuses on childhood viral hepatitis infection aiming at evaluating the serological and clinical efficacy of HBV vaccines, establishing regional baseline data on paediatric viral hepatitis infection and estimating the vertical transmission of hepatitis B infection. The second component focused on mothers and aimed at estimating the diseases burden of hepatitis infection (HBV/HCV/HDV/HEV) and to assess the presence of regional variation on magnitude of hepatitis in 5 selected regions. The third component focused on known chronic liver diseases and hepatic cellular carcinoma patients aiming at defining the role of viral hepatitis and their genotypes on these diseases.

Preliminary analysis of more than 2300 children involving 4 distinct regions of the country, suggests that the frequency of children with protective levels of anti-HBsAg antibodies specific for the HBV vaccine administered during infancy is generally low, but shows a marked variation ranging from 32.2% in Gondar, 54.3% in Addis Ababa, and 58% in Jimma to 97.5% in Haramaya. Among non-vaccinated children HBsAg expression, indicating active infection, was high in Jimma (7.0%), and Haramaya (3.1%), undetectable in Addis Ababa (0%) and among vaccinated children in Gondar, HBsAg expression was high (4.2%). Comparison of HBsAg prevalence among vaccinated and non vaccinated children yielded clinical efficacies of 64% (Haramaya) and 70% (Jimma).

Thus analysis to date indicates that both serological and clinical efficacy are substantially lower than the 90-95% protection observed in multiple studies in N. America, Europe and Asia. This suggests that the currently used vaccines are either less efficacious or less durable than expected, and without further modification in the vaccine program in Ethiopia are unlikely to achieve as efficient a reduction in hepatitis prevalence over time as has been seen in other regions of the world such as Asia.

On a more positive note, however, it is remarkable that the level of HBsAg positivity in unvaccinated children in Addis Ababa in the current study, as well as an independent pilot study completed recently at AHRI, is substantially lower than percentages obtained of similarly aged children in a 1994 study in Addis Ababa. In that study about 5% of children aged 5-9 years old were HBsAg positive, a value consistent with that seen in our current study among non-vaccinated children in peripheral regions of Ethiopia. Thus, at face value, assuming the 1994 and 2017 studies are comparable, this suggests that changes in Addis Ababa alone over that time interval had more impact on the decrease in disease prevalence than has the vaccine in any Ethiopian location, at least thus far. Although analysis is ongoing and the aforementioned results should be considered preliminary, this finding, if confirmed, suggests that more comprehensive studies are needed comparing cultural and health care practices between Addis Ababa and peripheral locations, and that such studies may reveal important non-vaccine interventions having major impact on hepatitis prevalence in the country.

Seroprevalence of hepatitis B and C virus infections among mothers ranges from 3.7% and 2.0 %, respectively in Addis Ababa, 5.7%, and 2.6% in Jimma, 5.9 % and 1.1% in Harar and 3.8% HBV infection in Gondar. These percentages are consistent with those of many studies of adults over the past 5-10 years. Importantly, several sites observed that there was strong age-related decrease HBsAg positivity among mothers even though percentages of anti-HBcAg, and thus exposure, were comparable across age. There are potentially multiple explanations for this, but perhaps the simplest is that with time individuals with chronic infection gradually improve control over the disease, an encouraging finding from a public health perspective, but one which requires further investigation.

Studies involved in estimating vertical transmission of HBV in Ethiopia are more fully described below, as are those investigating hepatitis virus etiologies and molecular characteristics associated with chronic hepatitis disease and hepatocellular carcinoma.

Manuscripts from all sites are under preparation.

Funding: Federal Ministry of Health, Ethiopia

II. Ongoing research projects

Estimating the vertical transmission of hepatitis B infection

Yayeherad Tasachew[1], Adane Mihret[2], Andargachew Mulu[2], Rawleigh Howe[2], Abraham Aseffa[2]

[1] Hawassa University; [2]Armauer Hansen Research Institute

While transmission through sexual intercourse and intravenous drug abuse are the major risk factors for acquisition of hepatitis B among adults, perinatal transmission is responsible for up to 50% of HBV infection worldwide, and is particularly common in Asia. It has not been well studied in Africa, but available data, including a study 20 years ago in Ethiopia, suggests it occurs but much less frequently. The current vaccination guidelines in the country are not optimal to block vertical transmission because the vaccine is administered several weeks after birth, rather than at birth, and does not include prophylactic anti-hepatitis globulin. The present study is an indirect approach--nested within the aforementioned

multisite study of hepatitis-- to estimate the magnitude of vertical transmission and the impact of the current vaccination program thereof. Paired samples from 5-9 year old children and their mothers have been screened for ongoing infection by the presence of blood HbsAg, and positive paired samples will be subject to sequencing to infer relatedness of isolates. Identical isolates from paired samples will be assumed to have occurred via vertical transmission. The magnitude of inferred vertical transmission will be compared among children with or without vaccination to address the efficacy of vaccination.

Funding: Federal Ministry of Health, Ethiopia

Molecular Epidemiology of Hepatitis B Virus Genotypes and pattern of mutations among HIV Co-infected and HBV Mono-infected Adults

Desalegn Admassu[1], Rawleigh Howe[2], Abraham Aseffa[2], Berhanu Seyoum[1], Adane Mihret[2], Andargachew Mulu[2]

[1] Haromaya University; [2]Armauer Hansen Research Institute

Hepatitis B Virus has a worldwide distribution and is endemic in many populations. HBV genotype and viral variants have clear implications for many clinical aspects of Chronic Hepatitis B and should become more routinely utilized to help predict likely clinical course. Understanding HBV genotype distribution is important for epidemiologic characterization of HBV transmission and to control infection. HBV and HIV represent the two most important blood-borne viral pathogens in terms of prevalence, morbidity and mortality in sub-Saharan Africa, where both viruses are endemic and the organisms also share transmission routes. Assessing the status of HBV infection in HIV co-infected or HBV mono-infected individuals using HBsAg may underestimate the actual prevalence of hepatitis B virus for many reasons including window period of infection, HBsAg negativity due to mutations in the S region and occult HBV infection. Thus, the objective of this research project is to investigate the molecular epidemiology of HBV, mutational patterns and pathways among HIV co-infected and HBV mono-infected adults in selected public hospitals in Eastern Ethiopia from August-November 2017.

A total of 2168 samples will be obtained, with equal numbers allocated to HIV+ and HIV- study groups. Samples will be tested for hepatitis serology and appropriate samples selected for further comprehensive molecular analysis.

The study protocol has been ethically approved and data collection has started in all the three sites

Funding: Federal Ministry of Health, Ethiopia

Role of viral hepatitis and genotypes in Hepatocellular carcinoma (HCC) and chronic liver disease (CLD) in southern and central Ethiopia

Yayehyirad Tassachew[1], Abraham Aseffa[2], Rawleigh Howe[2], Adane Mihret[2], Andargachew Mulu[2], Tamrat Abebe[3]

[1]Hawassa University; [2]Armauer Hansen Research Institute; [3]Addis Ababa University

Hepatocellular carcinoma (HCC), also called malignant hepatoma, is the most frequent type of primary liver cancer, and is responsible for more than 80% of the worldwide total liver cancer burden and for the death of more than half a million people a year. HCC is a disease with multiple aetiologies, though chronic hepatitis B or C infections are the principal risk factors for most parts of the world. Sub-Saharan

Africa is one of the geographic regions of the world with a high burden of HCC, and this is likely to be true in Ethiopia, though its magnitude in different regions of the country is unknown. The objective of the current study is to determine the etiological association of hepatitis B, C and D viruses, and other risk factors such as aflatoxin with in Ethiopia.

This is a hospital based case-control study, consisting of 127 clinically confirmed cases of HCC, 127 chronic liver disease (CLD) cases and 254 sex and age (± 5 years) matched apparently healthy controls free from any liver disease. The study sites include the gastroenterology units of five hospitals. Diagnosis of HCC, CLD and hepatic viral infection is carried out based on clinico-pathological, biochemical, and virological parameters.

The study has been ethically approved and in progress. So far demographic and clinical data, blood and urine samples from 90 HCC, 70 CLD and 50 controls have been collected.

Funding: Federal Ministry of Health, Ethiopia

Characterizing the Unmet HIV Prevention Needs and HIV Risk Vulnerabilities of Adolescent Girls and Young Women in Ethiopia

Taye Tolera[1], Andargachew Mulu[1], Stefan Baral[2], Sheree Swarz[2], Carly Comins[2]

[1]Armauer Hansen Research Institute, [2]Johns Hopkins University

Adolescent girls and young women (AGYW) aged 15-24 are an important key population that experiences a disproportionate level of new HIV infections. This population continues to sustain high incidence rates, thus preventing achievement of ambitious UNAIDS goals for an AIDS-free generation and epidemic control by 2030. Although the disparity in HIV prevalence among young women and their male counterparts is not new, there is increasing recognition that it is fueling the epidemic and must be addressed in high HIV burden countries. In Ethiopia, data characterizing unmet HIV prevention needs and HIV risk vulnerabilities among AGYW is lacking due to challenges in studying the needs of this population. This project proposes to conduct an integrated HIV bio-behavioral survey (IBBS) among AGYW to characterize HIV prevalence and to estimate vulnerability and risk factors for HIV in this population to guide future HIV programming.

The protocol has been approved ethically and its work is in progress. Data collectors have been recruited and trained. The first stage of the project that is venue identification and mapping is in progress at Addis Ababa.

Funding: USAID

Training on data collection for AGYW project

Malaria and Neglected Tropical Diseases Research

Summary

Malaria-NTD directorate is involved in research (basic and operational), capacity building and supporting national technical working groups. The directorate is proactive and productive in operational research: we are working on the challenges in the transition from malaria control to elimination with implications in Ethiopia and beyond. We are working to avail locally produced visceral leishmaniasis method.

I. Ongoing research projects

The prevalence and dynamics of asymptomatic malaria in Ethiopia: would it be a challenge to the transition from control to elimination?

Fitsum G Tadesse[1, 2, 3], Elifaged Hailemeskel[1,2], Surafel Tebeje[1,3], Wakwoya Chali[1], Girma Shumie[1], Sagni Chali[1], Robert Sauerwein[3], Taye T Balcha[1], Abraham Aseffa[1], Delenasaw Yewhalaw[4], Endalamaw Gadisa[1], Chris Drakeley[5], Teun Bousema[3,5]

[1]Armauer Hansen Research Institute), Addis Ababa, Ethiopia; [2]Addis Ababa University, Addis Ababa, Ethiopia; [3]Radboud Institute for Health Sciences, Radboud University Medical Centre, Nijmegen, The Netherlands; [4] Tropical Infectious Diseases Research Center, Jimma University, Jimm, Ethiopia; [5]Department of Immunology and Infection, London School of Hygiene and Tropical Medicine, London, United Kingdom

The majority of *P. vivax* and *P. falciparum* infections in low-endemic settings are asymptomatic. The relative contribution to the infectious reservoir of these often of low-parasite-density infections, compared to clinical malaria cases is currently unknown. Learning the importance of such reservoirs for onward transmission fills key gap in the move for malaria elimination.

Using membrane feeding, we assessed infectiousness to *Anopheles arabiensis* colony of self-presenting microscopy-detected symptomatic (n=41) and community-recruited asymptomatic (n=41), and infections detected by PCR- (n=82) only. Malaria incidence and prevalence data was used to model the relative importance of the respective population.

Adjusting for population level malaria prevalence, clinical cases, asymptomatic microscopy and PCR-detected infections were responsible for 4.9%, 78.8% and 16.3% of the infectious reservoir for *P. vivax*, respectively. And similarly, clinical cases, asymptomatic microscopy or PCR-detected infections were estimated to be responsible for 0.7%, 69.5% and 29.7% of the infectious reservoir for *P. falciparum*, respectively.

In the study setting asymptomatic infections are highly prevalent and are the main sources for the majority of onward mosquito infections. Thus, the Ethiopian malaria elimination program needs to design strategies to identify and treatment asymptomatic infections to avoid re-emergence/resurge of malaria.

Funding: NUFFIC and AHRI Core budget

Strengthening the malaria control and/or elimination efforts of the country: the value of reactive active case detection and G6PD prevalence

Mulualem Belachew[1,2], Getasew Shitaye[1,3], Wakwoya Chali[1], Girma Shumie[1], Sagni Chali[1], Taye T Balcha[1], Abraham Aseffa[1], Robert Sauerwein[3], Chris Drakeley[4], Teun Bousema[3,4], Fitsum G Tadesse[1,2,3], Endalamaw Gadisa[1]

[1]Armauer Hansen Research Institute, Addis Ababa, Ethiopia; [2]Addis Ababa University, Addis Ababa, Ethiopia; [3]Radboud Institute for Health Sciences, Radboud University Medical Centre, Nijmegen, The Netherlands; [4]Department of Immunology and Infection, London School of Hygiene and Tropical Medicine, London, United Kingdom

As the incidence of malaria decreases the distribution of malaria becomes highly heterogeneous and clusters in certain geographical areas and/or households. Due to this to maximum return for pre-elimination or elimination investment, strategies need be optimized. Thus, identifying hotspots and the related risk factors, and the use of radical cure would be critical to remove the remaining reservoirs of infection. Yet, the use of Primaquine as radical cure depends on G6PDd status, common genetic deficiency in malaria endemic regions, as it induces potentially life threatening hemolytic anemia.

Reactive active case detection was carried out following a total of 18 RDT-confirmed clinical malaria cases and 18 individuals that visited the same health-post for malaria unrelated cause between October and December 2016. Consenting, six immediate neighbors and their family members were screened using RDT and 18S ribosomal RNA based quantitative PCR. A community based cross sectional survey involving 1609 individuals from different eco-epidemiological settings was done using CareStart™ Rapid Diagnostic kit (RDT) to screen for G6PD enzyme activity and sequencing selected representative samples. *Plasmodium* blood-stage parasitaemia detection was performed using the CareStart™ Malaria Ag PLDH/HRP2 and nested Polymerase Chain Reaction. SaTScan for spatial clustering basement and STATA version 12 for summarization and comparison were used.

Individuals in HHs with RDT-detected *P. falciparum* and *P. vivax* infections were 28.8 and 5.0 fold more likely to have qPCR detected infections compared to individuals who lived in HHs without RDT detected infection, respectively. With in hotspots, age was found to be a strong predictor of parasite carriage and density of infections, with children under 15 years carrying the majority of the malaria burden. We detected 22 and 31 G6PDd individuals with CareStart™ RDT and sequencing respectively. Symptomatic and asymptomatic malaria cases clustered significantly around index cases compared to non-malaria control cases. Thus, malaria control and elimination strategies of the country could benefit implementing reactive case detection approach. We found more G6PDd individuals not detected by the RDT, thus to avoid hemolytic anemia upon using PQ in such individuals, we recommend further sequencing and CYP2D analysis in larger population.

Funding: AHRI core with support from Prof Teun

Serological and molecular tools, their implication in evaluating malaria control programmatic performance in Ethiopia

Migbaru Kefale[1,2], Wakwoya Chali[1], Girma Shumie[1], Sagni Chali[1], Taye T Balcha[1], Abraham Aseffa[1], Robert Sauerwein[3], Chris Drakeley[4], Teun Bousema[3,4], Fitsum G Tadesse[1, 2, 3], Endalamaw Gadisa[1]

[1]Armauer Hansen Research Institute, Addis Ababa, Ethiopia; [2]Addis Ababa University, Addis Ababa, Ethiopia; [3]Radboud Institute for Health Sciences, Radboud University Medical Centre, Nijmegen, The Netherlands; [4]Department of Immunology and Infection, London School of Hygiene and Tropical Medicine, London, United Kingdom

As a result of malaria prevention and controls interventions, Ethiopia has experienced a 66% decline in confirmed malaria cases between 2001 and 2011. Motivated by this decline, the country set a plan to eliminate malaria in selected low-transmission districts by the end of 2020. Therefore, this study proposal is designed with the objective of evaluating the effectiveness of malaria control program by using age specific seroconversion/reversion curve and estimates the prevalence using PCR based techniques. Multistage sampling technique was used to select study districts (Goro Gutu and Babile) based on the malaria control program stratification. Retrospective and scociodemographic, and a finger prick blood sample were collected. IgG antibodies against *Plasmodium* antigens and *Anopheles* salivary protein will be assayed using indirect ELISA and the presence of parasite genome will be detected by using nested PCR. Using reverse catalytic model, age wise malaria transmission intensity variation will be evaluated and the level of agreement between PCR and ELISA test in estimating the program success will be assessed by kappa test.

Sample collection finalized, a total of 1700 (762 males and 938 females) participants were interviewed and DBS sample collected. Retrospective malaria program data was collected from the district health bureau and health posts.

From the total sample 493 blood films examined microscopically 21(4.3%) were positive.

Funding: AHRI core with partial support from Prof Teun Bousema

The move to replace RDT by microscopy at health center and referral systems for malaria diagnosis in Ethiopia; does the system have enough and/or competent professionals?

Sagni Chali[1], Wakwoya Chali[1], Taye T Balcha[1], Abraham Aseffa[1], Fitsum G Tadesse[1, 2, 3], Endalamaw Gadisa[1]

[1]Armauer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia; [2]Addis Ababa University, Addis Ababa, Ethiopia; [3]Radboud Institute for Health Sciences, Radboud University Medical Centre, Nijmegen, The Netherlands

Microscopic diagnosis of Geimsa stained thick and thin blood films by skilled microscopists has remained the standard laboratory method for the diagnosis of malaria. However, diagnosis of malaria with this method is problematic since interpretation of results requires considerable expertise particularly at low parasite level.

The objective of this study is to evaluate the performance of laboratory professionals in the diagnosis of malaria in Oromia region, from April to June, 2017.

This is a cross-sectional study which will be conducted in 356 selected health facilities of the region. The data will be entered, cleaned and analyzed using SPSS for windows version 20. Association between the

outcome and the independent variables will be taken as significant at P<0.05. Mean, standard deviation, chi-square (for categorical data), sensitivity, specificity, proportion of errors, percent agreement, kappa score will be calculated to assess the performance of the laboratory professionals and results will be presented in tables and figures.
Data was collected from 87 participants; 53 of whom (61%) received training on malaria microscopy.

Funding: AHRI core with partial support from Prof Teun Bousema

In house production of diagnostic kit for the diagnosis of visceral leishmaniasis

Girma Shumie[1], Wakwoya Chali[1], Taye T Balcha[1], Abraham Aseffa[1], Endalamaw Gadisa[1]

[1]Armauer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia

Ethiopia is among the top 6 global high burden countries for visceral (VL) leishmaniasis. VL is the cause for up to 4500 hospitalization per year. Among the major challenges in VL control in Ethiopia are insufficient access to diagnosis and treatment. Thus our primary objective is to reduce cost and build capacity for local production to improve the diagnostics. The local production of DAT will enable us to retain the foreign currency, reduce the cost and improve accessibility. Moreover, strain variation is claimed to result in the inconsistency of VL diagnostics, therefore, having our own local production facility will avoid such problem. Also the improved diagnostic coverage will allow us in collecting the circulating parasites which intern be used for both drug resistance monitoring.

Liquid DAT was produced at AHRI and its performance was compared against standard kits.
Performance of the LQ-DAT, FD-DAT and rK39 for the diagnosis of VL was evaluated considering Smear Microscopy as Gold standard (N=74). Our in-house produced DAT showed equivalent performance as the commercial DAT and rK39 kits and had high agreement in its performance.

Funding: Ethiopia Biotechnology Institute

Non-communicable Diseases Research

Summary

Non-communicable diseases are increasing rapidly in prevalence in Africa, and in Ethiopia now contribute to half of all mortality. Although AHRI has for some time engaged in sporadic research projects, particularly in relation to cancer, we have now formalized a non-communicable diseases division. At present, we have focused research primarily on hematologic malignancies and breast cancer, but have projects under consideration for cervical cancer, and non-communicable diseases such as autoimmune skin diseases, diabetes and asthma.

I. Current research in Leukemia and Lymphoma

Hematological or leukocyte malignancies, which include acute and chronic leukemia, Non-Hodgkins Lymphoma, and Hodgkins Lymphoma, when summed in prevalence comprise the third leading cause of cancer and cancer mortality in Ethiopia, following only Breast and Cervical Cancer.

AHRI's entry into hematological malignancies originated in our expertise in flow cytometry, long an important technological asset of the institute since the purchase of our FACScan flow cytometer in 1991 (the first flow cytometer in Africa). Flow cytometry has since emerged as one of the key diagnostic modalities for hematologic malignancies, and the potential applicability of flow cytometry is underscored by the large number of flow cytometers present in the country as part of CD4 counts for HIV disease. We have planned a large number of student studies involving different subsets of patients with hematologic malignancies, and all have a flow cytometry component. Additional techniques of value for diagnosis include Fluorescent In Situ Hybridization (FISH) to characterize some of the genetic aberrations which are typical for certain malignancy subtypes. We have yet to implement this technique but have recently received a very highquality fluorescence microscope (capable of assessing up to 7 fluorescent colors), and at least two international collaborations (University of Leipzig and University of Michigan) in place for student training. Very useful phenotypic information on malignancies can also be obtained using immunohistochemistry and standard immunofluorescence, and application of this technique, again with ARHI's new microscope- will be particularly useful for one of our student's thesis work on Hodgkin's lymphoma in collaboration with the University of Lund in Sweden. Finally, we will enter the era of advanced molecular characterization of cancer by utilizing target gene sequencing as part of the characterization of projected resistance of some Chronic Myeloid Leukemia patients' response to tyrosine kinase inhibitor drugs and for guidance of improved therapy. A distinct feature of all these studies is that their immediate clinical relevance is high. The initial studies on flow cytometric phenotyping of acute leukemia have been completed, and in several instances, flow phenotyping has provided an alternate and more appropriate diagnosis guiding therapy for patients failing chemotherapy based on traditional morphology diagnosis. We have still much to learn in the field of hematological

malignancy research, but progress has been steady and promising.

1. Diagnostic utility of immunophenotyping by flow cytometry and comparison with morphology and cytochemistry for diagnosis and classification of acute leukemia

[1]Metasebia Tegegn and Kiya Desalegn

[3]All Africa Leprosy and TB Rehabilitation and Training Center

Immunophenotypic characterization of acute leukemia has become an important modality for diagnosis. There are three general types of acute leukemia: acute myelogenous leukemia (AML), B cell acute lymphocytic leukemia (B-ALL) and T-cell acute lymphocytic leukemia (T-ALL). Each general type can be further subdivided according to various criteria depending on the methodology used. In the context of treatment utilized in Ethiopia, it is particularly relevant to accurately distinguish between myeloid and lymphoid lineage acute leukemia because different treatment regimens are used. In the present study we analyzed 80 acute leukemia patients using a panel of antibodies specific for myeloid, B cell and T cell lineage markers. In parallel, blood smears from all 80 patients were evaluated by morphology and 40 by cytochemical stains. The goal of the study was to determine the feasibility of flow cytometry in the Ethiopian setting and to compare diagnoses by each of the three methods. Flow cytometric markers used to identify myeloid lineage cells included intracytoplasmic myeloperoxidase, CD13, and CD33; B lineage markers included CD19, intracytoplasmic CD79a and CD10; and antibodies specific for CD4, CD7, CD8 and surface and cytoplasmic CD3 were used as T lineage markers. Other markers defined early progenitor stages within lineages, including HLA-DR, CD34 and CD117, the latter more specific for early myeloid progenitors. Cytochemical stains included Sudan Black (SDB), a stain specific for granules of myeloid cells and Periodic Acid Schiff (PAS), useful in identifying blasts from acute lymphocytic leukemia cells.

Overall, there were 33 cases of acute lymphocytic leukemia (ALL), defined by flow cytometry, of which 17 were B-cell ALL and 16 were T-cell ALL, 46 cases of AML, and one bi-lineage leukemia expressing markers of both T cell ALL and AML. Conversely, by morphology (which cannot discriminate between B- and T-ALL), there were 32 cases of ALL and 43 of AML, with the other cases indeterminant. Despite nearly identical total numbers of ALL and AML cases diagnosed by either method, the concordance between flow and morphology was only 78%. Comparison with cytochemistry revealed that AML cases diagnosed by both flow and morphology were uniformly negative for the ALL cytochemical marker PAS (which again cannot distinguish between cells of B or T origin), and greater than 90% positive for the myeloid marker SDB. However, while greater than 80% of ALL cases diagnosed by flow or morphology were positive for the cytochemical ALL marker PAS, there was little concordance with the myeloid marker SDB, with 57% of flow diagnosed ALL cases positive for SDB and 47% of morphology defined ALL cases positive. These results, which are consistent with many other international studies, underscore the diagnostic dilemmas inherent in leukemia diagnosis, and reinforce the growing consensus that leukemia diagnosis requires a multidimensional approach.

Funding: Ministry of Health

2. Leukemia Characterization by Flowcytometry, Cytogenetic and Molecular Markers at TikurAnbessa Specialized Hospital, Addis Ababa, Ethiopia

Jemal Alemu, PhD Student

The leukemias are a heterogeneous group of malignancies due to transformation ofhaemopoietic cells. Although traditionally diagnosed by morphological criteria of blood and bone marrow smears, more recently increasing numbers of approaches have been utilized for diagnosis, including flow cytometry and methods to characterize the abundant genetic aberrancies. Whereas previous studies at AHRI and TikurAnbessa have begun to evaluate the usefulness of flow cytometry, this study, in addition to flow phenotyping, will begin the genetic analysis of acute leukemias. A comprehensive characterization of genetic aberrancies for each leukemia sample is beyond our current capacity; however, there are many common genetic aberrancies that are useful for diagnostic purposes and in fact compatible with current WHO guidelines for diagnostic criteria. Common translocations include the t(1:19)/E2A-PBX1 and t(12:21)/TEL-AML-1 associated with Pre-B cell leukemias; t(9;22) BCR-ABL1 associated with common B cell leukemias; t((4;11)/MLAF4 translocation common with Pre-Pre B cell leukemia; and t(10;24/HOX11) associated with T cell Acute Lymphocyte Leukemia. Among AML, common aberrancies include the t(8,21) translocation leading to the RUNX1-RUNX1T1 fusion transcript, the t(15,17) translocation leading to the PML-RARalpha transcript, and the inv(16,16) inversion resulting in the CBFbeta-MYH11 transcript. This study will characterize over 100 acute leukemia samples by standard morphology, by flow cytometry using a large panel of lineage specific markers, and using RT-PCR and Fluorescence In Situ Hybridization (FISH) for genetic analysis. For these purposes, we will take advantage of AHRI's newly purchased Olympus fluorescence microscope, which has capacity to analyze multiple immunofluorescent parameters. This will be undertaken in collaboration with TikurAnbessa hospital in Addis Ababa, as well as the pathology department from the University of Michigan, USA, who will provide training and guidance for FISH technology. (Funded by Ministry of Health)

3. BCR-ABL kinase domain mutations to Tyrosine Kinase Inhibitor Drugs in Ethiopian CML Patients Attending Tertiary Hospitals

Samuel Kinde[1,2], Markos Abebe[1], Kah Teong Soh[3], Daniel Seifu[2], Amha Gebremedhin[2], Paul K. Wallace[3], and Rawleigh Howe[1]

[1]Armauer Hansen Research Institute; [2]School of Medicine, college of Health Sciences, Addis Ababa University; [3]Norris Cotton Cancer Center, Dept. of Microbiology and Immunology, Dartmouth Medical School, Lebanon

Chronic Myelogenous Leukemia (CML) accounts for a significant fraction of all leukemias. Although it is typically diagnosed based on clinical criteria and abnormal blood and bone marrow smears, from a cytogenetics perspective it is very commonly associated with the presence of the so-called "Philadelphia Chromosome", thet (9;22) translocation resultingin the fusion of the ABL1 and BCR genes, both of which are protein kinases involved incellular regulation, and in CML are constitutively up regulated leading to uncontrolled cell growth. CMLtreatment was revolutionized by the development of a class of ABL1 kinase inhibitors, but resistance develops in about 10% of treated cases. Mutational analysis by

direct sequencing of the ABL1 gene is recommended for patients failing therapy; many of the common mutations are associated with different sensitivity and resistance patterns to current drugs so that such analysis guides alternate therapy. The goals of the CML project will be to apply protocols toquantitate the levels of the ABLBCR fusion products in a large cohort of patients receiving therapy at TikurAnbessa hospital by quantitative PCR, Fluorescence In Situ Hybridization, or a new branched RNA flow cytometry assay. Leukemic cells from patients with high levels of ABL1BCR fusion product--evidence of failed therapy--will undergo sequencing in order to further characterize the fusion products and accumulated mutationsin detail. This study will be performed in collaboration with the University of Leipzig.
Funding: Ministry of Health

4. Pharmacodynamic and pharmacodynamics study of TyrosineKinase Inhibitors (TKIs) with emphasis on Imatinib Mesylate in the treatment of patients with Chronic Myelogenous Leukemia

Yohannes Jorge[1,2], Eyasu Makonnen2, Amha Gebremedhin[2], Rawleigh Howe[1], Abraham Aseffa[1], Taye Tolera[1]

[1]Armauer Hansen Research Institute; [2]School of Medicine, college of Health Sciences, Addis Ababa University

This study also characterizes CML patients on therapy currently receiving imatinib, one of the tyrosine kinase inhibitors, but rather focuses on pharmacodynamic and pharmacokinetic properties of the administered drug. This includes measurement of the actual blood levels of imatinib attained within individual patients, and identification of factors which may contribute to such levels. Factors include patient compliance, concomitantuse of other medications, particularly those metabolized, like imatinib, by the **CYP3A4 and CYP**5 cytochrome P450 molecules, **CYP3A4**/5 polymorphism, as well as renal and hepatic function. In turn, such pharmacokinetic properties will be related to the patient's biological response to therapy, including recorded drug related toxicities and reductions in the imatinib target molecule, the BCR-ABL1 fusion product. This study thus complements the preceding one, and will aid future management of patients being treated with this and other related drugs. (Funded by AHRI core)

5. Diagnostic utility of FlowCytometry immunophenotyping for the detection of Hematolymphoid neoplasms from Peripheral Blood, Bone Marrow and disaggregated Lymph Node biopsies at TikurAnbessa Specialized Hospital, Addis Ababa - Ethiopia.

Mebratu Teshome, MSc student

Non-Hodgkins Lymphoma (NHL) is as common in Ethiopia as leukemia, and current diagnostic modalities in the country are limited largely to standard histological approaches. In that NHL is dominated by transformation of B cells and a lesser extent T and NK lineage cells, flow cytometry can play an important role in diagnosis. However, ready access of single cell suspensions of cells for analysis is not as straightforward as in leukemia. Although many NHL types frequently leukemize resulting in malignant cells identifiable in either peripheral blood or bone marrow cell preparations, other NHL are largely restricted to lymph node tissue. Increasingly, however, flow cytometry is being performed on

disaggregated cell preparations in lymphoma cases. Hence, the goal of the current study is to evaluate a series of cases of NHL using peripheral blood, bone marrow and disaggregated lymph node cells for preparative purposes. Markers that are particularly useful for NHL include CD5, CD23, FMC7, CD10, CD43, CD38, and others to distinguish between various B cell malignancies, including CLL, and follicular, mantle, marginal zone, and Burkitts lymphoma. This project has been initiated and to date we have analyzed 5 lymphoma cases. (Funded by Ministry of Health)

6. Lymphoma Phenotyping by Flow Cytometry and Fluorescence In-Situ Hybridization

Fitsum Daniel, PhD student

This project will be a more comprehensive evaluation of lymphoma, employing more extensive flow cytometry panels to gain greater precision in diagnosing subtypes, as well as genetic analyses. Characterization of chromosomal translocations is part of the current WHO classification system of lymphoma. Some examples of translocations with diagnostic value include: 1) the t(14;18) translocation, present in the majority of patients with follicular B-cell lymphoma, which results in overexpression of the anti-apoptotic BCL2 gene (on chromosome 18) to the constitutively active immunoglobulin (Ig) heavy chain gene (on chromosome 14); 2) the t(11,14) translocation, common in mantle cell lymphoma, resulting in juxtaposition of cell cycle regulator cyclin D1 (chromosome 11) to the heavy chain Ig locus; 3) t(2,8), t(8,14) or t(8,22) which leads to overexpression of the cMyc gene characteristic of Burkitt Lymphoma, and resulting from translocation of the cMyc gene on chromosome 8 to either the Ig lambda light chain on chromosome 22, the Ig kappa light chain on chromosome 2, or the Ig heavy chain locus; 4) the t(11,18) translocation common to some marginal zone lymphomas.
The objectives of this study will be 1) To characterize disaggregated lymph node and bone marrow biopsies from lymphoma patients with a panel of selected antibodies by flow cytometry; 2) To characterize such cells for common translocations using FISH approaches; 3) To compare the characteristics of lymphoma from HIV negative and HIV positive patients. Cells for analysis will be obtained from peripheral blood, bone marrow and disaggregated lymph node samples from greater than 100 NHL patients.
Funding: Ministry of Health

7. Immunologic, Virologic and Genetic correlates of Hodgkin's lymphoma in Ethiopia

Makka Adam Ali[1,2,3], Beyene Petros[2], Raweligh Howe[1], Mats Jerkeman[3], Amaha Gebremedhin[4], Yonas Bekuretsion[5]

[1]Armauer Hansen Research Institute; [2]Department of MCMB, Addis Ababa University, Ethiopia; Ethiopia; [3]Department of Oncology, Lund University, Sweden; [4]Department of Internal Medicine, Addis Ababa University, Ethiopia; [5]Department of Pathology, Addis Ababa University, Ethiopia

Hodgkin's lymphoma (HL), though less common that NHL is a distinct type of hematological malignancy featuring rare tumor cells of B cell origin, termed Reid Steinberg cells which comprise < 1 % of cells present in the affected lymph node. These cells occur in a background of numerous infiltrating reactive non-malignant cell populations of the immune system including B and T lymphocytes, plasma cells,

macrophages and histocytes, fibroblasts, mast cells and eosinophilic granulocytes. Multiple subtypes of HL have been histologically identified, based largely on the composition of reactive cells, but newerimmunohistochemical approaches aid specific diagnoses. Reid-Steinberg cells undergo extensive genetic variation, but do express characteristic molecules such as CD15, CD30, and CD138, and these together with an extensive array of other markers including CD40, CD20 and BCL-6 among reactive cells has improved diagnostic precision. Interestingly, the Reid-Steinberg cells commonly exhibitaberrancies associated with chromosome 9, frequently leading to significantly enhanced surface expression of molecules such as PD-1, a known negative modulator of cytotoxic T cells, and this may contribute to immune escape mechanisms. HL is strongly but not exclusively associated with EBV infection, most commonly identified immunohistochemically with LMP-1 expression, and this virus is well known to contribute to B cell transformation. Finally, the incidence of HL is 5-15 fold higher in HIV infected patients, not a surprising association given the immunocompromised state of such patients. (Funded by BSPP SIDA)

The cells within Hodgkins Lymphoma have been poorly characterized in Ethiopia.The goals of the current study will be to comprehensively evaluate HL from excised and fixed lymph nodes using primarily immunohistochemicaland RT-PCR approaches.The study will establish the distribution of HL subtypes in Ethiopia, the degree of association with EBV and HIV infections, and in addition characterize aberrancies in chromosome 9 which may lead to over expression of PD-1. This will help to establish more precise diagnostic modalities for this distinct lymphoma in Ethiopia. The study will be performed in collaboration with Mats Jerkeman of Lund University in Sweden.

II. Current research in Breast Cancer

1. Molecular characterization and assessment of viral tumorigenesis in breast cancer among women in Addis Ababa, Ethiopia

Endale Hadgu, PhD student

Breast cancer has not been well researched, but available evidence from the Addis Ababa City Cancer Registry indicates that it is the leading cause of cancer in Ethiopia. Like all cancers, breast cancer is heterogenous, and increasingly better definition of the various types of breast cancer helps to guide optimal therapy. While therapy has traditionally been based on cytotoxic chemotherapy, surgery, and radiation therapy, more recently, use of hormone receptor antagonists on appropriately expressing breast cancer subtypes has improved outcomes.Patients diagnosed at early or late stages and receiving hormone receptor antagonist therapy in combination with traditional modalities exhibit bettersurvival,often with improved conservation of breast tissue.Receptors of interest include those for estrogen (ER) and progesterone (PR), as well as the HER-2 (Human epidermal growth factor receptor-2). Newer molecular based classification thus divides breast cancer into 4 major types: Luminal A, expressing ER/PR without HER-2; Luminal B, ER/PR positive with either HER-2 expression or high proliferative markers; HER-2

enriched, expressing HER-2 but negative for other hormone receptors; and Triple Negative, expressing neither ER/PR or HER-2. The goal of the current study was to evaluate breast cancer tissue samples from 114 Ethiopian women according to multiple diagnostic and prognostic criteria. Traditional criteria included grade (related to markers of active proliferation), stage (extent of localization vs metastasis), histology (ie malignant cells of ductal vs lobular tissue origin in the breast). In addition to the aforementioned hormone receptors, other useful biomarkers such as Ki-67, an additional index of proliferation, and BRCA-1, a known tumor suppressor, mutations of which predispose to breast and other gynecological malignancies, were also characterized by immunohistochemistry approaches.

The results showed that the majority of breast cancer in this cohort fell into one of the hormone expressing categories, either Luminal A (40%), Luminal B (26%), or HER-2 (10%). Only 24% of the malignancies were triple negatives and without potential for improved therapy with hormone antagonists. This study is the first in Ethiopia to classify breast cancer by current international criteria, and further suggest that implementation of such improved immunohistochemical diagnostic procedures could contribute to improved outcomes for patients with breast cancer in Ethiopia.

Funding: BSPP (Sida)

2. Pharmacokinetic and Pharmacogenetic Studies of Cyclophosphamide among Patients with Breast Cancer in Ethiopia

Jemal Hussien[1,2], Eyasu Makonnen[2], Eleni Aklillu[3], Mathewos Assefa[2], Getnet Yimer[2], Daniel Seifu[2], Rawleigh Howe[1] , and Abraham Aseffa[1]

[1]Armauer Hansen Research Institute; [2]School of Medicine, College of Health Sciences, Addis Ababa Universit; [3]Division of Clinical Pharmacology, Department of Laboratory Medicine, Karolinska Institute, Sweden

Along with surgery and hormone therapy, cyclophosphamide based chemotherapy is a key component of treatment for many types and stages of breast cancer, particularly those which are not localized. Chemotherapeutic efficacy as well as toxicity is critically dependent on levels achieved within the body, and this in turn can be influenced by a number of factors, including hepatic metabolism, this in turn governed by the polymorphic cytochrome p450 system which is involved in breakdown to cyclophosphamide metabolites. The current study objectives are to recruit 350 breast cancer patients being treated with cyclophosphamide, and perform pharmacokinetic, to measure the serum levels of cyclophosphamide and its metabolites over time, and pharmacogenetic studies to evaluate the impact of metabolism related cytochrome P450 polymorphism on those levels. Additional variables, including age, body size, hematologic parameters, renal and hepatic function, and pre- vs post-menopausal status will also be assessed. Finally, the impact of cytotoxic therapy and associated drug levels on lipid peroxidation and anti-oxidant status will be determined. Sample collection has largely been achieved, and is currently being analyzed at both AAU and Karolinska institute.

Funding: BSPP (Sida)

3. Circulating Breast Cancer-associated MicroRNA Expression in Serum as Biomarker for Breast Cancer Detection

Tariku Sime[1,2], Markos Abebe[1], Fregent Tesfaye[1], Yonas Mulugeta[2], Dawit Hailu[1], Rawleigh Howe[1], Daniel Seifu[2],[1] *Armauer Hansen Research Institute;* [2] *School of Medicine, College of Health Sciences, Addis Ababa University;*

Considerable attention is being given to minimally invasive blood biomarker approaches for diagnostic and prognostic value. Such approaches may be particularly valuable either for early diagnosis of many cancers such as breast cancer at a stage when treatment is potentially curable, or to guide more appropriate therapy specific for a given subtype. As a first step, we have initiated a study evaluating expression of plasma miRNA among breast cancer patients compared with healthy controls. We have selected several miRNA which are likely to either be up or down regulated. These include: miRNA-16, often overexpressed in multiple types of cancers including breast cancer; miRNA-21, which inhibit phosphatases involved in down regulation of cell signaling pathways and one of the most frequently upregulated miRNAs in solid tumors; miRNA-145, hypothesized to be a tumor suppressor and known to be downregulated in breast cancer, colon cancer and acute myeloid leukemia; miRNA-195, known to target the cell signaling raf-1 gene and promote tumor cell apoptosis and decreasing breast cancer viability; and miRNA222, specific for the estrogen receptor alpha and known to inhibit expression of estrogen receptor alpha. 55 breast cancer cases and 55 age matched healthy women will be evaluated, and the magnitude of miRNA expression associated with breast cancer in general, or according to histopathological diagnosis, and clinical grade or stage. (Funded by AHRI core)

III. Future topics in non-communicable diseases

Although no additional projects have yet been submitted or approved, a number of projects in multiple areas are presently under development.

1. Leukemia and lymphoma diagnosis

At present two additional projects are being developed in collaboration with our hematology and pathology collaborators at TikurAnbessa. The first project involves use of flow cytometry to assess minimal residual disease in pediatric acute leukemia patients. This analysis aids clinicians in their assessment of the patient's response to a given chemotherapy or if such patients are beginning to experience relapse after prior remission. This approach is more advanced and demanding from a flow cytometric perspective because the leukemia burden may often be low and at this level will necessitate more marker analysis to clearly distinguish leukemia cells from normal progenitor cells in blood and bone marrow. However, flow approaches have been well established in multiple international laboratories for several years for detection of minimal residual disease particularly for pediatric B-cell ALL, and this patient group will be the focus in the planned study. The second project is a 1-2 year project aimed to transition full responsibility of basic flow cytometric phenotyping to the laboratory at TikurAnbessa. This will involve additional training of lab and pathology personnel, and will build on the experience we have already attained in our studies to date. Research questions will focus on quality control of laboratory procedure,

interpretive skill of pathologists as well as comprehensive assessment of patient outcomes. All flow cytometry performed will be independently assessed at AHRI to judge quality of performance. It is anticipated that after a 1-2-yearperiod, basic diagnostic capacity will be present at TikurAnbessa, and AHRI's role will be primarily oriented towards more advanced diagnostic evaluation such as for the aforementioned minimal residual disease.

2. Studies on Cervical Cancer

Cervical cancer is the second leading cause of cancer in Ethiopia, and presently there are no adequate screening methods in place for the country, despite the long-standing practice of PAP smear tests in developed countries. In part, the problem is a shortage of adequately trained pathologists in the country. Recently, a protocol evaluated in India involved a rapid cervical acetic acid stain which can easily be read by a health care worker with minimal training. Positive patients were subsequentlyreferred for colposcopy biopsy for definitive pathological diagnosis. Such an approach has been tried without success in Ethiopia. We are considering a trial which uses a two-step approach to screening; the first step consisting of acetic acid as in India, and if positive, a second screen using a Pap smear. Such a screening method is likely to be more feasible in the Ethiopian setting, and will place less demand on diagnostic pathologists than simply a Pap smear alone.

A vaccine is now available which is specific for some of the strains of human papilloma virus which cause cervical cancer. However, not all strains which cause cancer are covered by the vaccine, and there is only minimal information on the circulating strains in Ethiopia. In collaboration with the University of Lund in Sweden, we are considering a study to evaluate strain diversity within the country. This approach utilizes a self-swabbing technique which has been developed in Sweden and is considered more user-friendly ensuring privacy, and hence likely to lead to more random and representative recruitment of women into any given study. The swabs would then be evaluated by PCR methodology in Sweden for genotyping.

3. Studies on autoimmune dermatology disorders, diabetes and asthma

We have focused on three additional topic areas for study at AHRI, and for these topic areas have engaged in multiple discussions with leading Ethiopian clinicians in each specialty for their input on priorities. We are considering research studies into autoimmune skin disorders, not because these are particularly prevalent, but rather because of our proximity with the ALERT hospital which is a major referral center for skin disorders. Such diseases can be quite debilitating and AHRI has the facilities to provide more comprehensive immunological characterization, particularly with immunohistochemistry approaches useful for diagnosis.

Secondly, we have considered diabetes because the prevalence has increased markedly over the past 20 years and now approaches that of western countries. Because of its many chronic cardiovascular, renal, neurological, eye and infectious complications, is likely to become a major, if not the major cause of mortality in the country in the future. Future research topics under consideration include: 1) evaluation of the incidence of diabetes among patients presenting with acute coronary syndrome or cerebral

vascular accidents in local hospitals, 2) assessment of short term outcomes of women diagnosed with gestational diabetes, 3) characterization of malnutrition related diabetes, a disorder virtually unheard of in the western world and poorly understood, 4) Initiation of a program of comprehensive education for both health care providers and patients, and evaluation of such a program before and after initiation, 5) establishment of a large prospective cohort of diabetic patients to evaluate the incidence of chronic outcomes outlined above over a 5-10 year period, 6) characterization of maturity onset type I diabetes in Ethiopia.

Thirdly, we have considered asthma because, like diabetes, it is highly prevalent in the country, affects children and adults alike, and with inadequate attention over time can lead to permanent irreversible respiratory compromise. Like patients with diabetes, patients with asthma potentially have access to medications, yet long term outcomes are primarily influenced by health care and patient education, practices and adherence to guidelines.

In sum, these newer topic areas represent a new approach for AHRI, where research will depend less on relatively advanced laboratory techniques, and is less dependent on understanding pathophysiological mechanisms, but more on operations research. The advantage of such research is that it typically has high impact on patient care and is considerably less expensive to operate. It will necessitate close cooperation with clinicians.

4. HLA related activities

Chronic renal disease has multiple etiologies; most commonly it is the consequence of long standing hypertension and/or diabetes mellitus conditions which are dramatically increasing in Ethiopia. Consequently, it is likely that chronic renal disease as well will continue to increase in prevalence for many years. End stage renal disease requires either periodic dialysis, an expensive procedure not without its own additional risks, or kidney transplantation. Considering the increasing burden in Ethiopia, the Government has initiated a kidney transplantation program at St. Paul Hospital in close collaboration with University of Michigan (UM). With the latter's assistance, in the past 3 years, the program has been able to perform dozens of successful kidney transplantations.

HLA typing, flow cross-match and testing recipient's serum for anti-donor antibodies are key lab tests in the transplantation process to choose the optimal donor. Currently, these tests are being performed abroad as there are no local labs with all these capacities. Our goal is to establish a standard HLA lab at AHRI to support the transplantation program by providing service, building local capacity and supporting HLA related research. As part of this program, a staff member from AHRI (Kidist Bobosha) was trained in HLA typing and anti-HLA antibody detection techniques for 3 months at University of Michigan. Additional members of AHRI have also received training abroad recently.

HLA is part of the host immune defence mechanism that mediates antigen presentation to effector cells of the immune system, and is critical for adaptive immune responses of both cellular and humoral immunity. The HLA system is a challenge in the context of renal transplantation because T and B cells of

the recipient may recognize donor HLA molecules present on grafted kidneys as foreign antigens. Acute rejection is the most serious complication of solid organ transplantation, and this occurs if the recipient has pre-existing anti-HLA antibodies with specificity towards the donor. Pre-existing antibodies typically develop over several years prior to transplant, and are typically generated by either of two mechanisms. Women with multiple pregnancies are repeatedly sensitized to paternally derived HLA molecules of the fetus. Though such antibodies cause no harm during pregnancy, they persist for years, and even after declining to low levels can be rapidly produced at high levels upon re-exposure to relevant HLA antigens. Alternatively, anti-HLA responses can develop if the recipient has received multiple blood transfusions, which is often the case with end-stage renal disease. With each transfusion, HLA molecules expressed on transfused blood red cells, platelets and/or leukocytes potentially elicit an antibody response. HLA molecules are highly polymorphic which in essence means that it is extremely unlikely that any two given individuals will share common alleles, ie be HLA compatible or syngeneic. More likely, at least one or more of the HLA allelic molecules from one individual will differ from that of another, or be allogeneic. Since an individual is tolerant to his own HLA allelic products, when exposed to allogeneic cells will react only to those HLA molecules which differ from his own.

In the context of organ transplantation, there are two practical concepts to be emphasized from this. First, if the potential recipient has no anti-HLA antibodies at all, ie has never been sensitized—an example being a man or a nulliparous women who had never received a blood transfusion-- then s/he could receive any donor kidney regardless of HLA type, and not risk an acute rejection event. Although s/he will continue to receive immunosuppression indefinitely post transplantation, the likelihood of eventually developing a B or T cell response to the donor kidney does increase and s/he could reject the kidney after many years (chronic rejection). That risk could have been somewhat reduced if s/he had instead received a syngeneic HLA kidney, ie one completely HLA compatible, though even in that scenario s/he might eventually develop responses to non-HLA donor antigens leading to rejection (assuming the donor was not an identical twin). Practically speaking there is only slightly longer average graft survival in recipients of syngenic kidney grafts in comparison with fully allogeneic grafts, but this requires that the host have no pre-existing antibodies—at least for the donor-- at the time of transplantation. Again, the acute rejection due to pre-existing antibodies is the first and main obstacle to be avoided in renal transplantation.

On the other hand, this is the second key practical point if the potential kidney graft recipient does have pre-existing anti-HLA antibodies, the task becomes much more complicated because one now has to find a donor kidney which does not express HLA allelic products which those antibodies can recognize. This can be especially difficult if the potential recipient has received multiple blood transfusions (typical of one in need of a new kidney) or be a multiparous women, in particular one bearing children from more than a single father. In these scenarios, the recipient would have circulating antibodies to diverse HLA allelic products and the search to find a donor not reactive with those antibodies could be a long one taking months to many years even in countries with highly sophisticated organ registries.

There are two key tests which are used to identify potential donors. The first is HLA-typing, which is

a method to rigorously define all the HLA alleles expressed by an individual. This must be tested for both paternally derived and maternally derived alleles for each of the 6 major MHC gene products (3 class I and 3 class II), summing to a total of 12 allelic products. However, each gene is highly polymorphic with dozens or even hundreds of alleles present at the population level. Defining the exact HLA genotype, thus requires an extensive screen of many alleles which can be quite costly. In western countries, transplantation registries have lists of thousands of potential donors as well as potential recipients, all HLA genotyped so that potential matches can be found when needed. As alluded to above, often recipients must wait years before a potential donor is identified. The second key test is the so-called cross-match, and this is the test typically performed when a presumptive donor-recipient match has been found. The recipient's serum is mixed with the donor's blood cells. If no reactive antibodies are present, the match is confirmed. If the cross-match is positive, and this is typically measured either with a flow cytometry based or a complement lysis based approach, then the match is denied, and the search for a donor continues.

AHRI is in the process of developing an HLA-typing facility, and by this we mean a lab having the capacity to do all the aforementioned tests: the detection of anti-HLA antibodies with defined allelic specificity, HLA genotyping, as well as the flow cytometry based cross match. Because this technology takes some time to master and incorporate in this setting, multiple members have received overseas training. In addition, we have opted in the beginning for a semiconservative approach whereby, in addition to evaluating potential new donor-recipient matches, we develop a research program to address relevant questions, as we increase our capacity.

Training on HLA fusion software and Luminex application, Milan, Italy

Training on anti-HLA antibody detection assays, AHRI

A key principal which emerges from the aforementioned arguments is that the problem of organ transplantation in a developing country like Ethiopia becomes enormously simplified if one can find potential recipients with no or minimal anti-HLA antibodies at baseline-- it is then only a matter of finding a willing donor, and HLA genotypic compatibility is less relevant. Simultaneously, emphasis should be placed on implementing efforts to reduce sensitization—where possible— among other potential recipients. In fact, it is known that the primary sensitizing cells within blood are platelets, and

to a lesser extent leukocytes, whereas red cells have very little activity, except, unfortunately, if they bear the same HLA allelic products to which multiparous women have been exposed. It is possible to purify red blood cells prior to transfusions (depleting both leukocytes and platelets), but unfortunately this is not yet widely practiced in Ethiopia, though it should be a high priority. In any case, it follows that a research priority in the country should be to evaluate and monitor the degree of ongoing transfusion and pregnancy related sensitization.

In addition to transfusion and pregnancy, a third means of sensitization we have already alluded to, and that is the sensitization which may occur in post transplantation even as a patient is under immunosuppression, which occurs to allogeneic HLA (or non-HLA) molecules on the donor kidney. Many kidney recipients eventually reject their initial graft after many years and are in need of a second transplant. The etiologies underlying graft failure are many fold, not all are immune mediated; however, in many cases it results from eventual sensitization and development of donor specific antibodies. Evaluation of these three sensitization phenomena underly our initial research project being conducted by Tilahun Alelign as his PhD project entitled "Human Leukocyte Antigen Genotyping and Antibody Characterization among Chronic Kidney".

Human Leukocyte Antigen Genotyping and Antibody Characterization among Chronic Kidney Disease Patients in Ethiopia

Tilahun Alelign[1,2,5], Kidist Bobosha[1], Momina Ahmed[3], Yewondwosen Tadesse[4], Beyene Petros[2] Rawleigh Howe[1]

[1]Armauer Hansen Research Institute; [2]College of Natural Sciences, Department of Microbial, Cellular and Molecular Biology, Addis Ababa Universit; [3]Saint Paul Millennium Medical College; [4]School of Medicine, College of Health Sciences, Addis Ababa University; [5]Department of Biology, Debre Birhan University

In this PhD study, 100 study participants with renal failure, identified as potential candidates for transplantation and at risk for anti-HLA antibodies due to multiple transfusion or high multiparity. These subjects will be rigorously characterized for anti-HLA antibodies, and HLA genotyped (along with any potential identified donors). Levels and specificity of antibodies will be analyzed according to sensitization risk to clarify the relative impact of each. In a second cohort we will evaluate 25 recipients who are at least 5 years post transplantation. Anti-HLA antibodies and HLA genotypes will be determined among these patients and genotypes and cross matches defined with blood from their original kidney donors. Hence, development of antibodies of defined specificity can be matched with expression of appropriate allelic products of the donor. Such evaluation will contribute to the understanding of the post-transplant sensitization process, and provide some insight to such patients as to their potential risk for future graft rejection. To date, we have analysed anti-HLA antibodies of 22 subjects from the first cohort using the Luminex bead platform which allows rapid detection of antibodies with specificity for any one of more than 100 HLA allelic products. Many subjects exhibited high levels to some HLA allelic products. The HLA genotyping platform has not yet begun, but this is planned for early next year.

Funding: AHRI core budget

Bioinformatics and Biotechnology Research

The year 2017 was a time to emerge as a Directorate following incubation period under the Scientific Director for Research and Innovation office. Therefore, much of the activities during the past 6 months, as of the assigning of acting Director for the directorate in May 2017, have been to prepare the directorate's annual plan, establish a team, recruitment of staff, re-organizing laboratories including the sequencing facility, procurement of lab items and conducting training.

Establishment of the Directorate: According to the approved organogram of the Institute, Biotechnology and Bioinformatics is one of the 6 directorates of the research and innovation arm. However, it was under the Scientific Director for Research and Innovation for the past one year to be incubated. As the time comes to be formally established, Acting Director was assigned and the office started functioning as of June 2017.

Currently the directorate has only 5 staff members, namely; YonasKassahun, Tewodros Tariku, Elena Hailu, Dawit Hailu and MarkosAbebe. Advertisement for new staff recruitment is underway at different positions i.e., senior scientist in molecular biology and bioinformatics, research assistants and lab technologists. The Directorate has two case teams; biotechnology and Bioinformatics.

Projects: In 2016/2017 there are quite a few projects that have relevance to biotechnology and bioinformatics or products or services that might have direct or indirect contribution to patient care.

Projects include: Antigen preparation for DAT (Malaria NTD), HLA (NCD), Development of lateral flow assay for the diagnosis of TB (MDRD/BVDRD), Development of monoclonal antibody (BTBID), Blood fractionation (Malaria NTD), Laboratory scale production of PPD (MDRD), Cholera vaccine (BTBID), immune-phenotyping for leukemia diagnosis (NCD) most of which are at their initial phase.

Preparation and evaluation of in house monoclonal antibodies for clinical and research use

Markos Abebe[1], Abebe Animut[2], Martha Zewdie[1] and Rawleigh Howe[1]

[1]Armauer Hansen Research Institute; [2]Aklilu Lemma Institute of Pathobiology, Addis Ababa University

The production of monoclonal antibodies using hybridoma technology; which was invented by César Milstein and Georges Köhler dates back to 1975. Since then, monoclonal antibodies have been produced in large scale for research and clinical use.

Currently a number of monoclonal antibodies are already approved by FDA for the treatment of cancer. The first therapeutic monoclonal antibody, Orthoclone OKT3, for the prevention of kidney transplant rejection was approved in 1986. In 2014, the US Food and Drug Administration approved 47 monoclonal antibodies and by 2020 it is estimated that close to 70 monoclonal antibodies will be made available.

By 2020, the market share of different monoclonal antibodies will be expected to be close to 125 billion USD. AHRI, for example, purchases monoclonal antibodies every year with an amount of 60,000 USD

on average. The expansion of research in research centres and universities as well as diagnostic labs in the country require foreign procurement of a large amount of monoclonal antibodies.

There is now an interest to locally make monoclonal antibodies and therefore we want to determine the feasibility of creating a platform for local generation of mAbs useful for clinical diagnosis and national research. We propose that this feasible, and hence can be an attractive means of reducing costs in the country. The project is meant to transfer the technology of mAb preparation and fluorescence labelling by using local expertise and international partners. In particular, our initial focus will be to generate anti-CD4 and anti-CD38 reagents of comparable quality to commercial products. We will use three different but complimentary approaches to minimize the risk of the project i.e., generate hybridomas in house or purchase hybridomas and produce monoclonal antibodies or purchase unlabeled monoclonal antibodies and make the labelling in house.

So far, one researcher was trained in Germany, a dedicated lab is established, and reagents and consumables are ordered.
Funding: Federal Ministry of Science and Technology

Laboratory re-organization: One of the major challenges facing AHRI is shortage of laboratory and office space. This has slowed down our pace to expand in terms of developing critical mass of researchers and also arranging the laboratory as to the minimum standard. Nevertheless, through discussion with the management and directorates, it was possible to create some space for the sequencing lab and also Hybridoma lab. However, the space issue remains challenging to recruit more researchers/staff in order to implement the annual plan which was prepared with the assumption that adequate man power would be joining the directorate.

Illumina NextSeq 500 Sequencer installed at AHRI: As part of the new initiative to strengthen the biotechnology and bioinformatics capacity of AHRI, IlluminaNextSeq 500 Sequencer has been purchased and installed at AHRI. The installation was made by an engineer from Illumina, Mr Ahmed Khairy.

Installation of IlluminaNextSeq 500 Sequencer

Following the installation, test run and training was given for three days (November 6 - 8, 2017) to 7 AHRI staff members by FarazShaheed. The training includes: Basic concepts on NGS technology, sequencing test run using φX library on NextSeq 500, create sample information, data transfer protocols, routine machine washing steps, issue reporting for remote support, general safety and data quality checks.

Training on IlluminaNextSeq 500 Sequencer

The instrument is now ready and is expected to give service (whole genome, exome, RNA sequencing) not only to AHRI but also to all interested researchers/institutions in the country based on specific agreement. Dr Yonas Kassahun is in charge of the bioinformatics and sequencing facility.

Currently, the unit is in the process of acquiring accessories and reagents (which include Fragment analyzer, Server and storage, Spectrophotometer...) to avoid interruption in function.

Training: Technology transfer through training of AHRI staff and /or inviting experts to AHRI is the way forward as biotechnology and bioinformatics is a new area for the institute. This will help to build a critical mass of researchers on critical areas of expertise or skilled that is required to move towards R & D. To this effect, in 2016/2017 AHRI staff has received training on specific techniques relevant to their projects; which include:

***Reinstate Molecular biology and initiate biotechnology at AHRI*:** Molecular biology and Immunology have been corner stone in AHRI's research since its inception in 1970. However, basic molecular biology and preclinical studies including animal experiment faded away with time. With the interest of biotechnology in the country, in general, and at AHRI in particular, there is now a need to reinstate basic molecular biology capacity and health biotechnology. In order to strengthen this capacity, biotechnology and bioinformatics directorate has been recruiting senior scientist and research assistants with a skill in molecular biology techniques that would make AHRI occupy strategic position in the national health biotechnology. In order to identify AHRI's existing capacity and gaps in using molecular biology and biotechnology for the production of diagnostics and vaccines, a guest from Norway, Professor Audun HelgeNerland, Department of Clinical Science University of Bergen, was invited and visited AHRI and NVI in DebreZeit between February 28 to March 23, 2016) to evaluate the capacity of AHRI and NVI in the development of diagnostics and vaccines and submitted his recommendations to AHRI.

Clinical Trial Research

The establishment of the clinical trial Directorate (CTD) is one of the major milestones in the history of Armauer Hansen Research Institute (AHRI). Since its establishment in 2001, the clinical trial unit/ directorate conducted a number of GCP standard clinical trials on different drugs and vaccines.

I. ONGOING PROJECTS

The Evaluation of a Standardized Treatment Regimen of Anti-Tuberculosis Drugs for Patients with MDR-TB (STREAM)

Tesfamariam M.[1] Abraham A[1], MekonnenT.[1]Oumer A[1], Tsegaye H[1], EmawayishT[2], I D. Russen[3], Sarah Meridth[4] , Andrew Nunn[4]

[1] Armauer Hansen Research Institute; [2]ALERT Hospital; [3] Vital Strategies; [4] MRC-UCL

Despite the availability of an efficacious, affordable, six-month chemotherapy regimen and having an efficient strategy to deliver treatment under direct observation to the majority of TB patients, TB control worldwide is being hindered by the emergence of multidrug resistance (MDR). The existing treatment approach for MDR-TB has been based largely on expert opinion lacking good evidence on optimal management. Since May 2016, WHO has recommended a short term MDR TB regimen of 9-2 month duration based on observational cohort studies; STREAM is a multi-center, open label, non-inferiority trial undertaken in Ethiopia, South Africa, Vietnam and Mongolia. This study was started at AHRI in Feb 2013. The STREAM stage I recruitment target is already met, i.e. at least 400 participants in all four participating countries. In Ethiopia, both from ALERT and St Peter's hospitals, we achieved the recruitment target of 124 participants. All the laboratory (chemistry, hematology, sputum smear and culture), data management as well as clinical follow-up activities are proceeding smoothly.

The primary objective of the STREAM stage I trial is to assess whether the 9 month study B regimen based on that used in a study in Bangladesh is non-inferior to the control 20 month A regimen recommended by the WHO. A relapse-free cure rate of 88% was obtained in the Bangladesh study.
The practical, program-based study design will also ensure that if the results are favourable, they will be generalizable to routine program settings. In addition, health system and patient costs associated with implementation will be documented. These will be analyzed in association with the clinical outcomes of the trial using the TREAT TB Impact Assessment Frame work in order to provide as much information as possible for subsequent policy and practice decision-making.

In the stage I study, preliminary results to date show that the nine-month regimen (78.1% favourable outcomes) , has not been inferior to the control regimen (80.6% favourable outcomes). There have been very similar rates of grade 3-5 adverse events between the two arms, with 46% of participants in the nine-month regimen arm experiencing such events compared to 45% in the 20-month regimen arm. Preliminary health economic analysis indicates that the nine-month regimen reduced the cost of treatment for the health system, compared to the 20-month regimen. The cost reduction to the health

service per patient was approximately 2,900 USD in Ethiopia and 4,900 USD in South Africa. The entire followup period of STREAM stage I participants is nearly complete, with the STREAM stage 1 final report expected after the first quarter of 2018.

The second stage of the STREAM trial has three objectives: 1) To assess whether Regimen C (a 9 month MDR TB regimen with the new drug bedaquiline) is either superior or 2) non-inferior to Regimen B (the 9 month Bangladesh regimen), and 3) to determine whether a 6 month bedaquiline based regimen (D) is non-inferior to the Bangladesh regimen. All three objectives entail outcome assessment through week 76.

The STREAM stage II was started in June, 2016. So far 31 patients have been screened and 15 patients who fulfilled inclusion criteria have been randomized in the study. Out of the 15 randomized study participants 2 are on regimen A, 4 on regimen B, 5 on regimen C and 4 on regimen D. Among these enrolled patients, 2 have completed their treatment and 13 are on anti-TB treatment. To date, there have been no subjects lost to follow-up or withdrawal of consent.

STREAM Stage 2 SIV Training, Staff from AHRI /ALERT& St Peter's Hospital

Funding: The source of fund for STREAM stage I is from USAID & MRC, whereas for STREAM stage II- it is from USAID, MRC/DFID and Janssen Research & development/LLC

Bioequivalence Clinical Studies

MekonnenTeferi[1], Anteneh B[2], Zenebe A[1], Tesfamariam M.[1], Abraham Aseffa[1], Tsige G/mariam [2]

[1]Armauer Hansen Research Institute; [2]Regional Bioequivalence centre

Several efforts have been made to establish an internationally recognized and locally accessible and affordable bioequivalence (BE) testing facility over the last few years. This includes establishment of the Regional Bioequivalence Center (RBEC) and a clinical facility at AHRI since 2012 and 2013 respectively. AHRI took the initiative to strengthen its clinical facility so as to meet the requirements in the conduct

of standard BE clinical studies. Since the two pilot BE clinical studies were successfully completed with WHO inspection, preparations continue in the clinical trial site at AHRI and the bioanalytical facility at RBEC so that the sites can conduct pivotal studies for WHO pre-qualification application. Because the BE clinical study requirements are harmonized and unified worldwide, such clinical studies should fulfill international standards so that the results can be accepted and the tested generic products marketed universally.

Following the WHO inspection for the clinical and bioanalytical sites based on the pilot studies, training on Good Clinical Practice (GCP) regulation and CRO inspection was given to key staff and mock-inspection was made to the sites by WHO inspectors. Based on the feedback obtained, the sites are addressing gaps and have amended the developed protocol for its final approval by the regulatory authority. Therefore, a pivotal study will soon be conducted for possible WHO pre-qualification application once final approval is obtained. However, delayed performance for several reasons and limited resources has remained challenges to ensure sustainability of the project both at the clinical site facility and bio analytical lab.

Funding: AHRI and Regional Bioequivalence Center

Pharmacokinetics of Benzathine Penicillin G in Ethiopian Children with Rheumatic Fever and Rheumatic Heart Disease

Ayana Jaleta[1,2] , Mekonnen Teferi[1], Getnet Yimer[2] , Tamirat Moges[2], Maxwell T. CHihwa[4,] Etsegenet Gedlu[2] , Abraham Aseffa[1] ,Workineh Shibeshi[2]

[1] Armauer Hansen Research Institute (AHRI), Ethiopia [2]Addis Ababa University(AAU), college of health science, Ethiopia, [3]Cape Town University, South Africa

Background: Rheumatic fever (RF) and its major sequelae rheumatic heart disease (RHD) are autoimmune diseases that arise following infection of the throat by *S.pyogenes* in children and young individuals (3–19 years old) who present genetic components that confer susceptibility to the disease. Despite the 4 weekly regular prophylactic administration of BPG, in our country the annual mortality rate from RHD is as high as 12.5% which may be due to several causes. Since there is no data concerning the serum level of BPG, low serum level may be one of these causes and this gap motivated this study.
Hypothesis: The serum concentration of BPG will be less than the MIC 0.02mg/ml after two weeks of BPG administration.

Objective: Study seeks to evaluate the pharmacokinetics (PK) of BPG in RHD patients and specifically to evaluate the plasma level of intramuscular administered BPG at different time intervals and developing a population pharmacokinetic (PK) model of penicillin G concentrations to RHD patients.
Method: It is an open-label observational prospective study recruiting study patients from Tikur Anbessa Specialized Teaching Hospital, Department of Pediatrics and child Health, pediatric cardiac clinic in Addis Ababa, Ethiopia. Patients who are on BPG prophylaxis for documented RF/RHD, with age range of 5-17 years old and whose parents/guardians can give written informed were included in the study. On the other hand, patients who have history of allergy to penicillin, seriously ill, who took BPG within two

weeks prior to the study period, who have comorbid diseases such as renal failure and liver disease and who or whose parents are unwilling to take part in the study were excluded. Blood sample (4ml) was collected from eligible participants at baseline (day 0), days 1, 3, 7, 15, 21 and 28 after administration of BPG IM 600, 0000, 000IU for patients with <27kg and 1.2MU for >27kg body weight for determination of plasma BPG concentration. The participants will be followed for a total of 28 days and all relevant medical events were assessed. All samples were stored at -70 °C until bio-analysis is performed. DBS samples (30µl) were also collected at the same time as plasma PK samples by directly placing (spotting) whole blood from the venipuntured into DMPK FTA type C cards. A total of 98 subjects were targeted in the study. The plasma & DBS samples will be analyzed for BPG levels using HPLC/MS/MS. Thus, bioavailability of BPG will be determined by determining maximum plasma concentration (C_{max}), the time at which C_{max} achieved (T_{max}) and AUC of BPG. In addition, population PK parameters will be estimated using plasma samples alone as well as both plasma and DBS samples (NONMEM). In parallel, throat swabs were collected on day 0 from all participants and at day 21 from those who were postive for S. pyogens culture. This was primarily to assess the effectiveness of the BPG prophylactic treatment.
Significance of the study: There are no data in our country especially regarding the PK of BPG used to prevent the recurrence of the RF which accelerates the RHD or valve damage in the patients. Therefore the investigator believes that after the end of the present study, the PK data of BPG will be available. This may be useful for the policy makers to amend the treatment guideline and for health professionals to adjust the frequency of the drug administration and also to optimize the dose after performing simulation.

Status: A total of 80 patients have been recruited of which 74 have completed the study followup thus all the required samples and data were collected from these patients. Some throat swab culture results are available therefore more than 40% of the patients were positive for *S Pyogenes* at baseline. The bioanalysis of plasma samples is yet to be conducted and the study is still on-going.

Source of Budget: AHRI Core Fund & AAU

II. Projects under development

Efficacy and Safety of 'Locally Manufactured External Fixator' versus 'Conventional External Fixator' for Treatment of Long Bone Fractures in Ethiopia: A Randomized Controlled Trial.*

Zeynu zuber[1] , Yiheyis Feleke[1] , Zenebe Akalu[2] , Abraham Aseffa[2] , Ibrahim Ahmed[3] , Solomon Goshu[3]
[1] ALERT Hospital; [2]Armauer Hansen Research Institute; [3] St. Paul Hospital Millenium Medical College

The burden of trauma patients in Addis Ababa is very high. A simplified trauma registry in Tikur Anbessa Specialized Hospital has indicated that 77% of these injuries were unintentional and motor vehicle injuries accounted for 41% of all cases leading to different forms of orthopaedic injuries.

External fixation is used widely in the management of open tibia fractures, but delayed healing is common. There is concern that this might be due not only to the severity of injury but also to the mechanical conditions imposed at the fracture site by the fixator.The purpose of this trial is to evaluate

the efficacy of a locally manufactured external fixator for the management of long bone fractures. If this study shows promising result, it may contribute to saving hard currency used to import external fixators and in the long run may pave a way to export external fixators to neighbouring countries.

The objective of this trial is to assess the safety and efficacy of the locally manufactured external fixators compared with standard imported fixators in the management of long bone fractures in adults in Addis Ababa.

This is a two arm, individually randomized open controlled trial. However, outcomes assessors, data analysts and patients, will be blinded; however, blinding surgeons in a surgical intervention trial is not possible. Study participants will consist of trauma patients age 18 and above with a single open long bone fracture with no significant abnormalities in vital signs. Sample size of the trial will be 50 patients in each arm for a total of 100 patients, assuming a power of 0.8 and withdrawal rate of 15% for a non-inferiority randomized clinical trial. The trial will be conducted in ALERT Hospital and St. Paul Millennium hospital. The trial will be coordinated by the AHRI Clinical trial Directorate, and three orthopaedists will be dedicated for this trial after full training on Good Clinical Practice.

The protocol is submitted for Ethical review.

Funding: Grand Challenges Ethiopia

*Locally initiated clinical trial

The Safety and Efficacy of Ethiopian Highland Herb in the Treatment of Psoriasis-A Randomized Controlled Clinical Trial**

Endalamaw Gadissa[1], Adamu Bayissa[1] , Tesfamariam Mebrahtu[1] , Abraham Aseffa[1] , Taye Tolera[1] , Shimelis Nigusse[2] , Getachew Gebeyehu[3] , Bethelhem Gulelat[2]

[1]Armauer Hansen Research Institute; [2] ALERT Hospital; [3] Addis Ababa University

Psoriasis is a common immune related chronic inflammatory dermatological condition. It has serious emotional, social and economic implications. The treatment of psoriasis is still based on controlling symptoms. Topical and systemic therapies as well as phototherapy are being used. The current treatment modalities are usually life-long and are aimed at remission. So far, there is no curative therapy for psoriasis.

This study is an open label randomized controlled study to evaluate the safety of *U. simensis* as a topical remedy against Psoriasis. Participants will be adults with clinically psoriasis. Currently the protocol is under ethical review.

**This is a locally initiated clinical trial.

VALUE-TB Protocol

The End TB Strategy will require considerable investment in tuberculosis (TB) services and interventions. Given that current resources for TB are highly constrained, improving the allocation of resources both to and within TB programs has the potential to substantially impact the TB epidemic. Global and national funders are increasingly demanding sound investment cases before they provide resources to TB. Moreover, as new technologies are increasingly becoming available, it is critical that TB programs have the means to justify purchasing cost-effective technologies to their core funders. The cost data available for TB diagnostics and drug-susceptibility testing are scarce. The most commonly reported costs are for sputum smear microscopy, sputum culture and chest x-ray (CXR). ; However, only limited cost data is available for new technologies such as Xpert® MTB/RIF and loop-mediated isothermal amplification (TB-LAMP) and these may vary dramatically by placement and context. Hence, economic analyses of traditional and new technologies are urgently needed to justify funding for future National Tuberculosis Programmes (NTPs) goals such as enhanced or active case finding, the treatment of latent TB and social protection.

The primary aim of the study is to enable, the Ethiopian NTP and their funders, to allocate their resources, both to and within TB, in an efficient and fair way. To achieve this aim, we have defined two intermediate objectives:

1) To estimate the unit costs of a comprehensive set of TB services in Ethiopia

2) To develop a sustainable framework (in terms of tools and processes) for TB cost data collection at the country level

The primary output from this project will be a dataset of unit costs for all TB interventions in Ethiopia. Cost data will also be made available in a disaggregated form, in order to best facilitate future analysis and decision-making at the country level and by researchers. Breakdowns of data will include 1) prices and quantities of inputs by site, 2) costs broken down by ownership (public, private), activity and level (service-level vs. above-service level), and 3)economic and financial costs by payer (i.e. The Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM) vs. NTP funded interventions/resources). The protocol is on its final stage to be submitted for ethical review.

Funding: Bill and Melinda Gates Foundation

Participation in VALUE Protocol Development, Addis Ababa

III. Other Activities of the Clinical Trial Directorate

Organized Consultative Meeting on the Regulation and Clinical Trial of Traditional Medicine

The Clinical Trial Directorate at AHRI and the Directorate of Traditional and Modern Medicine at EPHI has jointly organized a consultative meeting on the Regulation and Clinical Trial of traditional medicine which took place at the training centre hall of Ethiopian Public Health Institute on September 5, 2017. The meeting provided an excellent opportunity and venue for the exchange of ideas among interdisciplinary group of researchers, academicians and regulators on the national effort for traditional medicine research and development and reviewing the global, national and regional experience on the regulation and clinical trial of traditional medicine.

A total of 63 participants attended the consultative meeting. The participants were delegates from EPHI, AHRI, traditional healer representatives, academicians and researchers from AAU, the University of Gondar, Jimma University, Mekelle University, St. Paulo's Millennium Medical College, the Ministry of Industry, the Food beverage Pharmaceutical Industry Development Institute, the Ministry of Science and Technology, EFMHACA, regional EFMHACA, professional associations, WHO, NSPA-Pharma, and other invited expertise.

Community Engagement (CE) in Clinical Trial at AHRI

When Clinical Trial research is undertaken in a community, there is a need to incorporate the community structures. The research that is proposed must fulfill health related needs within the community. In addition, community members need to have some understanding of research itself, ethics and regulatory requirements. To achieve this in a meaningful way, shared and trusted structure of communication needs to be established via a community engagement (CE) plan. AHRI has been engaged in several clinical research activities particularly clinical trials needs community consultation, education and engagement at different stages. To this effect a staff training and experience sharing event was held with South African colleagues in STREAM. AHRI Community Advisory Board has 20 members who were selected and delegated from different community representative groups like social based organizations (EDIR), district health offices (Health extension offices), women and youth affair offices, different stakeholders (hospital, challenge TB), Legal association representatives and AHRI research and human resource representatives. The CAB members have a regular meeting every month and trainings are arranged for the members every two month.

Trainings/Conferences Attended or Participated

The staffs in the clinical trial directorate has attended and participated in a number of pertinent forums and the complete list is shown in the table below.

Table-1: Local/International Trainings/Conferences Attended or Participated

S. No	Title of the Training	Period	Venue	No. of Participants	Organized by	Remark
01	Training on The Global Health Bioethics Network session (GHBN) from at	September 24 to 30, 2017	Durban, South Africa	01	Spring School, Durban, South Africa	
02	TB research methods course	12-16June, 2017	Montreal, Canada	01	McGill Institute in ID & GH.	
03	Ethiopian Medical Association Stakeholders` Consultative Workshop.	Oct 5/2017	Addis Ababa	01	EMA	
04	Global Health Course	Jul21/2017	St Paul's Hospital	01	St Paul's Hospital	
05	Consultative Meeting on the Establishment of Consortium for Regional Bioequivalence Center.	May 11/2017	Addis Ababa	02	MoH, MoI& RBEC	
06	The Third Korean Enterprise for Clinical Trial Conference	29 Oct to 03 Nov/2017	Seoul, The Republic of Korea	01	Korean Ministry of Health and Welfare	
07	Training on GCP regulation and CRO inspection	June 2017	WHO, USPQM & EAC.,	02	Addis Ababa, Ethiopia	
08	STREAM Investigators' meeting	15-16 Oct 2017	Mexico	02	Sponsor	
09	The Union conference on TB	11-14 Oct 2017	Mexico-City	02	The Union	
10	Research Ethics training	Jul 2017	AHRI	08	The Directorate	
11	VALUE-TB Protocol Development	02-03 Aug., 2017.	Addis Ababa	02		

The 3rd International Conference-Korean National Enterprise for Clinical Trials

One-Health Unit

Summary

The One-Health group at AHRI continues expanding in terms of research studies conducted since its formal creation, grants acquired and permanent staff. Two research assistants have joined the One-Health Unit this year. Eight post-graduate students have been associated with the Group in 2017, coming from national Universities (Haremaya, Addis Ababa and Jigjiga) as well from Swiss Institutions (Swiss TPH).

The mandate of the research group includes research on integrated human-animal health services (surveillance, diagnostics, health service delivery), research on zoonosis at the human-domestic animal-wildlife interface (including food-borne zoonosis) and the relationship of environmental health and public health.

The 3 major projects where this One-Health Unit is currently involved are the Ethicobots project that looks into control strategies of BTB in the dairy sector of Ehtiopia, the JOHI project (integrated health systems in ESRS) and the Brucellosis project looking at the disease at the human-animal interface in Afar and ESRS.

The group has currently a strong emphasis on One-Health in pastoral communities and gains increased experience in working in vulnerable settings. The Jigjiga One Health Initiative (JOHI) is a 12 year long research and development program partnership between AHRI, Jigjiga University and Swiss TPH funded by the Swiss Government that aims at improving the health and well-being of pastoralists and their animals in ESRS in an integrative manner. The project is in its second year, continuing capacity building in One-Health in ESRS. Baseline operational research results in the areas of mother and child health, nutrition, zoonotic diseases, environmental health among others will help developing and piloting integrated intervention strategies in the study area for year 3. The ultimate objective of JOHI is to provide evidence based research for a future sustainable integrated health service that can efficiently reach the remote mobile communities in ESRS, which will improve the prevention for instance of zoonotic diseases.

The One-Health team of AHRI is conducting in collaboration with Swiss TPH, a sero-surveillance of Brucellosis among pastoral communities in Afar and ESRS. The project funded by CDC will help mapping the disease at the animal-human interface and provide evidence based research results to initiate a potential pilot control program in a selected pastoral area in a phase 2. The results will stream into the 5 year national Brucellosis surveillance project.

The long standing experience of the One-Health Unit at AHRI has- and will continue to contribute to the One-Health in Ethiopia. A National One Health Steering committee (NOHSC) has been formally established this year, drawn from four core-government sectors namely Ministry of Health (MoH), Ministry of Livestock and Fisheries (MoLF), Ministry of Culture and Tourism/Ethiopian Wildlife Conservation Authority (MoCT) and Ministry of Environment Forest and Climate Change (MoEFCC)

in 2016. An Ethiopian One-Health strategic plan (2018-2022) is currently being drafted.

I. Ongoing research projects

ETHICOBOTS (WP6)

WP6 of the ETHICOBOTS project (ZELS project) described in details in other sections of the AHRI report is looking into the cost and feasibilities of various control programs to tackle BTB in dairy farms in Ethiopia. It is closely working together with all other WPs. Particularly; we are conducting a 3 year longitudinal study on animal productivity in order to assess ultimately the impact of BTB on productivity (loss in milk and meat production as well as impaired fertility rates). This information provides the base of future economic modeling for the economic impact of the disease for the livestock sector as well as cost-benefit analysis of different control strategies, such as vaccination, milk pasteurization, animal segregation, and test and slaughter programs that will be performed in 2018. In order to assess other factors that can impact on animal productivity and health, we performed a study on fodder quality and feeding management of dairy animals in collaboration with the School of Agricultural, Forest and Food Sciences (HAFL) in Switzerland.

Milk quality study in dairy farms

This AHRI funded study is investigating prevalence of clinical and sub-clinical mastitis and the quality of milk produced in 26 dairy farms in and surrounding Addis. Milk from mastitis positive animals is further assessed for E. coli, Stretococcusspp, Staphylococcus spp, Salmonella spp, and Listeria spp with particular emphasis on milk-borne zoonosis.

Vaccine development for haemorrhagic septicemia in cattle

This research project is at its initial stage and aimed at developing vaccine for haemorrhagic septicemia. This work is funded by IDRC and led by Calgary University (Canada). It will be a collaborative work between the One Health unit and the bacterial and viral diseases research directorate.

Tuberculosis in non-human primates

This is an on-going collaborative research between AHRI, Born Free Foundation and EWCA on tuberculosis in primates, mainly baboon spp, vervets and patas monkeys. It is illegal to keep primates as pets and they are confiscated by the Ethiopian Wildlife Conservation Authority and quarantined before release into the wild. Autopsies are performed in captive animals that died and tissue submitted for TB analysis at AHRI. In 2017, an olive baboon died suddenly after 6 years of captivity. The baboon had had a chronic cough but was negative for TB on quantiferon testing. Autopsy revealed multiple granulomas in the lung and *M. tuberculosis* was isolated from the samples.

Brucellosis surveillance in livestock and pastoralists in Afar and Somali Region

This project (July 2017-July 2018) is a collaboration between Swiss TPH and AHRI. We will conduct a sero-surveillance of brucellosis in livestock and pastoralists in selected areas of Afar and Somali Region.

In addition we will type the circulating brucella strains. The project will define sero-prevalence in animals and people, map the disease and investigate the possibility of a specific control program tailored to these communities (eg. Animal vaccination). This work will contribute to the national brucellosis surveillance program.

Funding: CDC in collaboration with MoH and MoFL

Jigjiga One Health Initiative (JOHI)

The Jigjiga One Health Initiative (JOHI) is a 10 year project (2015-2025) funded by the Swiss Government aiming at improving human and animal health in pastoral communities of ESRS. AHRI is equal partner of JOHI besides Jigjiga University and Swiss TPH and coordinates overall the project in Ethiopia through this One-Health Unit. AHRI will also take the important role of translating research results into policy advocacy, help train and support research particularly in the TB areas of research and training. The project is based on three pillars: 1) capacity building at academic and community level as well as improvement of lab and diagnostic capacity in ESRS, 2) operational research in animal and human health as well as environment, and 3) development of integrated animal-human health surveillance and delivery services.

So far, 2 MSc candidates successfully defended their thesis in Basel, Switzerland and are serving currently JJU as Deputy Dean of School of Graduate and Head of the Midwifery Department, respectively. Further, 3 MSC and 2 PhD from JJU as well as 2 PhD from Haremaya University are currently conducting their field work. Research topics are the following: mother and child health (nutritional status; endoparasite burden; prevalence of skilled delivery; gaps in health delivery systems); zoonosis at the livestock-human interface with emphasis on brucellosis, rift valley fever, Q fever and Corona-Mers); mental health (post natal depression in pastoral women), TB in pastoralists; water quality and diarrhea in children; integrated syndromic surveillance system and research in soil quality and associated livestock herd management. The information collected during the first phase of operational research will allow designing and piloting intervention strategies in the frame of integrated health surveillance and health delivery systems. Current research topics for 2017 are described briefly in table 1.

All the research components are conducted in a One-Health manner, with an integrative team of researchers from different academic background conducted in the same households and/or villages in AdadleWoreda.

Furthermore a One-Health curriculum has been developed with our partners and ratified by the MoE to be delivered this coming academic year at JJU as a MSc program. This is a two year, full-time, specialization program with intensive course work supplemented with relevant MOOCs.

HORN project (One Health Research Network for the Horn of Africa)

AHRI collaborates in the new HORN project (UK funded) lead by Liverpool University. The project will aim mainly at local capacity building and training in the Horn of Africa to undertake high quality One Health research. It aims also at developing national and regional networks on One Health.

Other activities in ONE Health

Contribution to the "Roadmoap for Zoonotic tuberculosis". WHO 2017

Contribution to the One-Health National platform

Contribution to the Ethiopian One-Health National Strategic plan (2018-2022)

3rd Nov 2017. Celebration of One Health Day hosted by EPHI, Addis Ababa.

8 Nov 2017. Conference on Health, Science and Higher education. AHRI

GizachewGemechu took the MOOC One-Health course offered by Swiss TPH

Table 1. Brief summary of research topics conducted in 2017

Research topic	Number of samples	Main results
Nutritional status in pregnant pastoral women (Feruza Aliya, MSc)	450 blood samples and household interviews.	Lab diagnostic pending; analysis of interview data
Drinking water quality and its correlation to diarrhea in children under 5 years (Abdifatah Muhumed, MSc)	465 water samples were analyzed with ChromAger; 450 household interviews	Study is on-going. So far, over 50% of the water samples for drinking contained E.coli and/or coliforms. High prevalence of diarrhea in children. They had no access to medication during the diarrhea episodes.
Rangeland management/Soil quality and livestock (Seid Mohammed, PhD)		Physical and chemical analysis of the quality of soil in Adadle has been undertaken in Basel. Traditional soil categorization of Adadle pastoralists was used in sampling process. This data will be combined with indigenous land unit as pastoralists in the study area traditionally recognize and used for exploring the connection with forage quality.
TB in pastoralists (Faisal Nooh, MSc; Fentabil Getnet PhD)	Data collection not started. Training of research staff + setting up of logistics	
Post natal depression (YonathanTegene, PhD)	Data collection not started. Training of research staff + logistics	
Integrated syndromic surveillance (Yahya Osman, MSc)	9 kebele leaders included in the surveillance since August 2017 (set up of the surveillance network; then start piloting the system)	504 illness cases in humans and 387 illness cases in animals reported so far. In people 41% of the cases were gastro-intestinal tract related problems and 35% respiratory problems. In addition cholera cases reported in humans, and epidemics in livestock (e.g. pox in goats; abortion storms in goats). Rapid reporting at community level allowed fresh samples to be taken and sent to the lab.
Zoonotic diseases (Mohammed Ibrahim, PhD)	920 blood samples collected in livestock and humans and 100 Household interviews in 6 kebeles in Adadle woreda	Overall seroprevalence of Q fever was 41%, RVF was 14.8%, MERS was 62.2%, RVF and Q fever was highest in camels and humans (up to 60%) 90% of households reported abortions in the last 6 month. However, sero-prevalence of brucellosis was very low (<1%). Main 2 diseases described by pastoralists were Anthrax and Trypanosomiasis Poor knowledge of zoonotic diseases among pastoralist.
Attitude of pastoralists towards wildlife and importance of wildlife at the animal-human interface in ESRS	To be started in November 2017	

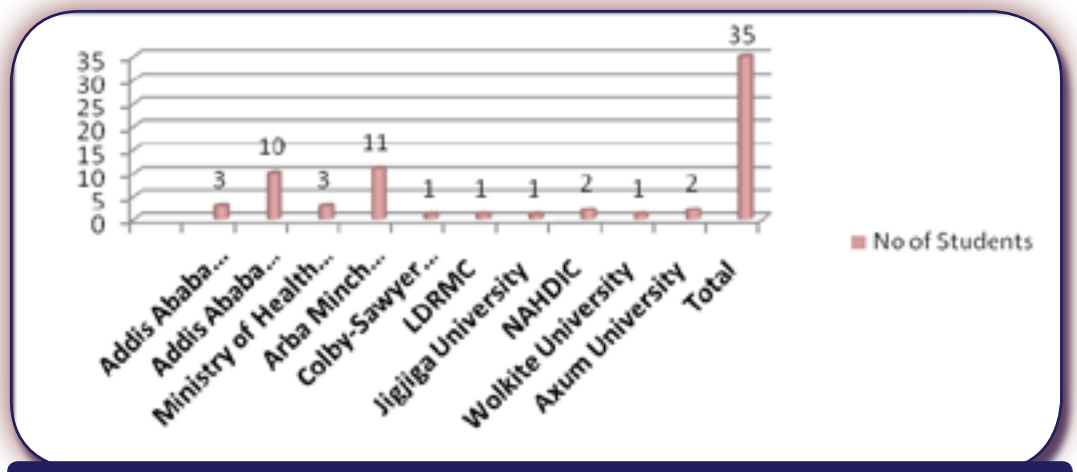
Laboratory Management Center

Summary

The Laboratory Management Center is organized in to Research Laboratory and Quality Assurance Case Team, Bio safety and Bio security Case Team and Laboratory Engineering Case Team. The Research Laboratory and Quality Assurance Case Team comprises of seven research laboratories namely clinical trial, P3 Tuberculosis, Immunology, Molecular Biology, Bacteriology, Pathology and Parasitology Laboratories. These laboratories are equipped with high tech biomedical equipment. All the seven laboratories have their own coordinator and the whole process is led by a Laboratory Manager. In addition to the listed laboratories, new TB, HLA and sequencing laboratories are established and are ready for specialized laboratory services. The Laboratory Engineering Case Team is primarily responsible for handling preventive, curative and maintenance equipment care and calibration of the biomedical equipment. The Bio safety and Bio security Case Team is recently established case team in order to facilitate the biosafety and bio security issues of the institute.

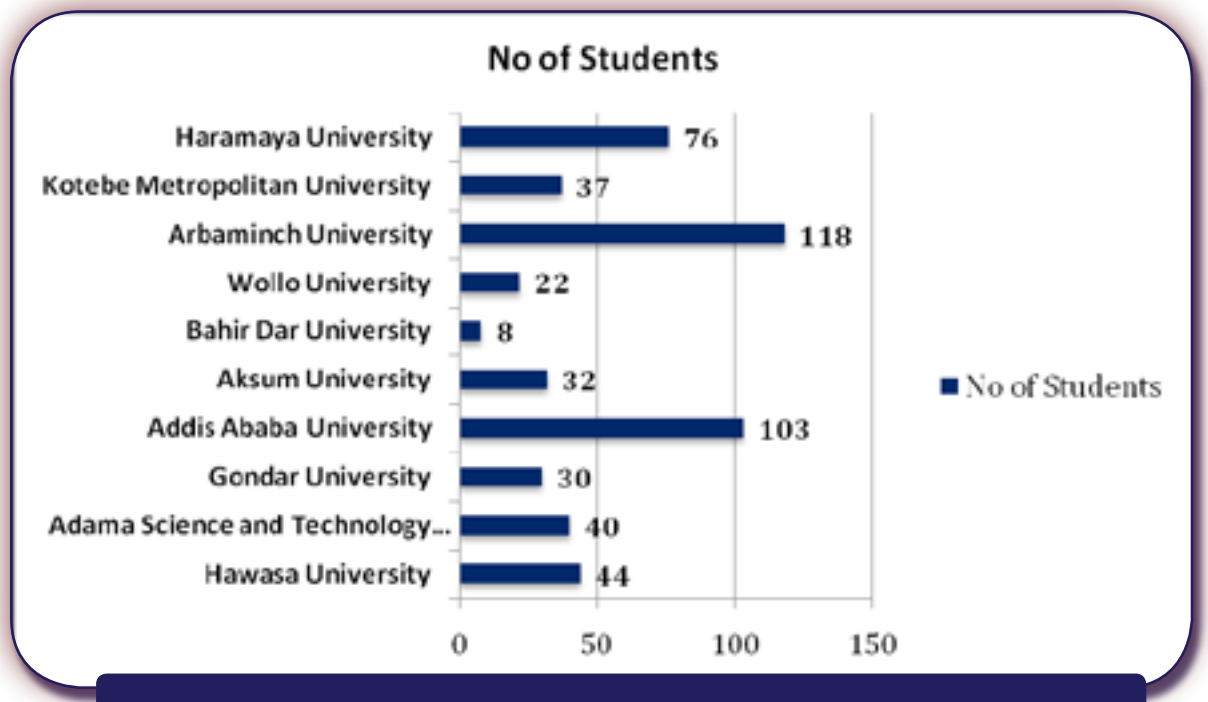
Major Activities

Laboratory Attachment: As one of its strategic objectives, the Laboratory management center provides training and lab attachment opportunity on a regular basis for postgraduate and undergraduate students coming from universities nationwide in the duration of two to six months. In 2017, 35 students were given the opportunity from 10 local and international universities and organizations.

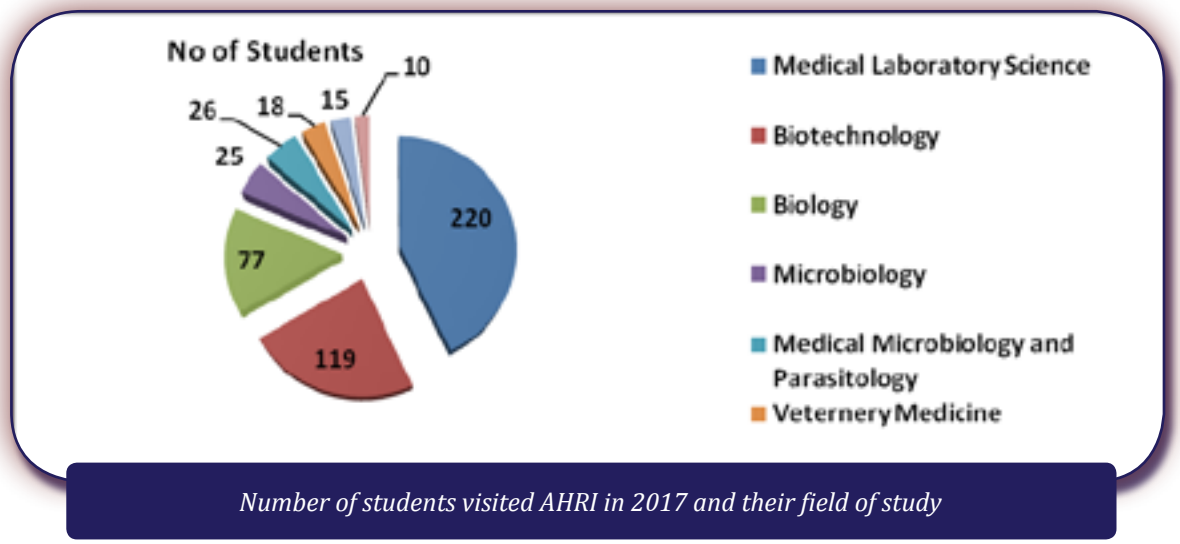


Number of students attached to AHRI and their home institutions

Laboratory Visit: The Laboratory management center also provides Laboratory Visit for one to five days stay from various universities to share experience in biomedical researches. The visiting candidates are registered their host universities on various disciplines of biomedical and health related biotechnology. In 2017, 510 students were visited our laboratory.



Number of students/university staff visited AHRI Laboratory in 2017



Number of students visited AHRI in 2017 and their field of study

Currently; various research activities are underway in these laboratories based on their technical specificity and scope of the research such as grant project research, Students (PhD or MSc projects) research, Individual Scientists' research, etc. In general, the lab team is always under continuous reform to cope with the increasing demand in services and standards.

Laboratory Service: In addition to the research activity the Laboratory Management Center provides pathological diagnostic service to 718 patients in the pathology laboratory. The diagnostic service includes Histopathology (H&E and other special staining) and FNA.

Biosafety and Biosecurity: 10 laboratory assistants were recruited, 14 laboratory assistant have been taken training on basics of bio safety and bio security. Laboratory safety manual for standard application

of safety procedure and practice in AHRI laboratory had been prepared and circulated for comment and approved by management. In collaboration with Mycobacterial disease research directorate freezer inventory is implemented to manage and prepare box map for isolate which are found in the TB laboratory. Expired chemicals and reagents are registered and stored in restricted place in order to dispose according to FMHACA guideline. For other biological waste materials segregation is implemented and regular disposition and follow up procedure have been applied. Emergency exits were prepared, Hepatitis B vaccination were given to researchers, laboratory staff and students.

Accreditation: The accreditation task started with the development of accreditation proposal. The proposal was developed through discussion with staffs and approved by AHRI management. After proposal approval the quality team was prepared work plan for the implementation of accreditation in AHRI laboratories. Brief discussion was conducted on the work plan with staff. Equipment list were prepared, and 45 equipments were calibrated by Ethiopian Metrologic Institute. Fourteen Biological safety cabinets were validated and certified by Clean Room Maintenance (CRM), South Africa. Test menu were prepared for each laboratory. Two day (half day for each) orientation training was prepared and delivered to research staff on document preparation, ISO and accreditation. Formats were prepared and implemented for different purpose like refrigerator monitoring and equipment usage forms. Quality Policy Manual (QPM) was prepared and circulated for comments and approved by management. Communication was established with Ethiopian National Accreditation Office (ENAO) and Ethiopian Standard Agency (ESA).

Grand Challenges Ethiopia

Summary

Armauer Hansen Research Institute (AHRI), is hosting Grand Challenges Ethiopia (GCE), a new initiative of Federal ministry of Health of Ethiopia. The aim of this initiative is to stimulatepromote and scale up of innovative ideas and concepts that are responsive to the defining health-related challenges and assist in the effective implementation of the health sector transformation plan (HSTP) and contribute to achieve the Sustainable Development Goals (SDGs).This initiative established under AHRI Director General's Office, with its own secretariat.GCE secretariat office run by a coordinator and a program assistant to carry out the day-to-day activities of GCE. In addition, the secretariat office will be supported by a national Taskforce to provide technical support and advice for the smooth implementation of the activities included in the initiative and, on demand, short-term technical assistances from Grand Challenges Canada.

Through competitive process, GCE together with national taskforce selected fourteen (14) health innovations and provide seed grant (300,000 ETB for each) in the preset priority areas of Child health, Maternal Health and pastoralist community health. The innovation winners are from public universities (Jimma University=7; Gondar University=1, Mekelle University =2; EPHI=1, St. Paul Hospital=1, Dilla University=1, Addis Ababa University=1). The recipient of this grant received orientation workshop and signed MoU. Currently the recipient has completed more that 50 % of their project task and budget. In this budget year, a new call is out in four thematic areas and plan to award about 20 new innovations.

Grand Challenges Ethiopia Orientation workshop

Development and Administration

Data Management and Biostatistics

Summary

Data management and Biostatistics unit is supporting various research projects in designing questionnaire and CRF, establishing database, double data entry, verification and validation, data analysis, output interpretation, data filing and archiving.

This year, the unit has been actively working in updating SOPs and in establishment of a system for documentation of databases at AHRI. In addition, the unit has been involved in training of students, staffs and partner employees on Data management and analysis with R, STATA, SPSS statistical software.

Data management and Biostatistics training

Research Training

AHRI has been engaged in postgraduate biomedical research training for over 30 years. The Training Directorate of AHRI is responsible for coordination of the postgraduate training program and capacity building of the postgraduate students as well as researchers through short courses on various topics. AHRI hosts Master's and PhD students from various universities in the country enabling them to conduct their research work through supervision and financial support. Students are enrolled into the program on by a competitive means and assigned an advisor to guide them through their graduate work in addition to their University advisor. Although AHRI does not grant degrees, it provides training and the necessary guidance and laboratory facility to conduct their research work. Some of the capacity building collaborations are as follows:

Emory-Ethiopia TB Research Training (EETBRT) is TB research training program involving Emory University, AHRI, AAU and EPHI. The program is funded by the National Institutes of Health Fogarty program with the objective of providing both short and long term trainings for promising MSc, PhD and post-doctoral trainees. Fifteen fellows from the above mentioned institutes are participating in this program. Of these, three are PhD students of AHRI.

Biomedical Sciences Postgraduate Training Program (BSPP) is a biomedicine training partnership between AHRI, AAU and various Swedish Universities. AHRI initiated this partnership with the objective of mitigating possible quality compromises associated with the rapid expansion of postgraduate training programs in the country and is funded by Sida. This program is currently supporting 12 PhD students where 80% of them have travel to Sweden to complete some of their lab. One PhD student from this program has already completed his dissertation.

Brighton-Sussex Global Health Centre is a postgraduate training partnership in the field of genomics of global health importance. The partnership is made between Brighton Sussex Medical School, AHRI and other African institutes and is funded by the Welcome Trust. This program has great potential for capacity building in the future. One PhD student is working on Podoconiosis as part of this collaboration.

FMoH-AHRI clinical research collaboration is postgraduate training program in the areas of maternal and child health, hepatitis B virus and arbovirus infection and antimicrobial susceptibility profiling. The program is supported by Federal Ministry of Health (FMoH) of Ethiopia and AHRI and conducted in partnership with local PIs at Adis Ababa University, University of Gondar, University of Jimma, Hawassa University and Haromaya University. This collaborative program aimed at bridging biomedical and clinical disciplines while strengthening institutions capacity at regional involved. Twenty one students, 2 PhD and 19 MSc, have been undertaking their research work as part of this collaboration. In 2017, all of the MSc students have successfully defined their thesis.

AHRI-Haromaya University joint PhD program is a postgraduate program where trainees are registered at Haromaya University and receive a joint supervision. Two PhD students withdrew from this program while two new candidates jointed in 2017. Thus, currently 5 PhD students are enrolled in this program and this model has a potential to expand to other partner universities.

In 2017, 4 PhD students and 28 MSc students defended their thesis successfully. We begin 2018 with 43 PhD students and 24 MSc students.

Congratulations to all Graduates of 2017!!

(A list of the students who graduated is found at the end of this section.)

Short term training

The Research Training Directorate has organized several short training courses and also facilitated other directorates training related activities. The trainings were attended by AHRI hosted MSc and PhD students and staff.

The short training courses organized by the Research Training Directorate are as follows:

- Research Ethics (**May and December 2017**)
- Data Management and Biostatistics **(April 2017)**
- Biosafety and Biosecurity **(February 2017)**
- Laboratory techniques in Immunology **(June 2016)**
- Laboratory techniques in Molecular Biology **(February 2017)**
- Policy Brief preparation **(June 2017)**
- Instruction Design and Curriculum Development **(September 2017, for AHRI researchers)**

Our plan for the future of the Training unit is to develop the short courses into structured modules that will be accredited and that contain a CME credit. This was initiated for selected modules as part of the Instructional Design and Curriculum Development workshop.

A list of current students and their research topics

PhD Students List

BACTERIAL AND VIRAL DISEASES RESEARCH	1	Getachew Ferede	Arbovirus infections and associated factors among febrile patients in Northwest.
	2	Tsegaye Sewenet	Molecular characteristics and Epidemiology of Extended Spectrum β-Lactamase (ESBL) producing gram negative bacteria (Enterobacteriaceae and Non-fermentative) among human and animals at Jimma, Ethiopia
	3	Yared Hailaye	Molecular characterization of microbial isolates from Injera and ersho: Evaluation of the role of ersho and injera on diarrheal diseases and formulation of defined starter cultures for improved injera
	4	Getachew Tadesse	Virulence genes and Integrons in Ethiopian Salmonella isolates
	5	Abel Abera	Phenotyping and Genotyping characteristics of streptococcus pneumoniae strains isolated from HIV seropositive and HIV negative pediatric patients with pneumococcal disease
	6	Yayeherad Tasachew	Hepatitis B, C and D genotype and subtypes: Distribution and contribution to the burden of HCC associated with CLD in Ethiopia.
	7	Desalegn Admassu	Molecular Epidemiology of Hepatitis B Virus genotypes, Disease profile and prognosis among HIV Co-infected individuals
	8	Mahlet Lemma Degefu	Impact of pediatric HIV infection on pneumococcal vaccination
	9	MelakuAdal	Role of HLA polymorphism in evolution of HIV viral diversity in Ethiopia
	10	Zelalem Petros	Genetic biomarkers for anti-tbc and ARV induced liver injury in Ethiopian TB/ HIV patients
	11	Berhanu Yitayew	Phenotypic and molecular characterization of potential bacteria in Akai river, Addis Ababa, Ethiopia
MYCOBACTERIAL DISEASES	1	Tsehayneh Kelemu	The impact of immune dysfunction and TB on adverse events during pregnancy
	2	Elena Hailu	Molecular EPIDEMIOLOGY, drug resistance pattern of M.tuberculosis and clinical outcome evaluation in Woldiya region, Ethiopia
	3	YaredMerid	Epidemiology of TB in South Ethiopia
	4	Fekadu Desta Tona	Immunophenotype and Functional Characterization of T Cell Subset and Monocyte-Macrophage Lineage cells within Lymph Node Granulomas and peripheral blood of calves Exposed to Natural Mycobacterium bovis Infection
	5	Meseret Habtamu	Molecular markers and mechanisms of T cell memory
	6	Ahmed Esmael Mussa	Potential of Antigen Specify Flow cytometry for the Diagnosis of Smear Negative TB and Pediatric TB
	7	Alem Alemayehu	Genetic Diversity and Drug Resistance Pattern of Mycobacterium Species among Smear Negative Pulmonary Tuberculosis Individuals, Eastern Ethiopia
	8	Ezra Shimeli	Epidemiologic determinants of TB disease and timeliness of obtaining diagnosis and treatment services
	9	Beker Feto	TB
	10	Hawlt Taye	Prevalence of Tuberculosis in Human living or working in settings with high dairy development in Ethiopia
	11	Kedir Urgesa	Leprosy
	12	Tsehaynesh Lema	Detection of new leprosy cases and tracing of household contacts at risk of developing leprosy by active case detection and assessment outcome of treatment in Kokosa Woreda, West Arsi zone, Oromia region: Clinical, molecular and immunological approach
	13	Wegene Tamene	Monocyte function in TB, HIV, TB/HIV co-infected patients

NEGLECTED TROPICAL DISEASES	1	GeremewTassew	Innate Immunity in visceral leishmaniasis
	2	Menberework Chanyalew	Innate Immunity in cutaneous leishmaniasis
	3	Fitsum Girma Tadesse	Understanding the dynamics and infectivity of low-density submicroscopic infections in low and moderate endemic settings in Ethiopia: are they challenges for elimination?
	4	Tewdros Tariku G/Silase	Discovering Podoconiosis Susceptability Genes: from molecular to disease control for a neglected tropical disease (NTD)
	5	Elifaged Hailemeskel Beshah	Transmission dynamics of submicroscopic malaria and contribution of secondary vectors in continuing transmission during dry and early in transmission seasons in P. falciparum and P. vivax co-endemic settings, Ethiopia
CLINICAL TRIAL	1	Jemale Hussien Ahmed	Pharmacokinetic and Pharmacogenetic Studies of Cyclophosphamide among Breast Cancer Patients in Eth
	2	Yohannes Jorge	Pharmacodynamic, Pharmacokinetic and Pharmacogenetic Study of Tyrosine Kinase Inhibitors (TKIs) Emphasis on Imatinib Mesylate in the treatment of the Ethiopian Chronic Myelogenous Leukemia(CML) patients.
NCD	1	Makka Adam Ali	Role of EBV in Hodgkins Lymphoma
	2	Samuel Kinde	Gene Expression Profile of Chronic and Acute Myeloid Leukemia patients: for improved diagnostic, classification and outcome prediction
	3	Fitsum Daniel Tulu_	Lymphoma Phenotyping by flow cytometry, Microscoping and Molecular Techincics
	4	Jemal Alemu Ibrahim	Leukemia Phenotyping by flow cytometry and Molecular Techincics
	5	Meron Talu	Determination of pollutant of Akaki Rivers, Residual Effect on Human and Animals and Transmission Pattern of Pollutant
	6	Asmamaw Abera	“Air pollution and its health effect”
	7	Tiahun Alelign Wassie	Characterization of Anti HLA Antibodies in Multiparous women and Reciepents of Blood Transfussion
	8	Nigatu Tuasha	Phytotherapeutic study of Sidama Medicinal Plants Lore: With Research Emphasis on Identification, Characterization, and phytopharmaceutical study of Antiviral Medicinal Plants
Biotechnology & Bioinformatics	1	Zelalem Petros	Genetic biomarkers for anti-tbc and ARV induced liver injury in Ethiopian TB/ HIV patients
	2	Abdella Gemechu	New PhD students from Haromaya University
	3	Ayichew	New PhD students from Haromaya University

MSc Students List

BVDRD	1	Tigist Girma	Peripheral B cells phenotype as biomarkers of mycobacterium tuberculosis infection and disease in endemic setting , Addis Ababa, Ethiopia
	2	Emawayish Andargie	Prevalence of Burkholderia pseudomallei and other bacterial pathogens in community acquired infections in different regions of Ethiopia
	3	Muluye Shimeles	Characterization of Measels virus in recent outbreaks.
	4	Adugna Tsehaye	Medical Laboratory Professionals Competence Assessment on Gram stain Examination and Interpretation in Hospitals in Addis Ababa, Ethiopia
	5	Wondimu Ashagrie	In vitro starvation model for assessing phenotypic drug tolerance on MTB Lineages in Ethiopia
	6	Belete Haile (DVM)	Effect of benzathine penicillin treatment on throat culture positivity and antibiotic susceptibility of beta-hemolytic streptococci in children receiving secondary prophylaxis after rheumatic heart disease
	7	Aminu Seman	Molecular characterization of Staphylococcus aureusand ESBLproducing isolates causing neonatal and Maternal infections
	8	Mulugeta Kiros	Real-time phylodynamic and phylogeography of HIV-1C transmission networks
	9	Alene Geteneh	Pyogenic and aseptic Meningitis
	10	Tewachew Awoke	The rate of multiple serotype carriage rate of Streptococcus pneumoniae in PCV10 completed under five children in Ethiopia
	11	Henok Andualem	Immunophenotyping of Innate immune cells in HIV positive patients who receive Hepatitis B vaccine
MDRD	1	Mahlet Osman	The correlation of LL37 Antimicrobial peptide expression among leprosy pateints and their contacts attending all Africa Leprosy Rehabilitation and Training Center
	2	Dareskedar Tsehaye	The role of Neutrophil Gamma Receptors in the pathogeneousis of Erythma Nodosum Leprosum
	3	Ousman Mohammed	molecular epidemiology of tuberculosis in Somali Region
	4	Yosef Tsegaye	Hormone profiles in different spectrum of TB disease
	5	Jagmar Worku Bizani	Occupational hazard of TB in different health facilities
	6	Wasihun Admassu	Humoral immunity in tuberculosis
	7	Derbie Alemu	The role of NTM in treatment failure cases of smear positive TB patients.
NTD	1	Migbaru Keffale	Serological conversion to evaluate malaria control program in Ethiopia
	2	Birhan Ayelign	
CLINICAL TRIAL	1	Ayana Jaleta Kinati	Pharmacokinetics of Benzathine Penicillin in Ethiopian RHD Children
NCD	1	Mebratu Teshome Abose	Comparison of Lymphoma Immunophenotyping by FCM, IHC/ICC with complementary cytomorphology study approach and its role in the diagnosis and lymphomas on adult and pediatric clinical specimen at Tikur Anbesse Specialized Hosptial Addis Ababa Ethiopia
	2	Tariku Sime	Circulatory breast cancer-associated mRNA expression in serum as biomarkre for breast cancer detection.
BB	1	Shemse Sebre	Microbiome and resistome assessment in selected Hospital Environment.

A list of 2017 graduates and their research topics

PhD Students

Wondossen Tsegaye	Epidemiology of Streptococus pneumoniae nasopharyngeal carriage: Serotyping, molecular characterization and Antibiotic sensitivity pattern in Ethiopia	Addis Ababa University
Wude Mihret	Bacterial Meningitis in Ethiopia: study of etiologies by use of real time PCR, MLST and cytokine detection	Addis Ababa University
Endale Hadgua	Molecular and Genetic Characterization of Triple Negative Breast Cancer (TNBC) among Ethiopian Women	Addis Ababa University
Sisay Getachew	Assessing the prevalence of Plasmodium vivaxchloroquine resistance by using in vivo drug sensitivity test and molecular characterization of parasite isolates in South Nations and Nationalities Peoples Regional State (SNNPR), Ethiopia	Addis Ababa University

Msc Students

Seifudin Usman	Level of antibody to hepatitis B surface antigen among hepatitis B vaccinated children during infancy in Harrar, Ethiopia	Haromaya University
Fitsum Abebe	Seroprevalence of HBV, HVC, HDV, and HEV infections and its associated risk factors among mothers of children livivng in Harar, Eastern Ethiopia	Haromaya University
Daniel Demissie	Prevalence, drug susceptibility pattern and associated factors of septicemia among women attending delivery in Dire Dawa, Eastern Ethiopia.	Haromaya University
Kenasa Tesema	Bacterial agents, drug susceptibility pattern and associated risk factors of neonatal sepsis in Dire Dawa, Eastern Ethiopia.	Haromaya University
Melat Woldemariam	Assessing monocyte subset frequency and surface marker expression level in cutaneous leishmaniasis patients.	University of Gondar
Teshger Dubie	Characterization of the Activation Status of Multiple Lymphocyte Subpopulations from Localized and Diffused Cutaneous Leishmaniasis Patients due to Leishmania aethiopica at University of Gondar Hospital, North West Ethiopia'	University of Gondar
Mohammed Adem	Evaluation of Regulatory and Gamma/ Delta T cells expression in different forms of cutaneous Leishmaniasis	University of Gondar
Mekuanint Geta	Sero-prevalence of hepatitis B, hepatitis C, hepatitis D and hepatitis E viruses among mothers in North West Ethiopia, 2015	University of Gondar
Getnet Ayalew	Immunogenicity and efficacy of hepatitis B vaccination among children in North-West Ethiopia.	University of Gondar

Tsehaynesh G/ Eyesus	Assessment of the bacterial etiologic agent causing neonatal sepsis and its antimicrobial susceptibility pattern at Gondar University Hospital, North -West Ethiopia.	University of Gondar
Abebaw Bitew Kifilie	Bacterial profile, antibacterial susceptibility test and associated factors during pregnancy and following delivery of mothers at University of Gondar Teaching Hospital, North-West Ethiopia.	University of Gondar
Bedru Argaw	HBV infection, pattern of transmission and efficacy of HBV vaccine among children in Hawassa, SNNPR, Ethiopia: A community based cross sectional study.	Hawassa University
Daniel Eshetu	Seroprevalence of dengue virus and its associated factors in ArbaMinch and Konso Districts, South Nation Nationalities Peoples Region (SNNPR)	Hawassa University
Eshetu Negussie	Seroprevalence of Yellow Fever Virus and associated risk factors in selected health facilities in Borena District, Oromia Region, Ethiopia	Hawassa University
Tsegaye Alemayehu	Nosocomial infedtion of aerobic bacteria, their drug resistance profile and associated fisk factors among pediatric ward admitted patients at Hawassa University Tecahing and Referral Hospital, Hawassa, Southerm Ethiopia.	Hawassa University
Azeb Bereket	Molecular detection of mycobacterium Leprea in stained slite skin smear from lepreosy pateints and their correlation of molecular and histopathological findings with clinical data	AAU
Getaneh Tegegne Afework	Comparative assessment of microscopy, malaria rapid diagnostic test (RDT) and polymerase chain reaction (PCR) as malaria diagnostic tools in Adama Woreda, east Shoa zone of Ethiopia.	AAU
Getasew Shitaye Ayalew	Glucose -6 - Phosphate Dehydrognase (G6PD) Genotyping and Enzyme Activity	AAU
Habtamu Biazin	Hepatitis, Addis Ababa University	AAU
Mulualem Belachew Shuba	Clustering of Malaria	AAU
Seifegebriel Teshome	Hepatitis, Addis Ababa University	AAU
Temesgen Menberu Kebede	Chloroquine Resistance Mapping P. falciparum	AAU
Zeleke Ayanaw	Nosocomial infedtion of aerobic bacteria, their drug resistance profile and associated fisk factors among pediatric ward admitted patients at Hawassa University Tecahing and Referral Hospital, Hawassa, Southerm Ethiopia.	AAU
Rebie Kedir	HBV vaccine efficacy and genotyping of HBV among children of Jimma Zone, Southwest Ethiopia.	Jimma University
Belayneh Dimah	Prevalence of Hepatitis B virus infection and Hepatitis E virus infection and associated factors among pregnant women in Jimma zone, South west Ethiopia	Jimma University
Milkiyas Toru	Antimicrobial, Jimma University	Jimma University
Zeleke Gizachew	Multi drug resistance and virulence phenotypes among uropathogenic Escherichia coli from urinary tract infection in reproductive age women, Jimma, South West Ethiopia	Jimma University

Finance and Procurement

Source of Finance: The institute core fund comes from Sida, Norad and government of Ethiopia. We also have other competitive grants.

Sida: The institute signed five year grant agreement with Sida on July 2016. The total grant amount is 49.97Million SEK, the grant components are: core support, BSPP and south to south collaboration. We received first disbursement 7 Million SEK on July 2016.

Norad: Norad grant started on 2014 and closed on July 2017. We submitted new five year grant proposal.

Government: From government source we secured ETB 19,581,000.00 for recurrent budget and ETB 85 Million for capital budget for 2010 budget year.

Competitive Grants: The competitive grants which continued from 2016 and new signed in 2017 are:

ETHICOCOBOTS:- Department of Veterinary Medicine, Cambridge University five years grant from 2014 to 2018 £ 384,390.0

STREAM Stage II: extension of Stream Stage I from International Union against Tuber Clause and Lung Disease, donor USAID sub grantee to AHRI, five years grant from 2014 to 2019 USD 1,322,817.00

Screen TB: EDCTP grant from European Union 5 years grant from 2014 to 2018, € 299,625.00

MoH: Minister of Healthy support for clinical trial USD 500,000.00

MoH Grand Challenge Project: and also MOH has signed an agreement with AHRI for grand challenge activities 1Million USD.

SOAR: Johns Hopkins University ("JHU")-Sub grant of USAID USD $200,291.00

Minister of Science and Technology/MoST/- three research grant agreements(ETB 8,981,820)

In addition to the above stated projects we do have also: Emory University, NIPH influenza, SETA, One-health, Ministry of Science and technology project grants, Podoconiosis grant are other projects.

Upcoming

H3Africa: USD 3,036,850.00 well come trust fund

GiZ: € 547350.00 funding initiatives of the Germen Federal Minister of Education and Research (BMBF)

Value TB project: London School of Hygiene US$65,850.00

Procurement of Laboratory equipment's and reagent and consumables

Continued from 2016 laboratory equipment procurement under UNOPS procurement system, in 2017 we received USD 887,652.27 equipment, only one item remaining USD 73,050.00

The reagent, consumables office equipment and other lab equipment and supplies we had procured in 2017 are USD 711,332.00

Property Administration

Annual property count conducted for 2017 June.

Properties that are out of use are separated to dispose according to government property administration guidelines.

External financial audit

Independent internal audit conducted for 2016 calendar year additionally due to AHRI become government organization in addition to December 2016 audit the audit also conducted from January 2017 to June 2017. Starting from 2017 July, the institute calendar year becomes from July to June according to the Ethiopian government financial regulation.

ARMAUER HANSEN RESEARCH INSTITUTE (AHRI)

Balance Sheet

As at 30 June 2017

	Notes	Birr	Birr	2016
CURRENT ASSETS				
Debtors & prepayments	3		25,519,716.40	33,705,058.03
Cash & bank balances	4		18,523,932.92	27,513,433.12
TOTAL ASSETS			44,043,649.32	61,218,491.15
LIABILITIES & FUND BALANCE				
CURRENT LIABILITIES				
Creditors & accruals	5	1,855,766.17		338,250.24
TOTAL LIABILITIES			1,855,766.17	338,250.24
FUND BALANCE				
General fund		7,566,501.70		4,819,160.34
SIDA and NORAD funds	6	15,874,480.99		38,686,192.79
Other projects funds	7	18,746,900.46		17,374,887.78
TOTAL FUND BALANCE			42,187,883.15	60,880,240.91
TOTAL LIABILITIES & FUND BALANCE			44,043,649.32	61,218,491.15

ARMAUER HANSEN RESEARCH INSTITUTE (AHRI)
Statement of income and expenditures
For six months ended 30 June 2017

	Notes	Birr	Birr	2016 Birr
GRANT RECEIVED				
SIDA core fund			14,698,533.00	12,649,747.91
NORAD fund			12,461,682.90	15,508,463.22
SIDA students fund			0.00	22,141,751.70
SIDA equipment fund			0.00	11,443,634.22
Government contribution	8		223,226.50	568,162.84
Other projects grants			10,277,490.00	20,892,492.65
Other income			22,322.79	13,161.99
Overhead			997,705.23	2,692,737.39
Exchange rate gain / loss			(2,427.90)	5,850.11
Total receipt			**38,678,532.52**	**85,916,002.03**
EXPENDITURES				
Management & administration		0.00		1,961,483.31
Common support		0.00		20,294,941.42
		0.00		**22,256,424.73**
Research units:				
Tuberculosis		0.00		1,838,242.57
Research equipment, reagents & supplies		0.00		4,063,543.17
SIDA equipment fund expenditures		0.00		11,443,634.22
SIDA Student fund expenditures		0.00		22,141,751.69
SIDA core fund		14,698,533.06		0.00
NORDA fund		12,461,682.89		0.00
		27,160,215.95		**39,487,171.65**
Other projects expenditures		10,277,490.00		20,892,492.68
Total expenditures			**(37,437,705.95)**	**82,636,089.06**
EXCESS OF INCOME OVER EXPENDITURES			1,240,826.57	3,279,912.97
FUND BALANCE brought forward		4,819,160.26		10,243,301.06
PRIOR YEAR ADJUSTMENT		1,506,514.87		(8,704,053.77)
			6,325,675.13	1,539,247.29
FUND BALANCE carried forward			**7,566,501.70**	**4,819,160.26**

page 4/7

Human Resources Development

The human resource directorate plays a significant part in executing the institute's strategy and is aligned to three overarching goals: transforming the institute in terms of its mission, capacity building and operations; resolving issues and shortcomings of the past; making the organization fit for the future.

A crucial aspect of the institute transformation includes the restructuring of all of its business divisions. In 2016, the focus was on successfully planning and implementing the significant people aspects of this restructuring in close collaboration with the respective divisions and functions. We hired/transferred 84 employees within the calendar year. This could be 40% boost compared to last year.

Qualification	F	M	Total
PhD	4	9	13
MA/MSc/MD	14	25	39
BSc/BA	35	28	63
Diploma	20	6	26
Level	15	6	21
Certificate		4	4
12 grade and below	28	6	34
Total	**116**	**84**	**200**

In line with its goal to strengthen employee capabilities in times of significant change, the directorate is providing training, coaching and cross-divisional exposure to a broad range of employee's. Throughout the year, 4 Phd, 7 second degree and 10 Bsc and Diploma candidates are recruited and support is set in 2017. This also includes a stronger focus on enabling managers to deliver a more active and more rounded approach to performance management to ensure they are well-equipped to lead the work unit to future.

Throughout 2017, the institute continued its efforts to advance women in the workplace .The percentage of women working is 116 which could be 56% .The attrition rate is reduced to 2% which is 5% in 2016.

Publications in 2017

1. Gebreegziabiher D, Adane K, **Abebe M**. ***A survey on undiagnosed active pulmonary tuberculosis among pregnant mothers in Mekelle and surrounding districts in Tigray, Ethiopia.*** Int J Mycobacteriol. 2017 Jan-Mar;6(1):43-46. doi: 10.4103/2212-5531.201889
2. **Tadesse FG**, van den Hoogen L, Lanke K, Schildkraut J, Tetteh K, **Aseffa A**, Mamo H, Sauerwein R, Felger I, Drakeley C, **Gadissa E**, Bousema T. ***The shape of the iceberg: quantification of submicroscopic Plasmodium falciparum and Plasmodium vivax parasitaemia and gametocytaemia in five low endemic settings in Ethiopia***. Malar J. 2017 Mar 3;16(1):99. doi: 10.1186/s12936-017-1749-4. PMID:28253867
3. Mariam SH, Zegeye N, **Aseffa A, Howe R**. ***Diffusible substances from lactic acid bacterial cultures exert strong inhibitory effects on Listeria monocytogenes and Salmonella enterica serovar enteritidis in a co-culture model.*** BMC Microbiol. 2017 Feb 15;17(1):35. doi: 10.1186/s12866-017-0944-3. PMID: 28202007
4. **Gadisa E, Tasew G**, Abera A, Gelaye W, **Chanyalew M**, **Abebe M**, Laskay T, **Aseffa A:** ***Serological signatures of clinical cure following successful treatment with sodium stibogluconate in Ethiopian visceral leishmaniasis***. Cytokine 03/2017; 91:6-9., DOI:10.1016/j.cyto.2016.11.016
5. **Tadesse FG**, Lanke K, Nebie I, Schildkraut JA, Bronner P, Tiono ABG, Sauerwein R, Drakeley C, Bousema T, Rijpma SR. ***Molecular Markers for Sensitive Detection of Plasmodium falciparum Asexual Stage Parasites and Their Application in a Malaria Clinical Trial***. The American Journal of Tropical Medicine and Hygiene. 24 April 2017. DOI: https://doi.org/10.4269/ajtmh.16-0893.
6. Gebreeyesus T, Moges, F, Eshetie S, **Yeshitela B**, Abate E. (2017). ***Bacterial etiologic agents causing neonatal sepsis and associated risk factors in Gondar, Northwest Ethiopia***. *BMC pediatrics*, *17*(1), 137.
7. Marks F, von Kalckreuth V, Aaby P, Adu-Sarkodie Y, El Tayeb MA, Ali M, **Aseffa A,** Baker S, Biggs HM, Bjerregaard-Andersen M, Breiman RF, Campbell JI, Cosmas L, Crump JA, Espinoza LM, Deerin JF, Dekker DM, Fields BS, Gasmelseed N, Hertz JT, Van Minh Hoang N, Im J, Jaeger A, Jeon HJ, Kabore LP, Keddy KH, Konings F, Krumkamp R, Ley B, Løfberg SV, May J, Meyer CG, Mintz ED, Montgomery JM, Niang AA, Nichols C, Olack B, Pak GD, Panzner U, Park JK, Park SE, Rabezanahary H, Rakotozandrindrainy R, Raminosoa TM, Razafindrabe TJ, Sampo E, Schütt-Gerowitt H, Sow AG, Sarpong N, Seo HJ, Sooka A, Soura AB, Tall A, **Teferi M**, Thriemer K, Warren MR, **Yeshitela B,** Clemens JD, Wierzba TF. ***Incidence of invasive salmonella disease in sub-Saharan Africa: a multicentre population-based surveillance study***. Lancet Glob Health. 2017 Mar;5(3):e310-e323. doi: 10.1016/S2214-109X(17)30022-0. PMID: 28193398
8. Abebe F, Belay M, Legesse M, **Mihret A**, Franklin KS. ***Association of ESAT-6/CFP-10-induced***

IFN-γ, TNF-α and IL-10 with clinical tuberculosis: Evidence from cohorts of pulmonary tuberculosis patients, household contacts, and community controls in an endemic setting. Clin Exp Immunol. 2017 Apr 3. doi: 10.1111/cei.12972. [Epub ahead of print]

9. Belyhun Y, Maier M, **Mulu A**, Diro E, Liebert UG. ***Hepatitis viruses in Ethiopia: a systematic review and meta-analysis***. BMC Infect Dis. 2016 Dec 19; 16(1):761: BMC Infect Dis. 2017 Feb 1; 17 (1):114.

10. **Abebe M**, Ali I, Ayele S, Overbo J, **Aseffa A, Mihret A.** ***Seroprevalence and risk factors of Hepatitis E Virus infection among pregnant women in Addis Ababa, Ethiopia***. PLoS One. 2017 Jun 26;12(6)
11. Tora A, Tadele G, **Aseffa A**, McBride CM, Davey G. ***Health beliefs of school-age rural children in podoconiosis-affected families: A qualitative study in Southern Ethiopia.*** PLoS Negl Trop Dis. 2017 May 25;11(5):e0005564. doi: 10.1371/journal.pntd.0005564. eCollection 2017 May PMID: 28542227
12. Leon ME, Assefa M, Kassa E, Bane A, Gemechu T, Tilahun Y, **Endalafer N**, Ferro G, Straif K, Ward E, **Aseffa A**, Schüz J, Jemal A. ***Qat use and esophageal cancer in Ethiopia: A pilot case-control study.*** PLoS One. 2017 Jun 8;12(6):e0178911. doi: 10.1371/journal.pone.0178911. eCollection 2017.
13. Bårnes GK, Brynildsrud OB, Børud B, Workalemahu B, Kristiansen PA, **Beyene D, Aseffa A**, Caugant DA. ***Whole genome sequencing reveals within-host genetic changes in paired meningococcal carriage isolates from Ethiopia***. BMC Genomics. 2017 May 25;18(1):407. doi: 10.1186/s12864-017-3806-3. PMID:28545446
14. Abaye GE, Abebe T, Worku A, Tolessa D, Ameni G, **Mihret A**. ***Detection of Mycobacterium tuberculosis from the stool of HIV sero-positive individuals suspected of pulmonary tuberculosis***. PLoS One. 2017 May 19;12(5):e0177529.
15. Ameni G, **Tafess K,** Zewde A, Eguale T, Tilahun M, Hailu T, Sirak A, Salguero FJ, Berg S, **Aseffa A**, Hewinson RG, Vordermeier HM. ***Vaccination of calves with Mycobacterium bovis Bacillus Calmette-Guerin reduces the frequency and severity of lesions of bovine tuberculosis under a natural transmission setting in Ethiopia.*** Transbound Emerg Dis. 2017 Feb 6. doi: 10.1111/tbed.12618. [Epub ahead of print] PMID:28168855.
16. Dembinski JL, **Mihret A,** Yimer SA, **Tessema B**, Trieu MC, **Tarekegn A**, **Getachew N**, Cox RJ, Oftung F, Haneberg B, **Aseffa A**, Mjaaland S**.** ***High Prevalence of Humoral and Cellular Immunity to Influenza Viruses in Preschool Children Living in Addis Ababa, Ethiopia***. Open Forum Infect Dis. 2017 Feb 11;4(1):ofx026. doi: 10.1093/ofid/ofx026. eCollection 2017 Winter. PMID:28480294
17. King HC, Khera-Butler T, James P, Oakley BB, Erenso G, **Aseffa A**, Knight R, Wellington EM, Courtenay O. ***Environmental reservoirs of pathogenic mycobacteria across the Ethiopian biogeographical landscape***. PLoS One. 2017 Mar 23;12(3):e0173811. doi: 10.1371/journal.pone.0173811. eCollection 2017 Mar 23. PMID:28333945
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carriage of Neisseria meningitidisgenogroups W and Y in the African meningitis belt. PLoS One. 2017 Aug 10;12(8)

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37. **Negera E**, Walker SL, **Bekele Y**, Dockrell HM, Lockwood. Increased activated memory B-cells in the peripheral blood of patients with erythema DN nodosum leprosum reactions. PLoS Negl Trop Dis. 2017 Dec 18;11(12):e0006121. doi: 10.1371/journal.pntd.0006121. [Epub ahead of print]. PMID:29253897

38. Reepalu A, **Balcha TT,** Skogmar S, Isberg PE, Medstrand P, Björkman P. ***Development of an algorithm for determination of the likelihood of virological failure in HIV-positive adults receiving antiretroviral therapy in decentralized care.*** Glob Health Action. 2017; 10(1):1371961. doi: 10.1080/16549716.2017.1371961.

In press

39. Huruy K, **Mulu A,** Maier M, Liebert UG. ***HIV-1C proviral DNA for testing of transmitted drug resistance mutations***. Jornal of Translational Medicine -D-17-00654

40. Tesfaye F, König J, Walles JK, Jansson MJ, **Balcha TT,** Winqvist N, Kefeni M, Garuma S, Belachew F, Sturegård E, Björkman P. ***QuantiFERON TB Gold-Plus for detection of latent TB infection in pregnant women living in TB and HIV endemic setting*** (in press)

41. **Tadesse FG**, Slater H, Chali W, Teelen K, Lanke K, Belachew M, Shumie G, Menberu T, Shitaye G, Okell LC, Graumans W, van Gemert GJ, Kedir S, Tesfaye A, Belachew F, Abebe W, Mamo H, Sauerwein R, **Tolera T, Aseffa A,** Yewhalaw D, **Gadisa E,** Drakeley C, Bousema T. ***The relative contribution of symptomatic and asymptomatic Plasmodium vivax and Plasmodium falciparum infections to the infectious reservoir in a low-endemic setting in Ethiopia***. Clinical Infectious Disease. 2017 (in press).

42. Benjak A, Avanzi C, Singh P, Loiseau C, **Girma S**, Busso P, Fontes AB, Miyamoto Y, Namisato M, **Bobosha K**, et al. Cole S. **Phylogenomics and antimicrobial resistance of the leprosy bacillus *Mycobacterium leprae*". Nature communications,** *in press.*

43. Mbilo C, M Léchenne, N Chitnis, R Tschopp, J Zinsstag. ***Rabies: A veterinary perspective. In: Rabies (Ed. AR Fooks)***. OIE *Scientific and Technical Review*, Vol. **37** (2), August 2018. In Press

44. Hussein J, **Zewdie M, Yamuah L, Bedru A, Abebe M, Dagnew AD, Chanyalew M, Yohannes AG, Ahmed J, Engers H**, Doherty TM, Bang P, Kromman I, Hoff S**, Aseffa A**. Safety and Immunogenicity of the adjuvanted tuberculosis subunit vaccine H1/IC31® in people living in a TB endemic area. Trials TRLS-D-17-00427.

45. **Alelign T**, Muhammed M, **Bobosha K**, Tadesse Y, **Howe R**, Petros B. Kidney Transplantation: The Challenge of Human Leukocyte Antigens And Its Therapeutic Strategies. Journal of Immunology Research

46. **Rawleigh C. Howe**. Peer review, critical review and writing of manuscripts. Ethiop Med J, 2018, Vol. 56, No.1

Training/conference/meeting participations

Wegene Tamene, Yared Merid, Markos Abebe, Liya Wassie, Kidist Bobosha	TB research advisory committee (TRAC) annual meeting	March 22-24, 2017	Addis Ababa
Wegene Tamene	annual EPHI TB/HIV research finding dissemination workshop		Dire Dawa
Selfu Girma	training on Loop Mediated Isothermal Amplification (LAMP) technique	June 27-30	Addis Ababa
Mesert Gebre, Liya Wassie, Wegene Tamene, Yared Merid, Markos Abebe, Kidist Bobosha	annual Ethiopia-Emory Tuberculosis Research and Training Program (EETB-RTP) conference	July 12-14	Debre Zeit
Meseret Gebre	hands-on training on meta-analysis	June 12-15	Bristol, UK
Elena Hailu	Training on Vaccinology	February 27-March 24	Paris, France
Elena Hailu	Keystone Symposia: New Developments in Our Basic Understanding of Tuberculosis	Jan 14 - 18	Vancouver, Canada
Meseret Habtamu	Global Health Summit	August 06 - December 20	Oslo, Norway (part of a PhD training)
Tsehainesh Lemma	Leprosy Awareness raising meeting	May 04	Addis Ababa
Yared Merid and Tsehainesh Lemma	Research dissemination and consultative meeting	June 22	AHRI
Tsehainesh Lemma	poster presentation	September 28 Oct. 01	Kilimanjaro Christian Medical Centre, Moshe, Tanzania
Tsehainesh Lemma	The Leprosy Mission in Ethiopia (TLMiE) meeting	November 23	Addis Ababa
Kidist Bobosha, Martha Zewdie, Million and Tilahun A	HLA fusion software	February 20-25	Millan, Italy
Dr Endalamaw Gadisa	preparation of antigen (DAT) for diagnosis of Leishmania donovani		
Menberework Chanyalew	training on blood fractionation/IgG purification	February 15-April 30	Paul Ehrlich Institute, Federal Institute f or vaccines and Biomedicines, Germany
Dr Markos Abebe	training on generation, screening, purification and labelling of monoclonal antibodies	September 12 to October 14	Fraunhofer Institute of cell therapy and immunology, Leipzig, Germany
Dawit Kebede	Control strategies for communicable and non-communicable disease	April 08 - May 10	Amsterdam, Netherlands
Sosina Ayalew and Wondimu Ashagre	Line Probe Assay, MGIT DST and LJ DST training	June 5th-16th	Kampala, Uganda
Wondimu Ashagre	Infectious Diseases: Biology to Intervention Strategies	September 7th-9th	Bangalore, India
Samuel Ayele, Tigist Beyene and Tsegaye Hailu	Bioinformatics and Statistical Genetics	Jan 15-24,	Khartoum Winter School in Khartoum, Sudan

Grants submitted

1. H3Africa – An integrated approach to unravelling susceptibility to tuberculosis in Africa (TBGENAfrica) (PI: Abraham Aseffa, 2.5 million USD) WT – Awarded

2. Accelerating Bovine TB control in Developing Countries. PI: VivekKapur. USD 5.5 million for 5 years to BMGF – Awarded (Coordinated by Adane Mihret)

3. PAVIA – Pharmacovigilance Africa. (PI: F Cobblens).EDCTP. 48 months. 3 million Euro. Awarded (Coordinated by Mekonnen Teferi)

4. IMPALA - Beyond drug treatment: immunoprophylaxis in leprosy patients in Africa. PI – C Kasang, DAHW. 5.3 million Euro – EDCTP – Pending. (Coordinated by Kidist Bobosha)

5. AHRI five-year core grant application to Norad (PI: Taye Tolera Balcha). 8 million NOK. Pending

6. Chemoprophylaxis for leprosy: comparing the effectiveness and feasibility of a skin camp intervention to a health centre based intervention. An implementation trial in Mozambique, Ethiopia and Tanzania.
Submitted: EDCTP in Oct 2017
Status: Pending
Coordinator: Netherlands Leprosy Relief, MsLiesbethMieras
Participants: NIMR, Tanzania (Dr John Changalucha) ; AHRI, Ethiopia (Dr. Kidist Bobosha); Dr Wim van Brakel (NLR); GLRA, Germany (Dr. Christa Kassang); EMS, The Netherlands (Prof. Jan H. Richardus, Mrs. Colette van Hees,) ; NTBLC, Tanzania, (Dr Blasdus Njako; Dr Deusdedit Kamara); NTBLC, Ethiopia (Mr. Taye Letta), GLRA Ethiopia (Mr. Ahmed Mohammed), ENAPAL, Ethiopia; NLR , Mozambique (Dr Alcino Ndeve); MOH, Mozambique (Dr Artur Manuel Muloliwa); Lurio University, Mozambique (Prof. Ferando Mitano); CUHAS, Tanzania (Prof. Paschalis Rugarabamu)

7. Modulation of C-type lectin receptor expression by helminth or HIV infection: setting the threshold for sensing of mycobacteria
Submitted: DFG, Germany in December 2017
Status: pending
PI/Co- Investigators: Markos Abebe and collaborators from National Biotechnology Development Agency, Nigeria and University Hospital Erlangen, Germany

8. Point-of-care feasibility, accuracy and potential impact of a rapid, serological triage assay for active TB (SeroSelectTB)
Submitted: EDCTP in October 2017
Status: rejected

PI: Carol Holm-Hanson (NIPH)
Participants: Dr. Kidist Bobosha (AHRI), Dr. Nyombi Balthazar (KCMC, Tanzania), Dr. Solomon Yimer, Dr. Abraham Alemayehu (TBLCP, Ethiopia)

9. Characterizing the Unmet HIV Prevention Needs and HIV Risk Vulnerabilities of Adolescent Girls and Young Women in Ethiopia
Submitted: USAID/JHU in June 2017
Status:Granted
PI /co-investigators: Stefan Baral, Taye Tolera, Andargachew Mulu, Samuel Ayele, Sheree Schwartz

10. A relative real time HIV transmission networks among AGYW in Addis Ababa
Submitted: USAID/JHU in November 2017
Status: Pending
PI /co-investigators: Andargachew Mulu, Stefan Baral, Taye Tolera, Samuel Ayele, Sheree Schwartz

11. Simple-low cost HIV-1 monitoring tools: In house HIV viral load and drug resistance testing assays
Submitted: Ethiopia Institute of Biotechnology Product Development in April 2017
Status: Rejected
PI /co-investigators: Andargachew Mulu, Taye Tolera, Dawit Aseffa, Melanie Maier

12. Simple-low cost HIV-1 monitoring tools: In house HIV viral load and drug resistance testing assays
Submitted: Global Fund/Federal HAPCO/MoHin November 2017
Status: Recommended for 2018 Call
PI /co-investigators: Andargachew Mulu, Taye Tolera, Abraham Aseffa, Adane Mihret

13. Whole blood HIV-1 Proviral DNA pol gene analysis for HIV drug resistance testing
Submitted: EDCTP Early Carrier Development in April 2017
Status: Rejected
PI: Andargachew Mulu

14. A relative real time HIV transmission networks: a population based study in Addis Ababa
Submitted:Global Fund/Federal HAPCO/MoH in November 2017
Status: Pending
PI /co-investigators: Andargachew Mulu, Taye Tolera, Abraham Aseffa, Adane Mihret

15. Establishment of HIV Cohort at ALERT Hospital for a Prospective Study of HIV

Infection: AHRI/ALERT Longitudinal Study of Anti-Retroviral Therapy (AALSART)
Submitted:Global Fund/Federal HAPCO/MoHin November 2017
Status: Pending
PI /co-investigators: Andargachew Mulu, TayeT olera, Abraham Aseffa, Adane Mihret

16. Could active tuberculosis infection leads to specific HIV drug resistance mutational pathways?
Submitted:TRAC, MoH Operational Research Grantin April 2017
Status: Rejected
PI /co-investigators: Andargachew Mulu, Adane Mihret, Markos Abebe

17. Transmission dynamics of Cholera in a dry region in Ethiopia
Submitted: MoH
Status: Pending
PI/Investigators: Taye Tolera, Adane Mihret, Sirajul Islam, Niyaz Ahmed, J. D. Clemens

18. Aligning Public Knowledge, Aptitude and Practice to Integrated Triple Drugs Mass Administration Targeting Soil Transmitted Helminth
Submitted: EDCTP
Status: Rejected
PI: Endalamaw Gadisa

19. A Randomized Controlled Trial On Radical Cure of P. Falciparum Malaria in Ethiopia
Submitted: EDCTP
Status: Rejected
PI: Professor Richard Price
Co/Investigator: Endalamaw Gadissa, Koen Peters, Chatherine Martel, Asrat Hailu

20. A Randomized Controlled Trial On Radical Cure Of P. Falciparum Malaria In Ethiopia
Submitted: MRC
Status: Rejected
PI: Endalamaw Gadissa

21. Safety of the Co-Administration of Azithromycin, Albendazole and Ivermectin Versus Standard Treatment Regimens During Mass Drug Administration (Mda) In Ethiopia: A Cluster-Randomized Trial
Submitted: ITI
Status: Third Round of Review
PI: Endalamaw Gadisa/Scott Mcpherson
Co-Investigator: Biruk Kebede

22. Health issues in Eritrean refugees
Submitted: SNF
Status: Accepted
PI: Rea Tschopp

Abraham Aseffa -

Presentations:

- ***Diagnosis of cutaneous leishmaniasis.*** IUIS-FAIS-IMMUNO-ETHIOPIA Course. 27 February 2017, University of Gondar, Gondar, Ethiopia
- ***Clinical Trials: Review of global, regional and local experiences for modern and traditional medicines.*** Consultative meeting on clinical trial and registration of traditional medicine: challenges and prospects. 5 September 2017, EPHI Training Center Conference Hall, Addis Ababa.
- ***Communication and Diffusion of Results: The AHRI Experience,*** GLOBVAC Student Conference, 13 March 2017, Trondheim, Norway.
- ***The Enigma of TB lymphadenitis in Ethiopia,* 2017** Global Health and Vaccination Research Conference, 14 March 2017, Trondheim, Norway
- ***National TB Research Plans and their strategic role in improving program performance: the experience of Ethiopia.*** Second TB Research Funders' *Forum*, 5-6 April 2017, NIH/NIAID Conference Center, Bethesda, Maryland, USA.
- **An integrated approach to unraveling susceptibility to tuberculosis in Africa.** H3Africa Applicant Interview. 14 September 2017, Nairobi, Kenya.
- ***TB Research in high burden setting: experience at AHRI, Ethiopia.*** TRIP Seminar Emory University. 8 November 2017, Atlanta, USA.

Conference participation:

- *First meeting of H3Africa (TBGENAfrica) grant principal investigators.* African Society of Human Genetics, 2017 Annual Conference. 15-20 Nov 2017, Cairo, Egypt.
- *Global Coordination Mechanism for R&D to prevent and respond to epidemics*. Wellcome Trust, London, UK, 28 March 2017
- Training: *Executive Program for Global Health Leadership*. 24-29 September 2017. LSHTM and Chatham House, London, UK


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Federal Democratic Republic of Ethiopia
Ministry of Health


Sida


Norad
Norwegian Agency for Development Cooperation


World Health Organization


EDCTP


ADDIS ABABA UNIVERSITY


Karolinska Institutet


EMORY UNIVERSITY


LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE


TDR
For research on diseases of poverty


The Research Council of Norway
With funding from
The Research Council of Norway


The African Academy of Sciences


giz
Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH


UiO : University of Oslo


LUND UNIVERSITY


ÖREBRO UNIVERSITY


UPPSALA UNIVERSITET


JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN


brighton and sussex medical school


Paul-Ehrlich-Institut


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CHALLENGE TB


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JIMMA ROAD, ALERT COMPOUND
ADDIS ABABA, ETHIOPIA
P.O. BOX 1005
TELEPHONE +251-113-483752

E-MAIL info@ahri.gov.et/ ahri@ahri.gov.et /
WEBSITE www.ahri.gov.et