Brief of INDONOR Research Proposals under joint initiative
"Indo-Norwegian (INDONOR) Co-operation on Antimicrobial Resistance (AMR)"

Background:

During the visit of Hon'ble President Pranab Mukherjee to Norway in October, 2014 a MoU for co-operation between Indian Council for Medical Research (ICMR) and the Research Council of Norway (RCN) was signed by India ambassador to Norway Norman Anil Kumar Browne and Arvid Hallen, Director General of RCN. This cooperation seeks to establish a health research relationship for encouraging research in a range of health-related areas of mutual interest, including human vaccines, infectious diseases and antimicrobial resistance. The agreement shall promote direct cooperation within the field being organized through joint calls and funding for research proposals/projects as well as facilitating exchange of scientists and scientific information.

Following this bilateral agreement, ICMR called for **INDONOR Research Proposals under Indo-Norwegian Co-operation on AMR in the first quarter of 2017.** A similar call for Proposals had been issued by RCN for Norwegian investigators under this program. The suggested projects would include principal partners from India and Norway. ICMR and the Norwegian Research Council (NRC) will jointly administer the program and fund the Indian and the Norwegian PIs respectively.

The following broad areas were covered in the call:

- Surveillance systems for AMR and antibiotic use in humans and/or animal population
- Design, implementation and evaluation of antibiotic stewardship programs including intervention studies to promote infection control and clinical practice guidelines in hospitals, primary care and veterinary medicine
- Novel strategies for diagnosis and treatment of infections caused by multidrug resistance bacteria
- Ecological, evolutionary and molecular properties of antimicrobial resistance

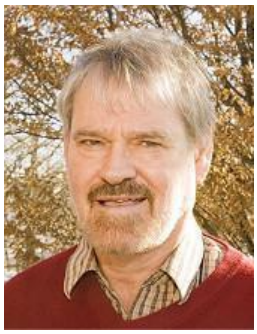
Six projects were shortlisted and selected for funding under this initiative by ICMR-RCN Joint Working Group.

Project 1. DRUG TARGETING FOR IMPROVED TREATMENT OF MULTI-DRUG RESISTANT TUBERCULOSIS (MDR TB)

Indian PI
Dr. Amit Misra, Ph.D
Pharmaceutics & Pharmacokinetics
CSIR-CDRI, Lucknow, India

Amit Misra is a pharmacist, and heads the Pharmaceutics and Pharmacokinetics Division of CSIR-Central Drug Research Institute, Lucknow, India. He is interested in formulation of low-cost and industrially-scalable drug delivery systems for infectious diseases and male contraception. He is the Vice President (India) for the Asian Federation for Pharmaceutical Sciences (AFPS). Amit is privileged to be associated with a large inter-disciplinary group spread across four continents, that addresses inhaled therapies for tuberculosis in a cooperative rather than competitive spirit.

Norwegian PI
Prof. Gareth W Griffiths
Dept. of Molecular Biosciences
University of Oslo, Norway



Gareth W Griffiths is a cell biologist, biophysicist and abiogenesis theoretician working at the University of Oslo, Norway. He has made seminal contributions to the field of electron microscopy, especially immunolabeling of cryo-sections to map intracellular traffic. He is interested in elucidating the fate of particles taken up by macrophages in the context of infection with *Mycobacterium tuberculosis*. Gareth Chairs the European Molecular Biology Laboratory (EMBL) Alumni Association. He has brought in the research groups of David Russell (Cornell), Andrew Thompson (Auckland), Mathias Barz (Mainz) and Bruno de Geest (Ghent) as contributors to the present project.

PROJECT SUMMARY

Multi-drug resistant (MDR) TB is a global public health issue. It requires treatment for over 2 years with extremely toxic drugs In order to propose a game-changing, novel strategy for treatment, it is crucial to have drug delivery systems with reasonable throughput and acceptable costs that validates claims of enhanced targeting to macrophages harboring Mtb. and also killing the bug more efficiently. The project

aims at synergizing these two chief areas by combining expertise of an Oslo group developing NP for injection and an Indian group focused on TB inhalation therapy using larger micro particles (MP). A New Zealand group specializing in derivatives of the new MDR-TB drug PA-824 and two NP specialist groups in Germany and Belgium will give crucial support. The primary objectives of the project are

1. To generate data on a series of diverse compositions and sizes of particulate drug delivery systems containing more potent analogues of pretomanid (PA-824), bedaquiline and linezolid to demonstrate “Superiority” of particle-incorporated drugs over “free” drugs in two animal models (zebra fish embryo model of granulomatous TB and aerosol infection mouse model of TB).

2. To develop a rational, comprehensive translation strategy for selecting injectable and inhalable particulate novel drug delivery systems for preclinical development. Improved delivery of existing drugs may not tackle the root cause of MDR but advantage of the encapsulation approach is to reduce toxicity, provide combination therapies and shorter treatment regimens leading to better compliance which in itself will reduce the threat of resistance development.

Project 2. ANTI-MICROBIAL PEPTIDES (BACTERIOCINS) AS ALTERNATIVE TO CONVENTIONAL ANTIMICROBIAL AGENTS - A NOVEL INTERVENTIONAL STUDY FOR TREATING INFECTED PLANTAR ULCERS IN LEPROSY AND DIABETES

Indian PI
Dr.Aparna Srikantam, M.D
LEPRA Society-Blue Peter Public Health and Research Center
Hyderabad, India

Aparna Srikantam is a clinical microbiologist by training. Her main research interests include antimicrobial resistance and its clinical implications. Her group is known for community based research on antimicrobial resistance in TB and leprosy. Also of special interest is the tackling of secondary infections on chronic foot ulcers in leprosy and diabetes. Secondary bacterial infections are studied through a multidisciplinary approach ranging from profiling of infections, molecular detection of antimicrobial resistance (through omics approach) to identifying suitable clinical interventions for tackling the same. The findings from these research studies are expected to be translated into clinical applications towards promotion of rational use of antimicrobials.

Norwegian PI
Prof. Dzung B. Diep, Ph.D
Dept. of Chemistry, Biotechnology and Food Science.
Norwegian University of Life Sciences
Oslo, Norway

Dzung Diep is a molecular microbiologist by training. The main research theme in his group is molecular microbiology. Lactic acid bacteria as well as pathogens are organisms being studied. An important and recognized area of research from this group is antimicrobial peptides (bacteriocins) produced by many bacterial species. Their mode of action, target recognition, immunity, resistance development as well as gene regulation are some of the topics being studied in great detail. Also of special interest is the development of bacteriocins into useful applications including food preservation and fighting infections and antibiotic resistance.

PROJECT SUMMARY

Plantar ulcers one of the major complications in leprosy and diabetes have significant socioeconomic impact on the patients and the health care settings. Plantar ulcers with community acquired infections

are almost treated on empirical antimicrobial regimen, which could potentially lead to emergence of antimicrobial resistance (AMR).

Proposed study aims are

1) To profile the secondary bacterial species infecting foot ulcers in leprosy and diabetes by conventional and metagenome analysis methods

2) To study *in-vitro* (microbiological tests) and *in-vivo* (mouse infection model) anti-biofilm activity of pathogen specific antimicrobial peptides.

3) To study the efficacy of topical antibacterial peptides as an alternate strategy in treating infected plantar ulcers.

This collaborative study will provide the wound care provider with information necessary to administer appropriate treatment for traditional antibiotic use. In addition the novel strategy of using bacteriocins could be an alternative intervention for treating bacterial AMR.

Project 3.STRUCTURE BASED TARGET EXPLORATION FOR THE DISCOVERY OF NEW LEADS FOR ANTIBIOTICS

Indian PI
Dr. Prathama S Manikar
Division of Medicinal Chemistry & Pharmacology
CSIR-Indian Institute of Chemical Technology,
Hyderabad, India

Prathama S. Mainkar is a synthetic organic chemist by training and has worked extensively in drug discovery. A molecule developed by her group has reached clinical trial Phase IIa. Another molecule is in pre-IND studies. Her expertise is in synthesis of complex molecules and library of molecules for SAR studies and pharmacophore identification. She has also worked on the identification of novel scaffolds for the treatment of tuberculosis. At present she working on total synthesis and analogue studies of the latest identified antibiotic teixobactin.

Norwegian PI
Professor Brenk Ruth
Department of Biomedicine
University of Bergen, Norway.

Ruth Brenk, a pharmacist by training, is an expert in virtual screening and structure-based ligand design, X-ray crystallography and drug discovery. In 2014, she took up a position as full professor at the University of Bergen in the Department of Biomedicine, where she is leading a research group in the area of structure-based drug design and molecular recognition. Currently, her group focuses on the exploitation of structural knowledge about protein and RNA targets for the design of new antibiotics.

PROJECT SUMMARY

There is increasing evidence that low clog P values and high affinities are crucial for drug penetration in to Gram negative bacteria. The project proposal will use that property as a driver for identifying and synthesizing new families of compounds targeting selected target enzymes.

Two more potential antibacterial targets have been identified. Pantothenate kinase from Pseudomonas aeruginosa (PaPanK) belongs to type III is structurally distinct from the human homolog which belongs to type II. A third target 4'- phosphopantetheine adenylyltransferase (PPAT) is inhibited by cycloalkyl pyrimidines which are active against PPAT from Gram-positive bacteria. The panel found the rational and preliminary results available for this project compelling. Work will be carried out on all three targets.

The main objectives are

1. To explore new scaffolds for targets for antibiotic activity against Gram-negative bacteria
2. Validate structure based druggability predictions.
3. To validate the HITs from screening assays.
4. To optimize at least one HIT series into a lead series

The approach will make use of the program DrugPred which was developed by the Brenk group to identify druggable pockets. Three enzymes have been identified as potentially suitable targets.

FabF seems to be a good antibacterial target especially as assays and X-ray structures are available in the Brenk group. The target has been validated in Gram-positive bacteria but Gram-negative bacteria do not respond to the inhibition of FabF as the molecules do not have accessibility to the target. Herein is a good opportunity to combine the synthetic capabilities of the Prathama group in India to develop large numbers of compounds with better penetration and biophysical and computational studies in Norway.

Project 4.AMR-DIAG: A NOVEL DIAGNOSTIC TOOL FOR SEQUENCE BASED PREDICTION OF ANTIMICROBIAL RESISTANCE

Indian PI
Dr. Punit Kaur
Department of Biophysics,
AIIMS, New Delhi

Punit Kaur is Professor and Head, Department of Biophysics, All India Institute of Medical Sciences, New Delhi. Her research interests are Bioinformatics, Genomics, and Structural Biology and Rational structure based drug design. She has experience in analysis of big data related to next generation techniques as well as X-ray crystallography and *in silico* structure- and ligand-based drug discovery. Her work involves structure determination of protein molecules to establish structure-function relationships and designing newer antimicrobial agents. Simultaneously she is exploring the data generated from NGS to arrive at the determinants of resistance in clinical isolates in the Indian scenario.

Norwegian PI
Dr. Rafi Ahmad
Bioinformatics & Drug Discovery,
Dept. of Natural Science & Technology
Inland Norway University of Applied Sciences, Norway

Rafi Ahmad is an Associate Professor in Bioinformatics and Drug Discovery at Inland Norway University of Applied Sciences. His educational background includes a Bachelor degree in Pharmacy from Jamia Hamdard, Delhi, Master of Science in Bioinformatics from University of Exeter, UK and a PhD in Bioinformatics from University of Tromsø, Norway. Rafi has 5+ years of experience working with Big Pharma including Astra Zeneca & Medivir. He is the project leader/WP leader of a number of projects, which have received research funding through various national and international grants in excess of 50 Million NOK. He is also an expert reviewer for EU H2020 CNECT HEALTH CALLS (2017 and 2018).

PROJECT SUMMARY

This project aims at developing a comprehensive tool/method for predicting antimicrobial resistance profiles using genomic signatures

Objectives

1. Development of a bioinformatics tool for rapid detection of AMR through
   a. Identification of variation landscape of selected bacterial isolates from India and Norway
   b. Development of algorithm for identifying bacterial strains and resistance profile using deep learning Techniques
2. Validation of developed tool using clinical isolates through genomics and proteomics approaches
3. Implementation and evaluation of developed tool in clinical settings of India and Norway for culture and culture-free identification of organisms and resistance profiles using clinical specimens

AMR-Diag tool will be designed for use by doctors and other health care professionals, providing information needed in order to choose the best treatment. The developed prediction tool will be fully automated, friendly and easy to run or interpret results. It will incorporate an appropriate and extensive user manual for ready reference by the user..Once the tool is developed, tested and validated, it will be designed and implemented on servers for online prediction as well as for personal computers/laptops/desktops for standalone/offline predictions.

Project 5. INHIBITION OF CLINICALLY RELEVANT CARBAPENEMASES (ICARBA).

Indian PI

Dr. Ranjana Pathania, Ph.D

Drug Discovery Group, Dept. of Biotechnology

IIT, Roorkee, India

Ranjana Pathania is currently an Associate professor in the Department of Biotechnology at Indian Institute of Technology Roorkee. Her research focuses on various aspects of clinically relevant Gram-negative bacteria. She leads the Molecular Bacteriology and Chemical Genetics group at IIT Roorkee which follows a two pronged approach towards antibiotic resistance. On one hand, she is trying to understand the mechanisms responsible for antibiotic resistance which led to identification of regulatory small RNA and drug efflux pumps; on the other she is developing novel strategies to counter determinants of drug resistance and drug resistant pathogens.

Norwegian PI

Dr. Hanna-Kirsti Schrøder Leiros

Dept. of Chemistry

UiT The Arctic University of Norway, Norway

Hanna-Kirsti Leiros is a scientist in the Dept. of Chemistry, The Arctic University of Norway, Tromso. She is a structural biologist and extensively works on determining biophysical interactions of antibiotic resistance proteins and their inhibitors. Her research intends to develop novel drugs and inhibitors of antibiotic resistance based on their structural properties.

PROJECT SUMMARY

Carbapenemases are the enzymes that confer the ability to resist the antibacterial action of carbapenems, the last resort antibiotics. Since the scattered earlier reports on identification of carbapenemases, these enzymes now have been isolated from all over the world and need immediate attention**.**

The overall aim of the study is to develop inhibitors against inhibitor resistant carbapenemases, OXA-48 and KPC-2. In collaboration, HKSL and RP have screened inhibitors for the metallo-betalactamase enzymes NDM-1, VIM-2 and GIM-1 and gained knowledge in inhibitor screening, complex structure determination and inhibitor development that will be valuable for this project.

Objectives:

1. To design current fragments into highly active carbapenemase inhibitors for use in cell based screening to identify lead compounds that revive the activity of carbapenems against the bacterial cells expressing either KPC-2 or OXA-48 carbapenemase.

2. To screen for putative inhibitors against clinically relevant OXA-48 and KPC-2 carbapenemases using small molecule libraries.

3. To validate and identify potential carbapenemase inhibitors by Structure Activity Relationship (SAR) and biophysical characterization.

4. To assess safety and efficacy of inhibitors *in-vivo* using mice models of infection to evaluate pre-clinical potential.

Project 6. BORN IN THE TWILIGHT OF ANTIBIOTICS: IMPLICATIONS OF ANTIBIOTIC USE TO THE PRETERM INFANT RESPIRATORY MICROBIOME AND RESISTOME DEVELOPMENT

Indian PI

Dr. Sushma Nangia MD, DM

Department of Neonatology,

LHMC & KSCH, New Delhi, India

A Neonatologist by training, **Sushma Nangia's** main research domains include neonatal sepsis, enteral feeding of preterm neonates and neonatal resuscitation. Her team's works in different aspects related to meconium have influenced resuscitation strategies. These include passage by the fetus and resuscitation-related research in neonates born through meconium stained amniotic fluid. Her centre has been part of a multi-site regulatory trial that has tested efficacy of affordable surfactant therapy. This is in addition to developing algorithms for rational surfactant therapy for preterm neonates with respiratory distress syndrome.

Rational antibiotic prescription, AMR and its clinical implications are her major recent focus areas. This research is an exciting area with emphasis on preterm infant's respiratory microbiome and its disruption with antibiotic therapy, while considering the possibility of resistome development. Findings from this research work are expected to be translated into clinical application towards promotion of rational use of antimicrobials in vulnerable preterm neonates.

Norwegian PI

Prof Fernanda Petersen

Department of Oral Biology,

University of Oslo, Norway

Prof Fernanda Petersen work focuses on understanding how microbes residing in the mouth and respiratory tract communicate with each other and with the human host. My team's research has identified and characterized cell-to-cell communication signals that enable microbes living in these sites to survive stress and exchange antibiotic resistance genes. We are now working on the impact of antibiotics on human microbial communities and how to mobilize resident microbes to design therapies that prevent infections. To achieve our goals, we have teamed up with international researchers with competencies that extend from microbial genetics and immunology to systems biology, public health, infectology and neonatology.

PROJECT SUMMARY

This project addresses the urgent need for improved evidence-based guidance for antibiotic use in preterm infants. The investigators aim to gain insights into how the respiratory microbiome and the collection of resistance genes carried by the microbiome, the resistome, develop in preterm infants in response to different antibiotic interventions. They will investigate differences in the microbial composition and resistome development in the upper respiratory tract, and identify risk factors for the development of respiratory microbiomes with enhanced pathogenic potential and antibiotic resistance in preterm infants in India and Norway. Moreover, using a mouse model, will investigate how different antibiotic treatment modalities may increase the risk of preterm, compared to term infants, to respiratory infections.

The project is based on already established successful collaborations between LHMC & KSCH hospitals in New Delhi and the Oslo University Hospital (OUS), and on ongoing collaborations between OUS, the University of Oslo (UoO), and the Norwegian Institute of Public Health (NIPH).