Indian Council of Medical Research

ICMR NIRT

National Institute for Research in Tuberculosis

ANNUAL REPORT 2017-18

WHO Collaborating Centre for Tuberculosis Research & Training
International Centre of Excellence in Research

NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS

Research Activities

April 2017– March 2018

CONTENTS

Cont'd…

PREFACE

In the year under review (2017-18), research was conducted in the clinical, socio-behavioural, bacteriological, epidemiological and basic science related aspects of tuberculosis.

A randomized controlled clinical trial was completed for comparing daily versus intermittent six month short course anti-TB chemotherapy for reducing the failures and the emergence of acquired rifampicin resistance in patients with HIV and pulmonary tuberculosis.

A multi-centric cohort study for the recurrence of tuberculosis among newly diagnosed sputum positive pulmonary tuberculosis patients treated under the Revised National TB Control Programme was completed. A study was carried out to determine whether administration of Evening DOTS was acceptable and improved treatment adherence among TB patients in India. A randomized clinical trial to study the efficacy and tolerability of 4-month regimens containing moxifloxacin in the treatment of patients with sputum positive PTB was completed and the manuscript submitted for publication.

A randomized clinical trial to study the efficacy and tolerability of a 4-month regimen containing ofloxacin compared to the standard 6-month regimen in the treatment of patients with superficial lymph node TB is ongoing.

As part of the C-TRIUMPh study, a cohort of tuberculosis patients and household contacts are being followed up and a repository of biological samples has been developed which could be utilised for studies related to development of TB related biomarkers.

A study is ongoing to determine the prevalence of TB infection and disease among pediatric household contacts of MDR-TB patients. The effectiveness of food supplement on treatment outcomes and nutritional status of adults with PTB on a retreatment regimen is being evaluated in another study being conducted in Vellore region. A study in collaboration with Cambrige University from UK is ongoing to study the antimicrobial resistance pattern in M. tuberculosis in the Indian setting together with studies related to efflux pumps and to T cell co-stimulatory pathways for controlling T cell exhaustion in DR and DS TB patients.

Among the ongoing clinical trials, a study was carried out to compare the feasibility, acceptability and effectiveness of pharmacologic therapy (using Bupropion SR) versus enhanced counselling package for cessation of smoking among TB patients initiating anti-TB treatment, under program conditions in India.

The Social and Behavioural Science Department at NIRT has conducted studies for improving adherence to anti-TB treatment, TB in tribal populations, HIV prevention among MSMs and various studies related to the TB Free Chennai project.

Various laboratory studies conducted at NIRT included studies related to diagnosis of tuberculosis, TB drug resistance (including NGS), pharmacokinetics, PK of second line anti-TB drugs and studies related to HIV and HIV associated TB and studies related to the immunology of tuberculosis.

The Department of Epidemiology at ICMR-NIRT has carried out a TB prevalence survey in the Tiruvallur area, which was completed recently. The UNOPS funded TB Free Chennai Project is ongoing as a collaborative project with the Corporation of Chennai, the NGO REACH and ICMR-NIRT and the various components of the project are included.

International Centre for Excellence in Research (ICER) has continued its research related to the immunology of parasitic infections as well research related to immunology of TB lymphadenitis. It has also studied the effect of diabetes on the immune responses in TB.

Training of MSc and PhD students has been an ongoing activity at NIRT. In addition, training programs are also conducted for MBBS and MD students as well as for nurses. Training of IRL staff as well as other staff of the RNTCP has also been conducted by NIRT.

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Sl. No	**Date of Lecture**	**Name of the speaker**	**Affiliation of the speaker**	**Topic of the Lecture**
1	April 13th 2017	Dr. Santha Devi	Ex-Staff of NIRT	Genesis of MDP
2	April 13th 2017	Dr. Rajeswari	Ex-Staff of NIRT	Operational Research Studies of MDP
3	April 13th 2017	Ms. Sudha Ganapathi	Ex-Staff of NIRT	Sociological Studies of MDP
4	April 13th 2017	Mr. P.G. Gopi	Ex-Staff of NIRT	Epidemiological Studies of MDP
5	July 11th 2017	Dr. Balamurugan. K	Staff scientist, NCI-NIH, Fredrick – USA	The CEBPD transcription factor at the cross roads of hypoxia, inflammation and cancer
6	July 27th 2017	Dr. Soumya Chatterjee	Assistant Professor, Division of Infectious Diseases, Allergy & Immunology, Departments of Internal Medicine & Molecular Microbiology, Saint Louis University, St. Louis, MO-USA	Discovery and Characterization of a Novel Mycobacterial-specific CD4+ Memory T cell
7	August 1st 2017	Dr. Bagavan Das	SRM University, Chennai	Big Data and its Applications
8	August 2nd 2017	Prof. Andrea Cooper	Professor of Cellular Immunology, Department of Infection, Immunity and Inflammation, University of Leicester, UK	T cells and TB: friends or foes
9	November 15th 2017	Dr. Santhosh Deshpande	Kanchi Kamakoti Childs Trust Hospital, Chennai	Dengue - The Myths and reality
10	November 29th 2017	Dr. Ranjani Namasivayam	Laboratory of Parasitic Diseases, NIAID, NIH Bethesda, USA	Conventional anti-tuberculosis therapy triggers a persistent intestinal dysbosis
11	December 5th 2017	Dr. Nimalan Arinaminpathy	Assistant Professor in Mathematical Epidemiology, Imperial College, London	Dynamics and control of tuberculosis: how mathematical modelling can help!

ABBREVIATIONS

AIC	Akaike information criterion
ARR	Acquired rifampicin resistance
ART	Anti-retroviral treatment
ATT	Anti-TB treatment
BDQ	Bedaquiline
BIC	Bayesian information criterion
BMI	Body mass index
CD	Crohn's disease
CFU	Colony forming units
CHV	Community Health Volunteers
CM	Conditioned medium
CVL	Cervicovaginal lavage
DBMS	Data base management system
DCS	D-cycloserine
DEL	Delamanid
DM	Diabetes mellitus
DMC	Designated microscopy center
DRMs	Drug resistance mutations
DR-TB	Drug resistant-TB
DST	Drug susceptibility testing
EBA	Early bactericidal activity
EMB	Ethambutol
FDC	Fixed dose combination
FGDs	Focus group discussions
FNAC	Fine needle aspiration cytology
Fqs	Fluoroquinolones
GIS	Geographical Information System
GTT	Glucose Tolerance
HC	Healthy controls
HCC	Hepatocellular carcinoma
HCV	Hepatitis C Virus
HHC	Healthy household contacts
HIVDR	HIV drug resistance
HUVEC	Human umbilical vein endothelial cells
IBD	Inflammatory bowel diseases
IC	Indeterminate colitis
IGRA	Interferon Gamma Release Assay
INH	Isoniazid
IRIS	Immune reconstitutiton inflammatory syndrome
LIS	Laboratory Information System
LPA	Line probe assay
LTBI	Latent TB infection
LRP	Luciferase reporter phage assay
LSP1	Leukocyte specific protein-1
MAP	*M. avium*subspecies*paratuberculosis*
MDR-TB	Multi-drug resistant TB
MERM	Medication event reminder monitor

MGCT	Microbial Genome Comparative Tool
MIC	Minimum inhibitory concentration
MOX	Moxifloxacin
MR	Molecular replacement
MSM	Men having sex with men
MTDV	Mobile TB diagnostic van
NACO	National AIDS Control Organization
NC	Non cultivable
NGS	Next generation sequencing
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
NSP	New sputum positive
NTM	Non-tuberculous mycobacteria
NVP	Nevirapine
OFX	Ofloxacin
PBMC	Peripheral blood mononuclear cells
PCR-RFLP	Polymerase chain reaction based restriction fragment length polymorphism
PIs	Protease inhibitors
PMDT	Programmatic management of drug-resistant TB
PPS	Probability Proportional to Size
PT	Proficiency testing
PTB	Pulmonary tuberculosis
PTLFU	Pretreatment loss to follow-up
PZA	Pyrazinamide
RBT	Rifabutin
RCT	Randomized clinical trials
RMP	Rifampicin
RNTCP	Revised national TB control programme
RPF	Resuscitation promoting factor
RTV	Ritonavir
SNPs	Single nucleotide polymorphisms
STI	Sexually transmitted infection
TBM	Tuberculous meningitis
TBDM	TB and DM
TDF	Tenofovir
TF	Transmitted founder
TST	Tuberculin skin test
TU	TB units
VCF	Variant calling file
VDBP	Vitamin D binding protein
WGS	Whole genome sequencing

CLINICAL STUDIES

DEPARTMENT OF CLINICAL RESEARCH

STUDIES COMPLETED:

CL-1: A randomized controlled clinical trial comparing daily vs. intermittent 6 – month short course chemotherapy in reducing failures & emergence of acquired rifampicin resistance in patients with HIV and PTB – Study XXV

Principal Investigator	:	Dr. G. Narendran (email:nareng@nirt.res.in)
Co-PI	:	Dr. Soumya Swaminathan
Collaborators	:	Govt. Hospital of Thoracic Medicine, Tambaram; Govt. Rajiv Gandhi General Hospital, Chennai; NJIL and OMD, Agra; Govt. Stanley Hospital, Chennai; Govt. Otteri Hospital, Chennai; Govt. Vellore Medical College and Hospital, Vellore; Govt. Rajaji Hospital, Madurai
Source of funding	:	USAID (Thro' WHO Model DOTS Project)
Study period	:	2009-2017
Trial Registry No.	:	476/09, NCT No. 933790

Background: HIV-associated TB poses various complications in management. One of the debatable issues is the periodicity of dosing schedule and when the study was started, no randomized clinical trials (RCTs) were available n this aspect. We undertook this study to evaluate not only the efficacy but also into the intricate aspects of dual infection like sputum conversion, radiological clearance, immune reconstitution inflammatory syndrome (IRIS), treatment of emergent adverse drug reactions and drug levels.

Aims:

Primary: (i) To compare daily vs. intermittent therapy of anti-retroviral treatment (ART) in reducing failures and emergence of acquired rifampicin resistance (ARR)

Secondary: Sputum conversion, IRIS, emergence of ARR, radiological improvement, drug levels of anti-TB drugs and toxicity profile with respect to dosing schedule

Methods: HIV-TB patients with smear positive or Xpert-MTB/ Rif TB positive patients were randomized to three regimens viz:(1) daily regimen (2EHRZ7/4HR7), (2) part daily (2EHRZ7/4HR3) and (3) a fully intermittent regimen (2EHRZ3 /4HR3), given for 6 months duration and followed up for a further period of one

year, with stratification based on CD4 counts and sputum smear grading. Blood samples at 2-hr post dosing are being collected at months 2 and 6 of anti-TB treatment (ATT). Toxicity was monitored using modified DAIDS criteria. Unfavorable responses in each regimen during treatment and follow-up were compared. Only culture positive rifampicin (RMP) sensitive patients were analyzed. Both intent to treat and per protocol analysis were performed.

Results: 331 patients were enrolled, 224 from Chennai and 107 from Madurai. Table 1 provides the baseline parameters of enrolled subjects. Sputum smear and culture conversion seemed better with a daily intensive phase compared to an intermittent intensive phase. (98% vs 87%, p=0.002). Overall incidence of IRIS was 28%. The second interim analysis showed daily regimen to be better in efficacy than the intermittent arm (91% vs 77%, $p < 0.05$), with the part daily regimen having a success rate of 80%. Figure 1 shows the survival among the three regimens. Hepatotoxicity was more with a daily intensive regimen especially with concomitant ART but was manageable under trial conditions. Overall toxicity grading is provided in Table 2.

Conclusions: Daily regimen for 6 months in HIV-infected TB patients on concomitant ART proved superior to an intermittent regimen, in terms of efficacy and prevention of ARR. A slightly higher incidence of hepatotoxicity, using daily regimen demanded closer monitoring of liver function especially with co-administered ART in the intensive phase. This mandates close monitoring of liver enzymes at least during the intensive phase of ATT. Contrary to the previous WHO guidelines, the part-daily regimen did not prove to be a substitute for daily regimen and conferred no added advantage over an intermittent regimen with regard to efficacy or tolerability but successfully prevented ARR. A higher CD4 cell count (≥ 150 cells) at the end of intensive phase rather at baseline, forecasted a successful outcome.

Table 1: Pretreatment demographic details of study participants

Variables	Daily regimen (n = 111)	Part-daily regimen (n = 110)	Intermittent regimen (n = 110)
Demographic details			
Age, mean(SD), years	38±9	39±9	40±9
Male No. (%)	86 (77%)	81 (74%)	84 (76%)
BMI, mean(SD)	16.79±2.71	16.87±2.66	17.36±2.74
Site of lesion			
Pulmonary (No.)	70	77	68
Pulmonary with Extra-pulmonary (No.)	41	33	42
Opportunistic infections at baseline (No.)	37	36	32
Pneumocystitis Carinii pneumoniae	8	2	4
Oral candidiasis	15	23	19
Herpes zoster/simplex	6	8	7
Hepatitis B and C	8	2	3
Mycobacterium Avium intracellulare (n)	2	1	1
History of alcohol intake (No.)	54	46	52
Smoker (No.)	40	37	47
Transmission (No.)			
Heterosexual	90	87	88
Homosexual	1	2	3
Intravenous drug user	7	2	3
Others	7	8	8
Biochemical and Haematological parameters			
RBC, mean(SD), 10^6cells/mm^3	3.55(3.01-4.12)	3.54(2.98-4.13)	3.62(3.14-4.15)
WBC mean(SD),10^3cells/mm^3	6.4(4.4-8.9)	6.7(4.7-9.5)	6.8(4.9-8.3)
Platelets, mean(SD), lakh cells/mm^3	3.09(2.36-4.07)	3.12(1.94-4.24)	3.15(2.44-4.23)
Haemoglobin, mean(SD),g/dl	9.81±2.14	9.43±2.08	9.88±2.05
Haematocrit, mean(SD),%	30.07± 9.97	28.96±8.8	29.66±6.66
Plasma Glucose, median(IQR), mg/dL	102 (92-120)	105(96-124)	102(90-125)
Serum Creatinine,	0.77±0.19	0.73 ±0.21	0.73±0.18

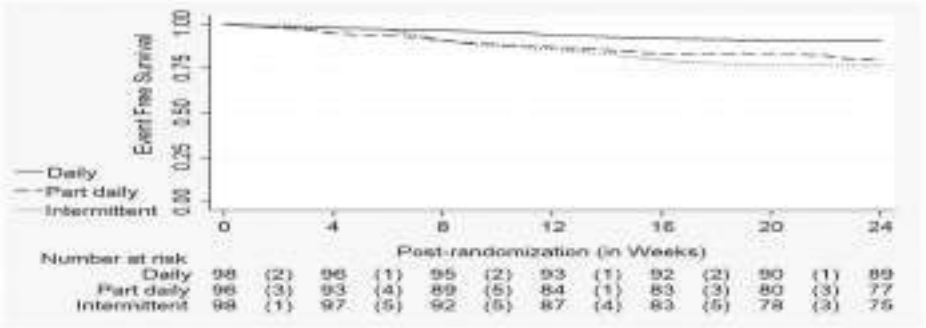
mean(SD), mg/dL			
Blood Urea, mean(SD), mg/dL	21.40±8.90	20.91±9.18	21.35±7.67
Serum. Bilirubin, median (IQR), mg/dL	0.5(0.4-0.7)	0.4(0.4-0.7)	0.5(0.4-0.7)
Serum AST, median (IQR), IU/L	37(24-66)	35(23-61)	34(25-52)
Serum ALT, median (IQR), IU/L	24(16-43)	22(13-33)	20(14-35)
Serum AP, median (IQR), IU/L	123(86-218)	108(74-189)	125(82-240)
Immunological parameters			
CD4, median (IQR), (cells/mm^3)	130(65-226)	144(71-261)	137(68-258)
CD8, median (IQR), (cells/mm^3)	600(334-957)	573(337-1048)	707(356-1018)
CD4/CD8 ratio	0.23(0.11-0.4)	0.23(0.16-0.40)	0.20(0.11-0.38)
Viral Load, mean (SD), log copies/mL	4.91±1.29	5.00±1.14	4.93±1.26
ATT-ART interval, median (IQR), days	16 (2-36)	17 (5-47)	15 (1-34)
CD4< 100, No. (%)	45 (41%)	38 (34%)	43 (39%)
Chest X-ray characteristics			
>3 zones, No./total (%)	71/111(64%)	82/110(75%)	67/110(61%)
Bilateral lesions (No.)	71	71	65
Lower lung TB (No.)	69	66	57
Cavitation (No.)	23	18	16
Pleural (No.)	24	18	17
Military (No.)	13	9	10
Mediastinal adenitis (No.)	31	30	25
Microbiological characteristics			
Sputum smear grade 2+, (No./total)	52/111	52/110	52/110
Culture grade 2+ or more, (No./total)	79/111	68/110	66/110
Sensitive to all first line drugs, No./total (%)	92/99(93%)	82/97(85%)	85/97(88%)
H-resistance, (No.)	5	11	11
MDR- TB at baseline (H and R-resistance)	4	4	2

Table 2: Treatment emergent adverse drug reactions among HIV-TB patients receiving the three ATT regimens

Toxicity type	Grades of toxicity	Daily regimen (n=111)	Part-daily regimen (n=110)	Intermittent regimen (n=110)
Gastrointestinal and hepatic	1 & 2	5	1	8
	3 & 4	11	11[b]	2
Neurological	1 & 2	11	8	8
	3 & 4	1	1	0
Cutaneous	1 & 2	1	0	1
	3 & 4	1[a]	0	0
Others	1&2	0	0	0
	3 & 4	0	2[c]	0
Overall Adverse drug reactions, No. (%)		30 (27%)	23 (21%)	19 (17%)

ATT was permanently discontinued in 4 patients (a - one case of Steven Johnson Syndrome;
b - two cases of jaundice; c- one case of thrombocytopenia)

Fig. 1: KM survival analysis of regimens during the 6 months of treatment



Time to unfavourable response was 22.76 (95% CI, 21.91 to 23.61) in the daily, 21.34 (95% CI, 20.11 to 22.57) in the part part-daily and 21.07 (95% CI, 19.91 to 22.22) in the intermittent regimen significant by the log rank test (P = 0.027).

CL-13: Multi-centric cohort study of recurrence of TB among newly diagnosed sputum positive pulmonary tuberculosis patients treated under RNTCP

Principal Investigator	:	Dr. Mohan Natrajan (email:mohan.n@nirt.res.in)
Collaborators	:	National Tuberculosis Institute, Bangalore, NITRD, New Delhi, RMRCT, Jabalpur, Thiruvananthapuram Medical College, Thiruvananthapuram, Mahatma Gandhi Inst. of Medcial Sciences, Wardha
Funding Agency	:	Central TB Division
Study period	:	2014-2017

Background: In the RNTCP, newly diagnosed smear - positive pulmonary tuberculosis (PTB) patients are treated with a 6-month thrice-weekly regimen. The country reports around 87% 'cure' among smear-positive patients. A TB recurrence rate of 10-12% has been reported from localized studies. There is minimal information on the proportion of patients who develop recurrent TB among those who have had a successful outcome at the end of treatment and on proportion of recurrent TB due to re-infection or endogenous reactivation.

Study Objective:

Primary objective:

(i) To estimate the recurrence of TB among newly diagnosed sputum positive PTB patients who have been successfully treated under Revised Naitonal TB Control Pogramme (RNTCP) with 6-month thrice-weekly regimen consisting of an initial intensive phase (IP) of INH (H), RMP (R), PZA (Z) and EMB (E) for two months followed by a continuation phase (CP) of H and R for 4 months ($2H_3R_3Z_3E_3$ / $4H_3R_3$)

Secondary objectives:

(i) To distinguish between *relapse* and *re-infection* among those who have recurrence of TB and

(ii) To identify risk factors for unfavourable treatment outcomes (treatment failed, lost to treatment follow-up and died) and recurrent TB

Methodology: Adult (aged $\geq$ 18 yrs) new smear positive PTB patients initiated on treatment under RNTCP were enrolled from sites in Tamil Nadu,

Karnataka, Delhi, Maharashtra, Madhya Pradesh and Kerala. Those declared as "treatment success" at the end of treatment were followed up at 3, 6 and 12 months after treatment completion. Study procedures included collection of relevant data from RNTCP records, patient interviews, sputum collection and examination by smear and culture, drug susceptibility testing (DST), HIV testing and measurement of capillary blood sugar. MIRU-VNTR genotyping was done to identify endogenous re-activation or exogenous re-infection at TB recurrence. TB recurrence was expressed as rate per 100 person-years (with 95% confidence interval [95%CI]). Regression models were used to identify the risk factors for unfavourable response to treatment and TB recurrence.

Results: Of the 1577 new smear positive PTB patients enrolled, 1565 were analysed. Majority, 1125 (72%) were males. The mean age $\pm$ SD was 41.05 $\pm$ 15.55 years (range 18 to 90 years) and the mean Body mass index (BMI) $\pm$ SD was 17.62 $\pm$ 3.44 kg/m^2 (range 10.22 to 38.93 kg/m^2). Of the 1565 patients, 1506 (96%) were non-reactive for HIV, 48 (3%) were reactive and HIV status was unknown in the remaining 11 patients. Two hundred and ninety three patients (19%) were found to have diabetes. There were 758 (48%) ever smokers and 806 (51%) ever alcohol users. The overall cure rate was 77% (1207/1565) and treatment success was 77% (1210 /1565). The cure rate varied from 65% to 86%. There were 158 of 1210 patients who had TB recurrence after treatment success. The pooled TB recurrence estimate was 10.9% [95%CI: 0.2-21.6] and TB recurrence rate per 100 person–years was 12.7 [95% CI: 0.4 – 25]. TB recurrence per 100 person–years varied from 5.4 to 30.5. Endogenous reactivation was observed in 56 (93%) of 60 patients for whom genotyping was done. Male gender was associated with TB recurrence.

Conclusion: A substantial proportion of new smear positive PTB patients successfully treated with 6 –month thrice-weekly regimen have TB recurrence under program settings.

CL-17: Improving treatment adherence among TB patients through evening DOTS in Chennai, India

Principal Investigator	:	Dr. Bella Devaleenal (email: bella.d@nirt.res.in)
Co-Principal Investigator	:	Dr.J. Lavanya, District TB Officer, Chennai district
Collaborators	:	Corporation of Chennai
Funding Agency	:	Global fund through Central TB Division and ICMR
Study period	:	Jan - Dec. 2017

Background: India has the highest burden of TB and multi-drug resistant TB (MDR-TB). Adherence to treatment plays an important role in improving treatment outcomes. A social assessment of RNTCP conducted in 2011 showed that in addition to socio cultural, economic and health system barriers in accessing care in RNTCP, there are additional difficulties such as fixed days of service, inconvenient fixed timings, waiting time etc. Studies conducted in different parts of India show that loss of wages and work timings are some of the reasons for non-adherence to ATT. Most of the urban populations are working in organized and unorganized sectors with fixed work timings. Chennai, where almost one fifth of the Tamil Nadu organized sector's working population lives has smear positive TB prevalence of 228/100000 population. We conducted a study to assess whether provision of DOTS in the evening improves treatment adherence and estimated the factors for treatment completion in Chennai district between 2016 and 2017.

Objectives:

(i) To compare the treatment outcomes among TB patients started on Category I treatment by evening DOTS and routine DOTS between 2016 and 2017 in Chennai district and

(ii) To determine factors for treatment completion among TB patients started on Category I treatment by routine DOTS and Evening DOTS in Chennai district between 2016 and 2017

Methods: In this prospective cohort study, adult TB patients aged ≥ 18 years diagnosed as Pulmonary or Extra pulmonary TB and initiated on CAT I in RNTCP in Chennai district between April 2017 to July 2017 were enrolled. A total of 7 TB units (TU) in Chennai

district were chosen for the study based on default rate and case load. All eligible participants in selected TUs were offered the option of taking DOTS in the daytime as currently being practised in the programme or from the evening DOTS centre (between 3.30 p.m. to 8 p.m.).

Those participants who opted for routine DOTS were given DOTS in the centres nearest to their residence. For those who opted for taking in the evening, DOTS was provided by identified treatment supporters in the evening DOTS centres established in already functioning hospitals, clinics and corporation centres between 3.30 p.m. and 8.00 p.m. As none of the patients opted for evening DOTS in one of the TUs, patients were given evening DOTS in 6 centres.

Patients were followed up at the end of intensive and continuation phases. The retrieval measures for patients who missed therapy were as per the RNTCP procedures.

Results: A total of 127 participants started on Cat I were recruited between April and July 2017. Fifteen percent (n=19) of the participants opted for taking DOTS in the evening. While almost every other patient opted for evening DOTS in one of the TUs and one third of the participants opted for evening DOTS in 2 TUs, there were no takers for evening DOTS in 1 TU. The incidence of Evening DOTS varied substantially and significantly between the seven clinics, the chi-square with 6 degrees of freedom being 20.9, P = 0.002. Treatment outcomes are indicated in Table 3. During baseline interview, 97% (n=105) of those who opted for regular timings chose the option because the centre from where they should take was near their residence and 68% (n=13) of them chose the evening time for DOTS because of the convenient timing and closeness to their work place. Factors influencing treatment outcome are given in Table 4.

Table 3: Treatment outcome in Category I patients who opted for routine and evening DOTS

Outcome	Routine DOTS n (%)	Evening DOTS n (%)	Total N (%)
Cured + Treatment completed	86 (79.6)	19 (100)	105 (82.7)
Death	3 (2.8)	0	3 (2.3)
Default	12(11.1)	0	12 (9.4)
Failure	1 (0.9)	0	1 (0.8)
Migrated	1 (0.9)	0	1 (0.8)
Private	2 (1.9)	0	2 (1.6)
Transferred Out	3 (2.8)	0	3 (2.4)
Total	108 (100)	19 (100)	127 (100)

Table 4: Factors influencing the treatment outcome among Cat I patients

Factors	No. of patients (N = 127) n (%)	Treatment success (N =105) n (%)	RR(95% CI)
Male#	89 (70.1)	69 (65.7)	0.82 (0.72,0.94)
≤ 40 years old	65 (51.2)	56 (53.3)	1.09 (0.93, 1.28)
Literate	104 (81.9)	88 (83.8)	1.15 (0.89, 1.48)
Employed	75 (59.1)	58 (55.2)	0.86 (074,1.00)
Married	88 (69.3)	72 (68.6)	0.97 (0.82,1.14)
Non Smokers	84 (66.1)	73 (69.5)	1.17 (0.96,1.42)
Non Alcoholic*	62 (48.8)	58 (55.2)	1.29 (1.10,1.52)

*p<0.01, #p<0.05

Conclusions: The proportion of patients who opted for evening DOTS varied in different TUs. This suggests that prior assessment of the need for Evening DOTS may have to be ascertained in each area before introducing Evening DOTS clinics. The purpose of Evening DOTS is to make DOTS more patient- friendly and thereby improve its outreach and improve treatment outcomes. These appear to be worth-while gains, however the cost of replicating this model under Programme conditions and the benefit of percent cured need to be ascertained before considering for implementation.

STUDIES IN PROGRESS:

CL-2: Smoking cessation strategies in TB patients

Principal Investigator	:	Dr.S. Ramesh Kumar (email: ramesh@nirt.res.in)
Collaborators	:	DTOs of Villupuram and Kancheepuram Dt; TCC – Adyar Cancer Institute.
Source of funding	:	USAID through MDP
Study period	:	2013-2018
Study Registry No.	:	CTRI/2013/07/003830

Background: Smoking prevalence among males (both rural and urban) has increased in India (NFHS III Vs NFHS II). Evidence for smoking as causal association for TB has been established with higher relapse, increased morbidity and mortality (4 times) in smokers with TB. A pilot study at NIRT (Madurai) showed, at month 1 that 35-45% of TB patients quit smoking when counseling/advice was given by Doctor or Social Workers.

Aim:

(i) To compare the feasibility, acceptability and effectiveness of pharmacologic therapy (Bupropion SR) versus enhanced counselling package in smoking cessation among TB patients initiating treatment, under program settings in India

Methods:

Study design: Cluster randomized effectiveness trial

Study Procedure: DMCs from two districts - Villupuram and Kanchipuram were randomly selected (cluster randomization) to receive T1 – Bupropion SR along with Standard counseling, or T2 – Enhanced Counseling arm including provisions of educative materials on smoking cessation, flip charts presentation, posters display, video presentation or C – Standard routine counseling/Control arm, so that each TB patient who were current smokers enrolled to the study received the intervention allotted to that study centre. Smoking cessation was assessed by self-reporting and confirmed by Carbon monoxide monitors, done at 0, 2 and 6 months (end of ATT). TB treatment outcome was recorded at the end of ATT for these patients. The sample size was calculated to be 600 patients. After recruiting 45 subjects to the pilot study,

recruitment to the main study was started in January 2014. A total number of 4290 patients were screened, of whom 517 patients were allocated to the study and the breakup of the recruitment between the 2 districts and the 3 arms are presented in Table 5.

Table 5: Number of subjects recruited in the three intervention arms, with district wise break-up

District	Drug	Enhanced	Standard	Total
Kanchipuram	62	73	75	210
Villupuram	90	97	120	307
Total	152	170	197	517

Interim analysis showed that out of the total 383 subjects for whom the quit status was available, the proportion of patients who have quit smoking in the Drug, Enhanced and Standard arms at the end of TB treatment was 67%, 83% and 51% (P= < 0.05) (Table 6).

Table 6: Quit status among the TB patients given different strategies for smoking cessation at RNTCP centres in Villupuram and Kancheepuram districts, Tamil Nadu, India

Final Status of smoking habit	Patients randomised to treatment arm			
	Bupropion n (%)	Enhanced Counseling n (%)	Standard Counseling n (%)	Total n (%)
Quit smoking	81 (66.9)	96 (82.8)	75 (51.4)	252 (65.8)
Still smoking	40 (33.1)	20 (17.2)	71 (48.6)	131 (34.2)
Total	121 (100)	116 (100)	146 (100)	383 (100)

As there was adequate power (>80%) to show the difference between the intervention arms, it was decided to analyse the study results. A complete analysis comparing the TB treatment outcome with quit status is under progress.

CL-3: Randomized clinical trial to study the efficacy and tolerability of 4-month regimens containing moxifloxacin in the treatment of patients with sputum positive PTB

Principal Investigator	:	Dr.V.V. Banu Rekha (Email: banurekha@nirt.res.in)
Source of funding	:	Intramural
Study period	:	2007-2019
CTRI Registration No.	:	PROVCTRI/2008/091/000024

Background: Shortening the duration of TB treatment from the currently recommended 6-month regimen for newly diagnosed PTB patients is a global research priority. The randomised clinical trial conducted by the ICMR-NIRT in Chennai, Madurai and Vellore compares relapse rates up to 24 months of follow-up after treatment in newly diagnosed smear and culture positive PTB patients treated with 4-month moxifloxacin (MFX) containing regimens with a 6-month regimen (control regimen). In this trial, the standard 4-drug TB regimen is supplemented with MFX, a fluoroquiniolone with potent bactericidal and sterilising activities against *M. tuberculosis*.

Methodology: HIV sero-negative, non-diabetic, newly diagnosed sputum positive PTB patients were randomised to 3-month or 4-month MFX regimens, or a 6-month control regimen (Table 7). Treatment was directly observed and response to treatment was assessed by monthly clinical evaluations and sputum examinations. The patients were closely monitored for adverse drug reactions. Patients with successful treatment outcome were followed up for 24 months after completion of treatment with monthly sputum and clinical evaluations for assessing recurrence of TB.

Table 7: Study regimens

Regimen	Intensive phase	Continuation phase	Duration (months)
Test regimen 1	3 RHZEM daily		3
Test regimen 2	2 RHZEM daily	2 RHM daily	4
Test regimen 3	2 RHZEM daily	2 RHM thrice weekly	4
Test regimen 4	2 RHZEM daily	2 RHEM thrice weekly	4
Control regimen	2 RHZE thrice weekly	4 RH thrice weekly	6

R – rifampicin; H – isoniazid; Z – pyrazinamide; E – ethambutol; M - moxifloxacin

Results: The enrollment to the study was completed in October, 2016, A total of 1371 patients have been enrolled in the study (May 2007 to October 2016). Forty two patients were excluded from the study as per the protocol criteria. The baseline characteristics of 1329 patients are shown in Table 8. Majority of the patients were males (75%), had advanced disease with 2+/3+ sputum cultures (95%) and radiological involvement of more than 2 lung zones (79%). There were 1162 (87%) patients who harboured bacilli susceptible to H, R, E and O.

Table 8: Baseline characteristics of new sputum positive PTB patients enrolled in the study (N=1329)

Patient characteristics		Regimen					Total N = 1329 n (%)
		Test Reg. 1 (N= 112) n (%)	Test Reg. 2 (N = 322) n (%)	Test Reg. 3 (N = 326) n (%)	Test Reg. 4 (N = 328) n (%)	Control Reg. (N = 241) n (%)	
Age years	< 35	56 (50)	136 (42)	162 (50)	162 (49)	125 (52)	641 (48)
	≥ 35 years	56 (50)	186 (58)	164 (50)	166 (51)	116 (48)	688 (52)
Gender	Male	89 (79)	249 (77)	245 (75)	233 (71)	184 (76)	1000 (75)
	Female	23 (21)	73 (23)	81 (25)	95 (29)	57 (24)	329 (25)
Pre-treatment sputum culture grading	≤ 1 +	3 (3)	13 (4)	19 (6)	23 (7)	7 (3)	65 (5)
	2 +	11 (10)	48 (15)	56 (17)	56 (17)	54 (22)	225 (17)
	3 +	98 (87)	261 (81)	251 (77)	249 (76)	180 (75)	1039 (78)
Extent of initial chest x-ray involvement (zones)	≤ 2	25 (22)	64 (20)	65 (20)	69 (21)	50 (21)	273 (21)
	>2	87 (78)	258 (80)	261 (80)	259 (79)	191 (79)	1056 (79)
Drug susceptibility profile							
Susceptible to H, R, E, O		98 (87)	289 (90)	277 (85)	289 (88)	209 (87)	1162 (87)
Resistant to any drug		14 (13)	33 (10)	49 (15)	39 (12)	32 (13)	167 (13)

R – rifampicin; H – isoniazid; E – ethambutol; O- ofloxacin

Sputum culture conversion at the end of 2 months of intensive phase of treatment was significantly higher in the MFX regimen (94%) [consolidated for all four test regimens] compared to the control regimen (77%) [p < 0.001]. This observation which was reported earlier (Annual Reports 2009-2010 to 2016-2017) suggests that significantly higher proportion of patients treated with MFX containing regimens become less infectious earlier compared to those treated with the control regimen.

At the end of treatment, 91% to 93% of patients treated with MFX regimens had a favourable response compared to 86% in the control regimen. [p<0.05 for Test regimens compared to control regimen] (intent-to-treat analysis). Of the 1193 patients with favourable response

at the end of treatment, preliminary analysis showed that 84 had recurrence of TB during MFX regimens, 16 (1.4%) required treatment modification. Follow-up of enrolled patients is ongoing.

CL-4: Randomized clinical trial to study the efficacy and tolerability of a 4-month regimen containing ofloxacin compared to the standard 6-month regimen in the treatment of patients with superficial lymph node TB

Principal Investigator	:	Dr. D. Baskaran (email: baskaran.d@nirt.res.in)
Collaborators	:	Govt. Stanley Hospital; Govt. Rajiv Gandhi Medical College Hospital, Kilpauk Medical College, Chennai; Govt. Vellore Medical College, Govt. Rajaji Hospital, Madurai and Corporation RNTCP centere in Chennai
Source of funding	:	Intramural
Study period	:	2013-2019
Trial Registry No.	:	CTRI/2013/03/003481

Background: TB lymphadenitis is the most common presentation of extra-pulmonary TB, accounting for 30–40% of cases in reported series. Under the RNTCP, patients with TB lymphadenitis are currently treated with a thrice weekly regimen (Category-I) with 4 drugs (RIF, INH, EMB and PZA) for the first two months followed by 2 drugs (RIF and INH) for the next 4 months. A study done at NIRT has shown that even less intensive regimens, viz. a 6-month regimen of $2RHZ_2/4RH_2$ and a 6-month regimen of RH daily were highly successful in patients with biopsy confirmed lymph node TB in Madurai, south India. Despite its efficacy, the regimen must be administered for at least 6 months to be fully effective. The delivery of TB chemotherapy in the field would be much easier if the duration of therapy could be shortened without sacrificing efficacy. This requires the development of agents with potent bactericidal and/or sterilizing activity against *M. tuberculosis*, which allows shortening the treatment duration.

In the current study we propose to investigate the 4-drug [RIF, Isoniazid (INH), Pyrazinamide (PZA) and Ofloxacin (OFX)] daily intensive phase of two months, followed by 3 –drug (RIF, INH and OFX) thrice weekly continuation phase. The control regimen for comparison of outcome measures with the test regimens proposed for this study will be the standard 6-month thrice-weekly regimen of RIF, INH, EMB and PZA for 2 months followed by RIF and INH for 4 months.

Objectives:

Primary objectives:

To compare the efficacy of the regimens in terms of

a. Response at the end of treatment;

b. Relapse up to 24 months of follow-up after treatment, in newly diagnosed superficial lymph node TB patients treated with 4-month OFX containing regimens, with the same outcome in those treated with a 6-month regimen (control regimen)

Secondary Objectives:

To compare the incidence of

a. "Paradoxical reaction" during treatment and follow-up;

b. Adverse drug reactions in newly diagnosed superficial lymph node TB patients treated with 4-month OFX containing regimens, with the same in those treated with a 6-month regimen (control regimen).

Study Design:

Type: Intervention

Design: Prospective, randomized (open-label) parallel arm, controlled clinical trial

Study Regimens:

Test regimen:

RIF, INH, PZA and OFX daily for 2 months followed by RIF, INH, and OFX thrice weekly for 2 months (2 RHZO daily / 2RHO thrice weekly) - Duration 4 months

Control regimen:

RIF, INH, EMB and PZA thrice weekly for 2 months followed by RIF and INH thrice weekly for 4 months (2 RHEZ thrice weekly / 4 RH thrice weekly) – Duration 6 months

Study Population:

Methodology: Patients attending the surgical, medical out-patient clinics of Govt. Stanley Hospital, Govt. Rajiv Gandhi Medical College Hospital, Kilpauk Medical College Chennai, Govt. Vellore Medical College, Govt. Rajaji Hospital Madurai and Corporation RNTCP Centre in Chennai in whom a

diagnosis of TB lymphadenitis is made by Fine needle aspiration cytology (FNAC) or clinical evidence of lymph node enlargement, are considered for the study.

After providing adequate information about the trial, consent is obtained for enrollment in the study. The patient is then referred to the surgeon for open biopsy of the lymph node (under general anesthesia). The open biopsy specimen of the lymph node, divided into 2 parts, is collected in 2 separate bottles for histopathology and bacteriological examinations. The specimens meant for histopathology are collected in formalin. Serial sections are made and the slides are read by an independent pathologist and graded.

The biopsy specimens are also examined by smear and culture methods for tubercle bacilli at NIRT. If the diagnosis of TB of the lymph node is confirmed either by histopathology or bacteriology the patient is considered eligible for enrollment to study if he/she satisfies the other inclusion criteria.

Those with lymph node histology not suggestive of TB are referred back for further management. Among this group of patients, if the lymph node culture yields *M.tb*, they are re-assessed and enrolled in the study if they have not been started on anti-TB treatment elsewhere. They are re-evaluated again for PTB before allocation to the study. The patients found ineligible for the study are referred back to the referring centre for appropriate management

Treatment: Patients allocated to test regimen (2 RHZO daily / 2 RHO thrice weekly) receive 60 daily doses in the intensive and 26 thrice-weekly doses in the continuation phase. Missed doses are compensated up to maximum of 15 days after the scheduled 2nd and 4th monthly examinations, so that the patients will complete the intensive and continuations phase each in a maximum of two months and 15 days.

Patients allocated to control regimen (2 RHEZ thrice weekly / 4 RH thrice weekly) receive 26 thrice-weekly doses in the intensive phase and 54 thrice-weekly doses in the continuation phase. Missed doses are compensated up to a maximum of 15 days after the scheduled 2nd and 6th monthly examinations so that the patients will complete the intensive phase in a maximum of two months and 15 days and the continuation phase in a

maximum of four months and 15 days respectively.

All patients undergo clinical evaluation every month including an assessment of the lymph node in the clinic. During these visits, patients are enquired about their general well being, evaluated for drug toxicity and information about any adverse events are recorded on a standardized toxicity form. All patients are followed up for a period of 24 months after completion of treatment; every month up to 12 months and then every 3 months for assessing recurrence of TB.

Study outcome:

The following outcome measures are compared between the test and control regimens:

Primary outcome:

(a) Favorable, Probably favorable but biopsy recommended, Unfavorable at the end of treatment

(b) Relapse during follow-up in those with favorable response at the end of treatment

Secondary outcome measure:

(a) Paradoxical reactions

(b) Adverse reactions to anti-TB drugs

The study is being conducted in Govt. Stanley Hospital, Govt. Rajiv Gandhi Medical College Hospital, Kilpauk Medical College Chennai, Govt. Vellore Medical College, Govt. Rajaji Hospital Madurai and Corporation RNTCP Centre in Chennai. The estimated sample size for this trial is 330 patients; so far 104 patients have been enrolled to the trial.

Study progress: Data relating to number of cases admitted in the study, the number of cases analysed and DST are given in the following Tables 9 and 10.

The study is ongoing.

NUMBER OF CASES ADMITTED IN STUDY = 201 (as on 30th March 2018)

Table 9: Recruitment details

		2EHRZ3/4RH3 (n = 100)	2OHRZ7/2OHR3 (n = 101)
Sex	Male	27	33
	Female	73	68

Table 10: Outcome details of patients (n=178)

	Outcome	n
2EHRZ3/4RH3 (n = 87)	Died	1
	Favourable	84
	Unfavourable	2
	Total	**87**
2OHRZ7/2OHR3 (n = 91)	Died	0
	Favourable	87
	Unfavourable	4
	Total	**91**

CL-5: Evaluation of newer diagnostic tools and feasibility of consensus case definition in the diagnosis of intrathoracic TB in children

Principal Investigator	:	Dr. Syed Hissar (email: syed.hissar@nirt.res.in)
Collaborators	:	Govt. Stanley Hospital (GSH), Chennai; Institute of Child Health (ICH), Chennai; Christian Medical College (CMC), Vellore; Govt. Vellore Medical College (GVMC), Vellore; Govt. Rajaji Hospital (GRH), Madurai
Source of funding	:	USAID (Model DOTS Project)
Study period	:	2013-2019

Background: The lack of a gold standard for diagnosis is a major obstacle to accurately quantifying the true burden of childhood TB which is probably both over and under-diagnosed among children in different settings. The need for improved TB diagnostics in children is consistently acknowledged. Promising novel techniques (Xpert® MTB/RIF, urine LAM) that have been developed for the diagnosis of TB need to be tested and validated in children. Xpert® MTB/RIF (Cepheid, Sunnyvale, USA) is an automated user-friendly real-time PCR assay designed for the rapid and simultaneous detection of *M. tuberculosis (M.tb)* and RMP resistance. A group of international experts have developed a consensus reference standard and case definition for PTB in children, for use in research and clinical settings. This study will provide an ideal opportunity to test the feasibility and clinical relevance of this consensus case definition.

Aims:

(i) To determine the diagnostic accuracy of Xpert® MTB/RIF (Cepheid, Sunnyvale, USA) in the diagnosis of intra-thoracic TB in children and to study the feasibility of utilizing the newly developed consensus case definition and

(ii) To compare the yield of *M.tb* from different specimen collection methods (expectorated / induced sputum, gastric lavage) in various age groups in the diagnosis of intra-thoracic TB

Methodology: All children aged < 15 years attending the pediatric out-patient department with any of the following are screened for the study - (a) cough (b) weight loss/ failure to thrive

(c) persistent unexplained fever (d) persistent, unexplained lethargy or reduced playfulness. Symptom screening, a detailed general and clinical evaluation are done. Chest X-ray, tuberculin skin test (TST), collection of gastric lavage / induced / expectorated sputum for Xpert® MTB/RIF, AFB smear, culture and DST if culture positive, are done.

In addition, in infants (i.e. aged < 1 yr), stools is collected for 2 consecutive days which is examined by Xpert® MTB/RIF for acid fast bacilli (AFB) smear, culture and DST if culture positive. Blood investigations and FNAC is done if needed. TB diagnosis in children is made and classified into the following groups based on smear result, chest radiograph and TST as confirmed TB, probable TB and others. Follow-up will be done at 2 weeks, 4 weeks, 8 weeks, and end of treatment.

Current status: The study was initiated in August 2013. Required sample size is 2761 children with respiratory symptoms. The study is currently enrolling children from five centres, namely Govt. Stanley Hospital (GSH), Chennai; Institute of Child Health (ICH), Chennai; Govt. Vellore Medical College (GVMC), Vellore; Christian Medical College (CMC), Vellore and Govt. Rajaji Hospital (GRH), Madurai.

As of March 2018, we have screened 4558 children, of whom 2201 children were successfully enrolled in the study (Table 11). Baseline characteristics of the enrolled children are detailed in Table 12. Overall 7.2% of children were bacteriolgoically positive by smear and/or Xpert and/or MGIT/LJ culture. Children positive only for AFB smear were 2.1% and children positive only for Xpert Mtb were 4.2% (Table 13). The study is ongoing.

Table 11: Site-wise recruitment status

	GSH	ICH	GVMC	CMC	GRH	Total
Number of children screened	1200	1245	334	239	1540	4558
Number of children enrolled	835	877	135	60	294	2201
Number of children on ATT treatment follow-up	130	163	30	14	20	357

Table 12: Baseline characteristics of children (n=2192)

		No. of children*
		N (%)
Age (n = 2192)	0-1 yr	88 (4.0%)
	2-5 yrs	896 (40.9%)
	6-10 yrs	889 (40.6%)
	11-15 yrs	319 (14.6%)
Sex (n = 2192)	Male	1196 (54.6%)
	Female	996 (45.4%)
TST (n = 1927)	Positive	488 (25.3%)
	Negative	1439 (74.7%)
Previously treated with ATT (n = 2015)	Yes	80 (7.0%)
	No	1062 (93.0%)
Contact with TB case (n = 2017)	Yes	495 (43.3%)
	No	647 (56.7%)
Chest X-ray abnormality (n = 1969)	Yes	273 (23.9%)
	No	869 (76.1%)
HIV (n = 1702)	Yes	12 (1.1%)
	No	1130 (98.9%)

* Table shows the data entered in the database

Table 13: Bacteriology results

	Total (n = 2088)
No. of Children positive for smear and/or LJ and/or MGIT and/or Xpert	152 (7.2%)
No. of Children Negative for all microbiological investigations	1937 (92.8%)
No. of children with smear results available	2088
No. of children with positive smears	45 (2.1%)
No. of children with atleast one available LJ culture result	2060
No. of children with LJ culture positive	88 (4.2%)
No. of children with atleast one available MGIT culture result	1990
No. of children with MGIT culture positive	108 (5.4%)
No. of children with atleast one available Xpert result	1771
No. of Xpert positive cases	76 (4.2%)
No. of RIF resistant cases	5 (6.7%)

CL-6: A prospective study to determine the incidence of TB among patients with type 2 diabetes mellitus

Principal Investigator	:	Dr.M. Makeshkumar (email: makeshkumar.m@nirt.res.in)
Collaborators	:	Govt. General Hosiptal, Chennai; Govt. Rajaji Hospital, Madurai; MV Hospital for Diabetes, Royapuram, Chennai.
Source of funding	:	ICMR
Study period	:	2013-2018

Background: The diabetes epidemic has a major impact on the epidemiologic dynamics of TB and poses several challenges in the control of TB in a resource-poor country like India. Diabetes/TB burden can be brought under control by timely diagnosis of TB among diabetics by intensified case finding, by adequate and effective treatment of detected cases and possibly preventive therapy. Given the serious threat posed by the diabetes epidemic on control of TB, and the current gaps in knowledge related to diagnosis, prevention and treatment of TB among diabetes persons in the Indian population, this cohort study was stated in a representative population to establish the Incidence of TB among Type 2 diabetes patients.

Aim:

Primary objective:

(i) To determine the incidence of TB among people with Type 2 diabetes mellitus

Secondary objectives:

(i) To identify risk factors for TB among people with Type 2 diabetes mellitus

(ii) To study the diagnostic accuracy of sputum smear for diagnosis of TB among people with Type 2 diabetes mellitus

(iii) To correlate clinical and radiographic features of TB with severity of Type 2 diabetes and

(iv) To evaluate the diagnostic accuracy of Gene Xpert MTB/RIF among Type 2 diabetes mellitus patients with suspected TB

Methods: This was a Multicentric prospective cohort study among Type 2 diabetic patients to study the incidence of TB. Study participants were recruited

from patients who attended Diabetic OPD at Government General Hospital Chennai, Government Rajaji Hospital Madurai, and MV Hospital for Diabetes, Royapuram, Chennai.

Study progress: The study recruitment was stopped in June 2017 and follow up of the recruited patients is ongoing. The recruitment details are shown in Table 14.

Table 14: Recruitment details

	Chennai	Sex	Madurai	Sex	Total
Screening	495	M -109	258	M-73	753
		F - 386		F - 185	
Admission	406	M-83	170	M-44	576
		F - 323		F- 126	
Refered Back	89		88		177
Discharged Cases	185		130		315
Drop out	47		11		58

Details of patients recruited to the study are shown in Table 15.

Table 15: Patient details

Parameter	Value
Mean age with SD	50.46 (±8.60)
Mean weight with SD	61.62 (±10.01)
HBA1C	8.18 (±1.63)
Fasting blood glucose	166.6 (±65.06)
Post-prandial blood glucose	276 (±91.20)

Two cases were smear positive during screening and those were referred to RNTCP for treatment.

Four cases were smear positive during follow up. Those patients were evaluated for further smears and X rays which were found to be normal. Cultures were negative in all 4 patients. One patient has isolated one culture positive and was further evaluated with smears and X ray, which were found to be normal and the subsequent smears and cultures were negative.

The study was reviewed by the Pre-SAC of NIRT in July 2017. The Committee recommended that the study be discontinued since not a single case of confirmed TB was detected in 2 years. No further recruitments have been done. Patients recruited to the study are being followed up.

CL-8: C-TRIUMPh: Cohort for TB research by the Indo-US medical partnership multicentric prospective observational study

Principal Investigator	:	Dr.C. Padmapriyadarsini (email: padmapriyardarsinic@nirt.res.in)
Funding Agency	:	DBT
Study period	:	2013-2018

This is a prospective multi-centric observational cohort study at two sites, Chennai and Pune, that is enrolling TB patients and their household contacts, to study host and microbial risk factors associated with progression of TB disease, response to treatment, progression from TB infection to disease as well as transmission. A repository of biological specimens is being created, that can be used for future basic science research including biomarker discovery, and be made available to investigators of this Partnership on request. The study is currently enrolling and as of 31st March 2018, 391 TB patients (Cohort A) and 551 household contacts (Cohort B) have been enrolled to the study. Table 16 shows the current status of patients in Cohort A.

Table 16: **Recruitment details**

Enrolment Update as on 2nd April, 2018								
Period		Upto 14		15 - 18		> 18		Total
		M	F	M	F	M	F	
Upto 2017								
	PTB	4	5	7	10	163	68	257
	EPTB	24	14	6	10	33	35	122
Jan - Feb, 2018								
	PTB	0	0	0	0	0	0	0
	EPTB	2	1	0	2	0	2	7
Mar-Apr, 2018								
	PTB	0	3	0	0	0	0	3
	EPTB	2	0	0	0	0	0	2
A	Total	32	23	13	22	196	105	391

The study is in progress.

CL- 9: **An open-label, non-randomized, two stage, dose-finding study of Verapamil [IR] tablet formulation in adult TB patients in continuation phase of anti-TB treatment**

Principal Investigator	:	Dr.C. Padmapriyadarsini (email: padmapriyardarsinic@nirt.res.in)
Collaborators	:	NITRD, New Delhi
Funding Agency	:	DBT
Study period	:	2013-2018

This is a phase 2 open-label dose-finding pharmacokinetic study of verapamil given in conjunction with RIF. The goal of this study is to determine the contribution of the efflux pump-mediated tolerance mechanism in delayed or incomplete sterilization in active PTB, i.e, whether verapamil

when added to standard TB therapy will accelerate sputum clearance of *M. tb*. The study, after obtaining DCGI approval, completed Stage I at NITRD, New Delhi in March 2017. Six patients were enrolled and underwent intensive PK for verapamil and RIF. A 50 – 90% reduction in the blood level of verapamil was found when given along with RIF in drug sensitive PTB patients. Results were submitted to it and DCGI has approved to proceed with Stage II of the study that targets at finding the optimal dose of Verapamil that can be given safely with RIF. The study will be initiated after obtaining funds from DCGI.

CL- 11: Species identification and response to appropriate treatment of symptomatic pulmonary non-tuberculous mycobacterial disease among patients treated for TB in Tamil Nadu

Principal Investigator	:	Dr.C. Padmapriyadarsini (email:padmapriyardarsinic@nirt.res.in)
Collaborators	:	Chennai Corporation, GHTM
Funding Agency	:	ICMR Task Force
Study period	:	2013-2020

This is a descriptive study to identify the various species of pathogenic non-tuberculous mycobacteria (NTM) causing symptomatic pulmonary disease and to evaluate their response to treatment, initiated based on ATS guidelines among patients with symptomatic pulmonary NTM disease in Tamilnadu. The study is currently enrolling pulmonary NTM patients from Chennai and Kanchipuram district. NTM species are identified and appropriate treatment given for the entire duration of 12 months of culture negativity. As of March 2018, 34 patients (22 males and 12 females, with mean (SD) age of 50.2 (10.02) years) have been recruited to the study. All patients are on treatment as per ATS guidelines. Three patients have

completed 18 months of treatment & have remained culture negative till date. We also had 1 each of *M.kyorinense* and *M.simiae* identified by sequencing and both have responded well to treatment. Table 17 describes the different NTM species identified.

Table 17: Description of NTM species

NTM Species	Total	Female	Male
M. kansasii	18	4	14
M. avium intracellulare	8	5	3
M.abscessus	5	1	4
M.fortitum	1	1	0
M.kyorinense	1	1	0
M.simiae	1	0	1

All patients are on appropriate treatment. The study is ongoing.

CL-15: Prevalence of TB infection and disease among pediatric household contacts of MDR-TB patients – A multicentric prospective cohort study

Principal Investigator	:	Dr. Dina Nair (email: dinanair@nirt.res.in)
Collaborators	:	NJIL, Agra, Govt. Hospital for Thoracic Medicine; Tambaram; Govt. Medical College & Hospital, Vellore; Govt. Rajaji Hospital. Madurai; Sarojini Naidu Medical College, Agra
Funding Agency	:	ICMR-Task Force
Study period	:	2015-2018

Background: In countries worldwide, the reported percentage of all TB cases occurring in children exposed to TB in the household varies from 24.2% to 69.2%. These children become the reservoir of future disease in adulthood,

thus perpetuating the epidemic. The transmission, dynamics of DR bacilli among the household contacts of MDR-TB patients is uncertain. Contact investigations though recommended to aid early diagnosis and prevention of further transmission, thereby reducing the disease burden, morbidity and mortality in children is not prioritized in TB control programmes. The data on the rate of TB infection and subsequent risk of active disease among MDR-TB contacts have not been consistent. Studies done in Brazil have reported that the prevalence of TB infection and progression to active TB was comparable in close contacts of MDR-TB and drug-susceptible TB patients, where as in Romania a study on pediatric population observed that TB infection rates were significantly lower in contacts of MDR-TB patients. A study done in South Africa concluded that MDR-TB is readily transmissible and is associated with a high risk of infection and active disease in children less than 5 years. Tuberculous infection with or without disease was present in 78% of children by 30 months, 95% of whom were already infected by 12 months. In India, the data on screening and prevalence of latent TB (LTB) Infection among child contacts of MDR-TB is sparse. The value of contact tracing and the prevalence of LTBI require more exploration in India.

Objectives:

Primary objective:

(i) To measure the prevalence of LTB infection among pediatric household contacts (less than 15 yrs of age) of MDR-TB patients

Secondary objectives:

i) To estimate the prevalence of active TB and DR-TB disease among the pediatric household contacts of MDR-TB adults

ii) To describe and compare the drug resistance pattern and *M. tuberculosis* genotype in adult-child contact pairs and

iii) To identify the risk factors for disease progression in the pediatric house hold contacts

Methodology: Eligible MDR-TB index cases with their pediatric contacts (<15yrs) are enrolled into the study. Symptom screening, TST, Chest radiograph are done for all contacts.

Three groups are identified –

i) LTBI group - Asymptomatic, TST positive;

ii) TB exposed group – asymptomatic, TST negative and

iii) Confirmed TB group

The LTBI and TB exposed groups are followed up once in 3 months for 24 months. Those with confirmed TB are referred to the RNTCP for initiation of treatment.

Progress: The enrolment is completed and the participants are being followed up (Table 18).

Table 18 : Study progress as on 31st March 2018

	NIRT Chennai	NJIL & OMD
No. of Index cases screened	1104	371
No. of Index cases recruited	160	64
No. of Pediatrics contacts recruited	296	142
No. of Pediatric contacts infected	90	-
No. of Pediatric contacts with abnormal Chest X-ray	5	-
No. of Pediatric contacts with active disease (MDR)	1	-
No. of contacts who completed 24 months of follow-up	155	

CL-16: A study on the effectiveness of food supplement on treatment outcomes and nutritional status of adults with PTB on a retreatment regimen

Principal Investigator	:	Dr. Devarajuly Reddy (email: devarajuly.s@nirt.res.in)
Collaborator	:	DTO, Vellore
Funding Agency	:	Intramural
Study period	:	2017-2019

Background: TB remains one of the major infectious causes of morbidity and mortality worldwide. Diabetes mellitus, under nutrition, HIV, immune-suppression, smoking, alcohol etc are a few known high risk factors for the disease. Under nutrition is an important risk factor and is a common consequence of TB; it is therefore a common co-morbid condition for people with active TB and is associated with increased risk of mortality and poor treatment outcome. Nutrition supplementation to TB patients has not only shown weight gain but also shorter time to sputum conversion, higher cure rate, better quality of life and functionality.

Aims:

Primary:

(i) To assess the effectiveness of food supplement on the treatment outcomes and loss to follow-up, among adults with PTB on retreatment regimen, attending RNTCP centers in Vellore district of Tamil Nadu

Secondary:

i) To evaluate the impact of food supplement on the nutritional status of adults with sputum smear positive PTB patients on retreatment regimen attending RNTCP centers in Vellore district of Tamil Nadu and

ii) To assess the quality of life, lung health and return to normal functionality among patients receiving food supplement along with anti-TB treatment

Methodology: PTB patients aged >18 years, with sputum smear positive for AFB (treatment failure/relapse/default for ATT) are screened and clinical examination is done. Sputum smear reports are collected from RNTCP cards. Blood samples are collected for complete hemogram/biochemistry/Vit.D/ proteins at baseline, end of intensive hpase and end of treatment.

Nutrition supplement (500 gms. powder packets) is supplied by the field

investigators to the eligible patients, every fortnightly, for a period of eight months. Patient will consume the supplement, 30 gms per day along with milk. Adherence to ATT and nutrition supplement is noted down by the Field Investigator (surprise home visits).

Sputum smear for AFB reports is noted from the RNTCP cards at 0, end of 3rd and 8th months.

Sample size: 435

Progress of the study: The study was initiated in March 2017. Patients have been enrolled from 7 TUs/40 DMCs of Vellore District, Tamil Nadu, India. As on 31st March, 2018, 242 patients are enrolled (145- intervention group and 97- control group). The study is ongoing.

CL-18: Cambridge-Chennai Centre Partnership on antimicrobial resistance in TB: Focus on novel diagnostics and therapeutics

Principal Investigator	:	Dr. Mohan Natrajan (email: mohan.n@nirt.res.in)
Collaborators	:	NIRT; University of Cambridge, MDR, UK
Funding Agency	:	Global fund through Central TB Division and DBT
Study period	:	2016-2018

Objectives:

(i) To establish and maintain 2 longitudinal prospective cohorts -- adults with DR-PTB (DR-TB Cohort / Cohort-I) and drug sensitive PTB (DS TB cohort / Cohort-II) to generate a clinical database and provide specimens to all the five projects

(ii) To create a database of *M. tuberculosis* genomes for isolates from India and to define the accuracy and clinical applicability of genetic DST

(iii) To study the structural and functional implications of novel mutations identified from whole genome sequencing (WGS) of Indian strains for protein structure and function

(iv) To develop assays to detect bacteria that express efflux pumps in patient's sputa and to assess directly the effect of verapamil over sputum bacteria singly or

in combination with anti-tuberculous treatment and

(v) To identify the T-cell co-stimulatory pathways responsible for controlling exhaustion in DR and drug sensitive TB patients, and to perform *in silico* screening for existing pharmaceuticals that limit or reverse exhaustion at transcriptional level

Five projects:

Project 1: Bacterial genomics as a diagnostic tool in DR-TB;

Project 2: New drug targets for TB through prediction/investigation of impact of resistance mutations;

Project 3: Population based study of gene repertoire associated with drug tolerance and their *in vivo* expression;

Project 4: Host directed therapy through autophagy stimulation and

Project 5: Manipulating T-cell exhaustion: new therapies to improve outcomes in resistant TB

Clinical Cohorts Methodology

Clinical Cohorts & sample size

COHORT-I - 50 patients with pulmonary MDR-TB (with and without additional drug resistance)

COHORT-II - 100 patients with newly diagnosed drug-susceptible PTB (DS-TB)

Patient enrolment, sample collection/ processing, & follow-up: Two new longitudinal prospective cohorts are being recruited based on the above mentioned criteria in order to support all the five proposed projects. Following recruitment, all patients in the cohort II are followed up for a period of 12 months (6 months of treatment and 6 months of post treatment follow-up). Clinical examination and 2 sputa are collected every month. Blood samples are collected at specific time points (Cohort-I – 0, 2, 6, 12, 18, 24, 30th month; Cohort-II – 0, 2, 6, 12th month). Two sputa and blood samples are collected in the event of failure or relapse. All patients receive anti-TB treatment from the respective RNTCP DOTS centre. They are requested to attend NIRT / NIRT subcentres for the investigations at pre-specified time intervals.

Sputum samples are being utilised in the first 4 projects, and blood samples are utilized in project 5. Sputum samples are subjected to smear, culture by LJ/MGIT media, and whole genome sequencing / DST (only baseline samples). Blood samples are subjected to safety lab tests, antigenic stimulation

with different peptides, flow cytometry analysis of different markers of T-cell anergy, flow sorting, RNA isolation from specific T-cell subsets (only baseline samples), etc.

Progress in recruitment and follow-up: Fifty patients in Cohort-I and 100 patients in Cohort II have been recruited (Tables 19 & 20). In Cohort-I, 50 patients have completed 6th monthly visit, 30 patients have completed 12th monthly visit and 7 patients have completed 18th monthly visit, respectively. In Cohort-II, 87 patients have completed 12th monthly visit.

Table 19: Outcome status as on April 2018

	COHORT-I (DR-TB)	**COHORT-II (DS-TB)**
Patients screened	268	384
Patients recruited	50 (35 Males / 15 Females)	100 (85 Males / 15 Females)
Defaults	0	4
Deaths	3	3
Failures	1	13
Relapse	0	2

Table 20: **Baseline characteristics**

	COHORT-I (DR-TB) No. of cases - 50	COHORT-II (DRS-TB) No. of cases - 100
Age (years) (Mean)	36	38
Weight (kg) (Mean)	44	43
Smear Grade 0,1+	33	58
Smear Grade 2+,3+	17	42
Culture Grade 0,1+	25	36
Culture Grade 2+,3+	25	64

CL-19: Optimizing treatment to improve TBM outcomes in children: The TBM-KIDS trial

A Phase I/II Randomized, Open-label trial to evaluate the pharmacokinetics, safety, and treatment outcomes of multidrug treatment including high dose RMP with or without Levofloxacin versus standard treatment for pediatric tuberculous meningitis

Principal Investigator	:	Dr. Bella Devaleenal
Collaborators	:	Johns Hopkins University School of Medicine, USA, Institute of Child Health (ICH) and Hospital for Children, Chennai.
Sponsored by	:	The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)
Clinical Trial Registry Number	:	CTRI/2017/03/008004
Study period	:	2016-2019

Background: Paediatric tuberculous meningitis (TBM) is associated with high mortality and severe morbidity. Children diagnosed with TBM are managed with anti-tuberculous therapy and steroids. They are at risk of severe functional and neurodevelopmental impairment and any improvement in treatment not only reduces the risk of death and functional disability but improves the quality of life in young children by improving long-term neurocognitive outcomes. There are studies reporting low concentrations of anti-TB drugs, especially of RMP and EMB in CSF. At the current recommended doses, CSF concentrations of rifampicin barely exceed the MIC against *M. tuberculosis*. Since RMP is the key sterilizing drug for TB and drives treatment response, higher doses might be required for optimal TBM therapy. INH, PZA and levofloxacin have excellent CSF penetration. In this trial, we are evaluating the PK, safety, and treatment outcomes of regimens containing higher dose of RIF with or without levofloxacin for the treatment of paediatric TBM compared to standard TBM treatment. The functional and neurocognitive outcomes are also being assessed. This is a multi-centric trial done in Pune, Malawi and Chennai.

Objectives:

Primary objectives:

(i) To characterize the pharmacokinetics (PK) [plasma and cerebrospinal fluid (CSF)] of RIF given at model-derived optimal daily doses and levofloxacin in children aged 6 months to 12 years of age with TBM

(ii) To assess the relationship between RIF concentrations and longitudinal functional outcomes, adjusting for factors known to affect treatment response, such as stage at presentation, leukotriene A4 hydrolase (LTA4H) genotype, levofloxacin co-administration and

(iii) To evaluate the safety of TBM treatment over eight weeks, by Arm

Secondary objectives:

(i) To describe neurocognitive outcomes among children aged 6 months to 6 years of age treated for TBM, longitudinally over 18 months, by Arm and

(ii) To describe TBM treatment outcomes at 12 months

Methods: This is an open-label, randomized clinical trial conducted in three treatment groups of children with TBM. Patients with probable or definite TBM will receive INH and PZA at standard doses for 8 weeks. Participants in Arm 2 will receive high-dose RIF for 8 weeks plus EMB at standard doses. Participants in Arm 3 will receive high-dose RMP plus levofloxacin for 8 weeks. Arm 1 participants will receive RIF plus EMB at standard doses for 8 weeks (control Arm). Patients will be screened to confirm TBM diagnosis, will receive 8 weeks of study treatment, and then will complete 12 months of standard TBM treatment. All participants will receive oral steroids. PK sampling will be performed within the first week and at 6 (+/- 2) weeks following treatment initiation. Participants will have scheduled follow-up visits to assess safety and clinical status. In addition, functional and neurocognitive outcomes up to 18 months following treatment initiation will be assessed. Interim PK and safety analyses will be performed to ensure whether dosing is producing predefined PK targets, and if safety is acceptable. A total of 120 children will be enrolled from all three sites.

Progress: Study was initiated in NIRT in June 2017. So far, 173 children were pre-screened for the study, 12 were screened and 2 children were enrolled

in NIRT Overall, 15 children have been enrolled from all the sites.

The recruitment for the study is ongoing.

CL-20: A phase IIB Open Label Randomized trial to evaluate the anti-bacterial activity, pharmacokinetics, safety and tolerability of Metformin when given along with RIF, INH, PZA and EMB in adults with newly diagnosed sputum positive PTB: an 8-week study

Principal Investigator	:	Dr.C.Padmapriyadarsini (email:padmapriyardarsinic@nirt.res.in)
Funding Agency	:	ITRC-India TB Research Consortium
Study period	:	2018-2020

This is a phase IIB open-label randomized clinical trial with an aim to evaluate the anti-bacterial activity of Metformin, by measuring the time to sputum culture conversion in liquid media, when given daily for 8 weeks along with standard first-line anti-TB treatment in adults with newly diagnosed sputum positive PTB. This is a multicentric trial and will enrol 320 new sputum smear positive PTB patients in New Delhi, Pune and Chennai. The study has got all regulatory approvals, training of staff completed and will be shortly initiated.

CL-22: The evaluation of a standardized treatment regimen of anti-TB drugs for patients with MDR-TB- STREAM Stage II

Principal Investigator	:	Dr. G. Narendran (email: nareng@nirt.res.in)
Collaboratoring institutions	:	Govt. Hospital of Thoracic Medicine, Tambaram; Govt. Rajiv Gandhi General Hospital, Chennai; Govt. Otteri Hospital, Chennai, B.J.M.C., Ahmedabad
Source of funding	:	The United States Agency for International Development (USAID); UK Medical Research Council (MRC) / Department for International Development (DFID); Janssen Research & Development, LLC; Liverpool School of Tropical Medicine, UK
Study period	:	2018-2023
Trial Registry No. if available	:	CTRI/2017/09/009693, 18148631 ISRCTN

Background: The prolonged duration of the current regimen (24-27 months) used to treat MDR-TB results in multiple and cumulative side effects and high loss-to-follow-up rates. This results in further amplification of resistance to additional drugs. The STREAM-II study aims to test a more effective regimen that reduces the treatment duration to six-to-nine months, which could significantly enhance the treatment success rate among MDR-TB patients. This could potentially save thousands of lives and reduce the MDR-TB burden in India.

Primary objective:

(i) To assess the efficacy of shorter treatment regimens at 76 weeks and to compare safety during treatment and follow-up upto 132 weeks

Methodology: The STREAM study is an international, multi-centre, parallel-group, open-label, randomised, controlled trial, enrolling patients with MDR-TB including patients with RMP-resistant and INH-sensitive TB without resistance to quinolones or aminoglycosides at baseline.

Trial interventions include three arms enlisted below and allocated in the ratio of 1:1:1

Regimen B (control regimen):

Regimen B consists of clofazimine, EMB, MFX and PZA given for 40 weeks, supplemented by INH, kanamycin, and prothionamide in the first 16 weeks (intensive phase).

Regimen C:

Regimen C is a 40-week all-oral regimen consisting of bedaquiline, clofazimine, EMB, levofloxacin, and PZA given for 40 weeks supplemented by INH and prothionamide for the first 16 weeks (intensive phase).

Regimen D:

Regimen D is a 28-week regimen consisting of bedaquiline, clofazimine, levofloxacin, and PZA given for 28 weeks supplemented by INH and kanamycin for the first 8 weeks (intensive phase).

Sample size: 1155, with 300 cases from India.

Study progress: This is a regulatory trial monitored by the FDA, DCGI and the Quintiles which is the CRA for the trial. The study was approved by the HMSC on 15.04.16 and the DCGI on 23-05-16. The study commenced in December 2017 and we have recruited 12 cases out of the 34 cases screened, as per study criteria. Centralized data collection allows for blinding. An independent data safety management committee for STREAM, closely scrutinizes the trial data.

The study is in progress.

CL-23: A randomised trial of therapy shortening for minimal TB with new WHO recommended doses/fixed-dose-combination of drugs in African and Indian HIV+ and HIV- children (SHINE Study)

Principal Investigator	:	Dr.S. Syed Hissar (email: syed.hissar@nirt.res.in)
Collaborators	:	Govt. Stanley Hospital, Chennai; Institute of Child Health, Chennai
Source of funding	:	DFID-UK, Wellcome Trust, MRC-UK and TB Alliance
Study period	:	2017 – 2020

Background: TB in children is frequently paucibacillary and non-severe forms of PTB are common. Evidence for TB treatment in children is largely extrapolated from adult studies. Trials in adults with smear-negative TB suggest that treatment can be effectively shortened from 6 to 4 months. New paediatric, fixed-dose combination anti-TB treatments have recently been introduced in many countries, making the implementation of WHO-revised dosing recommendations feasible. The safety and efficacy of these higher drug doses have not been systematically assessed in large studies in children, and the pharmacokinetics across children representing the range of weights and ages should be confirmed.

Aims:

(i) To determine whether the standard 6 month regimen (8 weeks HRZ (E) followed by 16 weeks HR) can be reduced with similar efficacy, to 4 months (8 weeks HRZ (E) followed by 8 weeks HR), in HIV-infected and uninfected African and Indian children with minimal TB, using recently revised dosing guidelines for anti-TB drugs and

(ii) To determine whether the higher WHO-recommended doses of daily first-line anti-TB drugs, given as new WHO-recommended, fixed-dose combination (FDC) dispersible tablets, and prescribed according to weight bands, result in appropriate drug exposures when compared with historical paediatric and adult pharmacokinetic (PK) data (PK substudy 1)

Methodology: This is a multicentric, open-label, parallel-group, non-inferiority, randomised controlled, two-arm trial comparing a 4-month vs the standard 6-month regimen using revised WHO paediatric anti-TB drug doses. Children aged less than 16 years with

non-severe TB, with or without HIV infection will be tested. The primary efficacy and safety endpoints are TB disease-free survival 72 weeks post randomization and grade 3 or 4 adverse events. Nested PK studies will evaluate anti-TB drug concentrations and will provide model-based predictions for optimal dosing. Socio-economic analyses will evaluate the cost-effectiveness of the intervention and social science studies will further explore the acceptability and palatability of these new paediatric drug formulations.

Current status: This study was initiated in August 2017. The required sample size is 70 children with minimal TB. The study is currently enrolling children from two centers, Govt. Stanley Hospital (GSH), Chennai and Institute of Child Health (ICH), Chennai. As of March 2018, we have screened 70 patients and enrolled 42 in the study.

The study is ongoing.

Towards a TB free Chennai: An Innovative Intersectoral Partnership

Principal Investigator : Dr Srikanth P.Tripathy
Study Co-ordinator: Dr S Sriram

The concept of TB free Chennai evolved from discussions with a group of public and private sector partners who together designed a comprehensive model for urban TB care. These partners include Chennai Corporation, National Institute for Research in Tuberculosis, REACH, Stop TB Partnership, World Health Organization (WHO) India Country office, Partners in Health, Central TB Division, Tamil Nadu State Health Department, Indian Medical Association, Clinton Health Access Initiative (CHAI) and Foundation for Innovative New Diagnostics (FIND). Each of the partner is committed to bringing in technical and/or financial resources towards the vision of a TB-free Chennai.

The technical approach of the project is structured in line with the key outputs recommended in the THALI project framework. The applicants propose to build on the strengths of both the public and private sectors to create a comprehensive end-to-end approach for fighting TB. Core to this effort will be establishment of a Public Private Interface Agency (PPIA) to develop and coordinate a strategic partnership between the private sector and the free, quality treatment and diagnostic tools available in the public sector. Innovations and partnerships are the backbone of this project with focus on reaching the vulnerable populations in Chennai. It is clear that rapid progress on TB control requires bold measures to disrupt the chain of transmission. The broad consortium of partners has enabled extensive cross-fertilization of ideas from other settings in order to inform program design. The research expertise of partners will also be used to generate learnings from this project that can be applied elsewhere.

Challenges to effective TB management in Chennai : The NIRT prevalence study done during 2010-2012 demonstrated a TB prevalence of 259/lakh (95% CI: 217-299). However, case detection in the program ranges

from 70 to 135/100,000, translating into a considerable number of patients who are unaccounted for. Programme data from the last year also showed that while death among new smear positive patients was 5%, mortality among drug resistant patients was much higher (12-25%). High loss to follow up, both initially (~14%) and during treatment (10 and 24% for new and retreatment patients respectively) are a matter of concern as these patients continue to transmit TB.

As expected in an urban landscape, there are several vulnerable populations such as slum dwellers, migrants, fishing community, pavement dwellers and construction workers. These groups are exposed to conditions such as over-crowding, poor sanitation, indoor air pollution, malnourishment, problems related to alcohol, smoking, thus increasing their risk of TB. Additionally, other vulnerable groups such as contacts of TB patients, diabetics, HIV-infected persons, antenatal women and health care workers would also need enhanced screening. It has been reported that 50-70% of patients access care from private facilities. The private sector is highly complex and fragmented with varying standards of quality of care, including prescription practices and poor tracking mechanisms. Furthermore, there is a lack of patient and provider awareness, delay in seeking care, timely diagnosis and poor patient support systems.

Overall Vision: To create a TB-free Chennai.

Objectives: To strengthen urban TB control in Chennai using innovative approaches and multi-stakeholder involvement

Strategic Approach

1. Build an expanded multi-stakeholder partnership for a TB-free Chennai.
2. Private health care provider engagement for quality TB care.
3. Enhanced case finding strategies with a special focus on vulnerable populations
4. Incubate and implement innovative patient-centred treatment support models
5. Newer modalities of Treatment and Prophylaxis of TB
6. Create an unique multifunctional IT enabled platform to provide “smart” solutions for existing challenges

7. Evidence based decision making and policy change
8. Monitoring the project effectively and efficiently

Role of NIRT in Evidence based decision making and policy change

NIRT will undertake quantitative and qualitative research on identified priority areas. This will provide evidence on effectiveness of the interventions proposed, to enable translation to the programme and make suggestions for policy changes. Key research areas included in Phase 1 are as follows:

1. **Utilization of school students as ambassadors for TB sensitization in an urban slum, Chennai**
2. **Social Network analysis as a tool to improve active case finding at community level in Chennai**
3. **Patients' Perception on Quality of Care in TB Care Settings in Chennai**
4. **Utility of 'Household Contact register and card' in ensuring adherence to screening and follow-up of household contacts of TB patients under RNTCP in Chennai District**
5. **Role of mobile phone intervention in improving adherence to chemoprophy - laxis for Paediatric contacts of sputum positive adult TB patients**

Monitoring the project effectively and efficiently

The Project Management Unit will be based at NIRT and will be headed by the Project Director. Monitoring (M) and Evaluation (E) team will be formed with members from the Chennai Corporation and NIRT along with the project M and E officer. M and E team will develop a log frame with input, process, output and outcome indicators for this project. The methodology, source of data, frequency of data collection and data analysis will be planned. Periodic evaluation will be done and findings presented to the Advisory Committee, which will be setup to oversee the implementation of the Project. Key learnings and findings will be disseminated to all the stakeholders.

The project is in progress.

Department of Socio Behavioral Research

STUDIES COMPLETED:

SB-3: Investigating pre-treatment loss to follow-up of smear-positive TB patients in the RNTCP in Chennai city and Tiruvallur district, Tamil Nadu

Principal Investigators	:	Dr. Beena E Thomas (Indian PI) (email: beenathomas@nirt.res.in) Dr. Ramnath Subbaraman (US PI)
Source of Funding	:	NIHGHES Fellowship via Brigham and Women's Hospital, USA
Study Period	:	2016-2017

Background: Pretreatment loss to follow-up (PTLFU) is a major barrier to TB control in India's RNTCP. PTLFU studies have not been conducted in India's mega-cities, where patient mobility may complicate linkage to care.

Methodology: We first collected registers of May 2015 from 22 RNTCP designated microscopy centers (DMCs) in Chennai and audited addresses and phone numbers of patients evaluated for suspected TB, to understand how missing contact information may contribute to PTLFU. From November 2015 to June 2016, we then audited one month of records from each of these 22 DMCs and tracked newly diagnosed smear-positive patients using RNTCP records, phone calls and home visits. We defined PTLFU cases as including: (i) Patients who did not start TB therapy within 14 days and (ii) Patients who started TB therapy but were lost to follow-up or died before RNTCP registration. Multivariate logistic regression was used to identify factors associated with PTLFU.

Main findings: In the audit of May 2015 DMC registers, out of 3,696 patients evaluated for TB, 1,273 (34.4%) had both addresses and phone numbers that were illegible or missing. Out of 344 smear-positive patients tracked from November 2015 to June 2016, 40 (11.6%) did not start TB therapy within 14 days and 36 (10.5%) started therapy but were not officially registered, for an overall PTLFU rate of 22.1% (95%CI: 17.8%—26.4%). Of all PTLFU patients, 55 (72.4%) were lost to follow-up, while 21 (27.6%) died before starting

treatment or before official RNTCP registration. In the regression analysis, age >50 years (OR 2.9, 95%CI 1.4—6.5), history of prior TB (OR 3.9, 95%CI 2.2—7.1), evaluation at a tertiary DMC (OR 3.2, 95% CI 1.7—6.3), and absence of legible patient contact information (OR 4.5, 95%CI 1.3—15.1) were significantly associated with PTLFU.

Conclusion: In an Indian mega-city, we found a high PTLFU rate, especially in patients with a prior TB history, who are at greater risk for having drug-resistance. Poor quality of patient contact information, seeking care at high-volume tertiary centers, and delays in patient registration were critical health system barriers contributing to PTLFU.

SB-7: Identifying costs contributing to catastrophic expenditure amongst TB patients registered under RNTCP in two metro cities in India

Principal Investigator	:	Dr M Muniyandi (email: mmuniyandi@yahoo.com)
Source of funding	:	Global Fund
Study Period	:	2017-18

Background: TB patients often incur huge costs on account of illness and in accessing healthcare. Such costs can create access and adherence barriers which can affect treatment outcomes and increase risk of transmission of disease. These costs can also contribute to the economic burden of households. The RNTCP, based on the DOTS strategy was introduced in India, to address the increasing burden of TB. RNTCP provides free diagnostic and treatment services to all the patients registered under it. Even when TB treatment is free under RNCTP, hidden costs incurred by patients and their households may worsen poverty and health. The costs could also turn catastrophic (>20% of income) leading to adverse treatment outcomes. Identifying costs leading to this catastrophic expenditure will help in the

design of interventions to reduce such expenditure.

Objectives:

(i) To estimate the proportion of households that experience catastrophic expenditure due to TB and to find out the various costs contributing to catastrophic expenditure due to TB

Methods: This was a prospective cohort study to follow TB patients at different time points. This study was conducted in two metropolitan cities in India - Chennai and New Delhi. National Institute of TB and Respiratory Diseases (NITRD) Delhi had conducted this study in the RNTCP area allotted to it with population of 0.8 million. NIRT (ICMR) Chennai had carried out the study in Chennai amongst the patients registered under RNTCP centers at Tondiarpet, East Cemetery Road, Elango Nagar and Pulianthope, which were selected based on the number of cases diagnosed and treated in a quarter. All pulmonary and extra-PTB patients consecutively registered under RNTCP and treated with Category I / II ATT were included at each site. The patient and parent /guardian of pediatric TB cases were interviewed at three time points after obtaining written consent/ assent within one week of initiation of treatment, at the end of intensive phase of treatment and at the end of continuation phase of treatment. WHO Generic questionnaire modified to suit the local condition was used to collect information on direct medical, non-medical and indirect costs.

Results: TB patients often incurred large costs related to illness, as well as in seeking and receiving healthcare. Despite TB treatment being free under RNCTP, out of pocket expenditure incurred by patients impoverished the households. This study was conducted in two metropolitan cities in India (Chennai and New Delhi) amongst the TB patients (N=950) including children (N=140) registered under RNTCP. The significant finding from this study was that among adult TB patients, 44% in Chennai and 8% in Delhi experienced catastrophic expenditure (more than 20% of their family annual income spent for TB treatment). Among children, 24% in Chennai and 7% in Delhi experienced catastrophic expenditure. In both cities, indirect costs due to work absenteeism were a major contributing factor for high catastrophic expenditure. It was also observed that a higher proportion of

persons with lower annual income (less than Rs.100,000) experienced catastrophic expenditure due to TB.

Conclusion: Our findings suggest that there is an urgent need to monitor health and social protection coverage in the context of TB care and prevention. This information will be useful for policy makers to design an intervention to provide financial protection to TB patients. In addition, this information will provide baseline details and help to periodically measure progress towards the 'End TB Strategy' so that no TB patient or their household should face "Catastrophic Expenditure".

SB-3: Comparative study of economic burden at the household level for TB patients detected through active and passive case finding strategies in Tiruvallur district

Principal Investigator	:	Dr M Muniyandi (email: mmuniyandi@yahoo.com)
Source of funding	:	Intramural
Study Period	:	2016-18

Background: The RNTCP, based on the DOTS strategy was introduced to address the increasing burden of TB. RNTCP provides free diagnostic and treatment services to all the patients registered under it. The current policy under RNTCP is passive case finding and the programme expects patients to come to the health facilities for diagnosis. Recognizing that TB services delivered through public health systems alone cannot reach all TB patients, there is a need to reduce barriers to access through more patient friendly services, explore the use of incentives and enablers via existing social welfare schemes for the poor and vulnerable, engage other health care providers and implement new ways of engaging health care providers in other sectors. It expects that active case finding strategy will also aim to reduce delay in

diagnosis, improve access to chest symptomatics and reduce out-of-pocket expenditure to the patients.

Objectives:

(i) To measure and compare the pretreatment costs of TB in the private and public sectors among patients diagnosed through active and passive case finding strategy and

(II) To measure and compare the time taken for diagnosis and start of treatment through active and passive case findings in the private and public settings

Methods: This cross-sectional survey was conducted in Tiruvallur district of Tamil Nadu, south India. Study survey was planned as a nested sub study of an ongoing community prevalence survey and was conducted among TB patients aged 18 years and above. This study had two groups; one from TB patients diagnosed through active case finding strategy during NIRT prevalence survey. The other group was TB patients diagnosed through the government health facilities under RNTCP and private health facilities in the study area. Semi-structured and a pre-coded interview schedules used in previous studies was utilized for data collection. The information on direct medical costs (which includes fees, investigations, drugs), direct non-medical costs (travel, special food) and indirect cost (loss of income), time taken for TB diagnosis and initiation of treatment were collected.

Results: A total of 338 TB patients, 228 diagnosed through passive case finding (RNTCP) and 110 diagnosed through active case finding (NIRT survey) were included in the study. It was estimated that 29% of TB patients were diagnosed through passive case finding and 10% of patients were diagnosed through active case finding.

Conclusion: The study finding provides insights on costs to TB patients diagnosed through active and passive case finding strategies. Also this study provides estimation of the proportion of TB patients facing the catastrophic health expenditure. The results show that active case finding is a strategy which could provide financial protection to TB patients. This information will be useful for policy makers to scale up this intervention to provide financial protection to TB patients.

SB-8: A multi-component health system strengthening intervention to reduce pre-treatment loss to follow-up of smear positive TB patients in Chennai

Principle Investigator	:	Mr. S. Senthil (email: senthil.s@nirt.res.in)
Source of Funding	:	Global Fund through ICMR and CTD
Study Period	:	2016-2018

Background: India has 24% of the global burden of TB cases and 27% of the world's "missing" cases, representing an estimated one million patients yearly who may not have received health services through Indian's RNTCP. A major factor contributing to missing cases is PTLFU (formerly referred to as "Initial Defaulter"). PTLFU refers to patients diagnosed with smear positive TB at government DMCs who fail to start treatment. These-positive patients spread infection in the community and have high mortality rates.

The Department of Social and Behavioural Research at the NIRT has conducted a study to estimate the prevalence of and reasons for PTLFU in Chennai. Based on our pilot fieldwork and the findings of prior studies, we hypothesize that the following health system deficiencies contribute substantially to PTLFU:

- Poor quality of TB patient record
- Patients commonly "lost" during the referral process
- Substantial proportion of diagnosed smear-positive patients live outside of Chennai

We implemented and tested a multi-component health system strengthening intervention aimed at addressing the three key health system deficiencies described above, as well as patient-related barriers that may emerge from a qualitative study conducted with PTLFU patients.

The primary objective of thi study is

(i) To test whether a multi-component health system strengthening intervention could decrease the proportion of smear-positive TB patients diagnosed who fail to start TB treatment within 7 days (i.e., PTLFU) under RNTCP, Chennai

The secondary objective of this study is:

(i) To identify if these interventions will help in reducing the delay in starting the treatment of diagnosed TB patients. These interventions also would help to obtain a performance feedback initiative (one of the

intervention components) on the following indicators:

(a) The proportion of correctly recorded phone number and address in DMC and referral registers;

(b) The proportion of patients with suspected TB who complete two sputum smears; and

(c) The proportion of smear-positive patients with a history of prior TB (i.e., retreatment cases) who undergo drug resistance testing with a line probe assay, which is currently mandated for retreatment cases in Chennai.

Methodology:

Study Design: We have adopted a *quasi-experimental study* with a *pre-post- design,* which will compare the PTLFU prevalence rate prior to the intervention with the PTLFU prevalence rate after the intervention has been implemented for 6 months at all DMCs.

Study setting: This study was implemented in 22 DMCs in Chennai city where >90% of smear-positive TB patients are diagnosed.

Study population: The study was conducted among RNTCP staffs from the selected 6 districts (Chennai, Thiruvallur, Kanchipuram, Villupuram, Vellore and Thiruvannamalai) included that HVs, STSs, LTs, STLSs and others (who are all supporting in the DMC work). A total of 223 RNTCP staff (50.7% were males and 49.3% were females) participated in this study with their informed consent.

Implementation strategy: This intervention study was implemented in 22 DMCs at Chennai district in Tamil Nadu where overall 90% of smear-positive patient gets diagnosed. We used PRECEDE (Predisposing, Reinforcing, Enabling, Construct in, Educational, Diagnosis, and Evaluation) for designing the multi-component intervention to strengthen the health system in reducing PTLFU rate in the 22 DMCs in Chennai district (Flow chart and Figs. 3 & 4).

Main findings:

Flow Chart : The overall impact analysis of intervention in the PTLFU rates in 22 DMCs at Chennai District

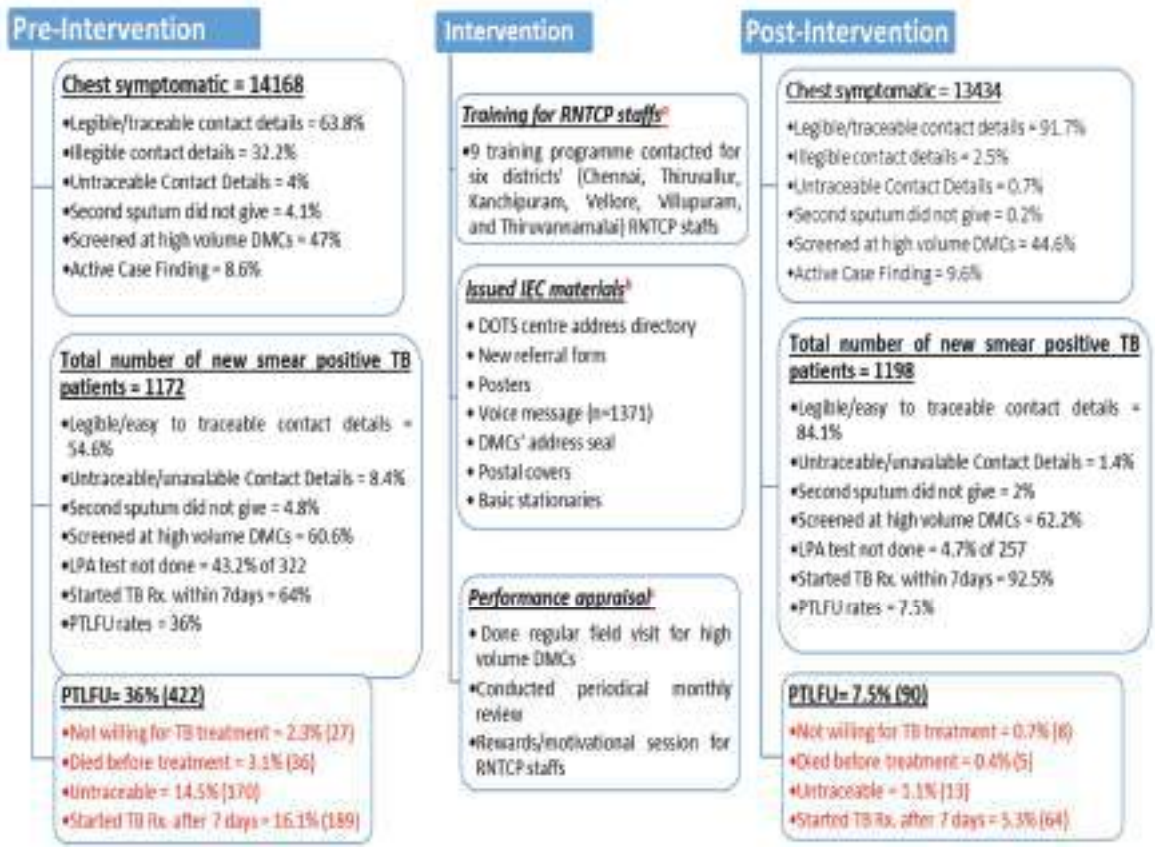


Fig. 2: **Baseline Vs End line outcome (chest presumptive cases)**

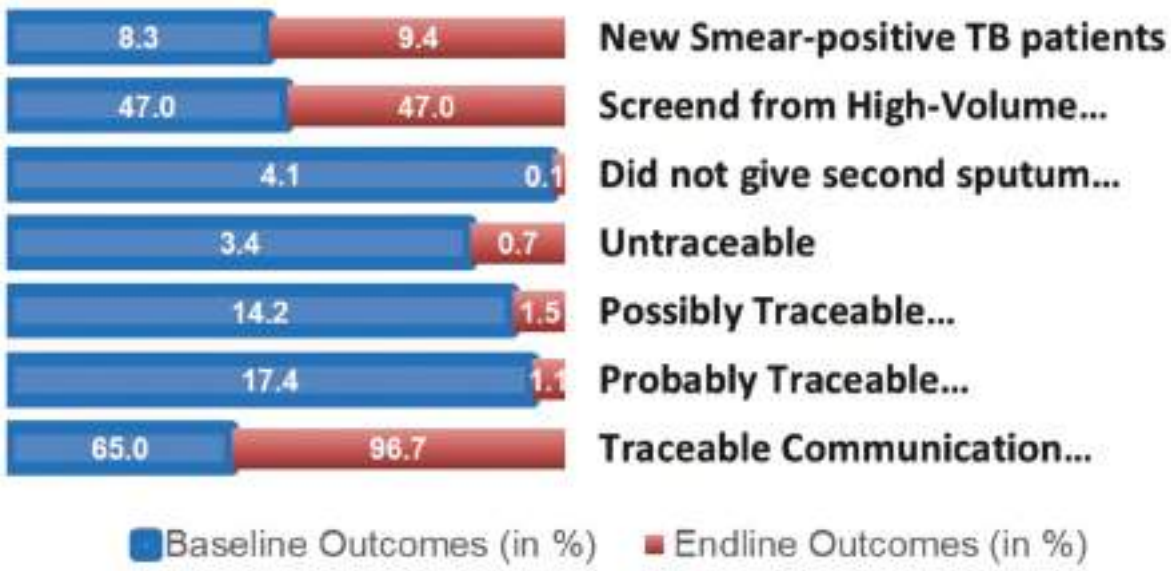
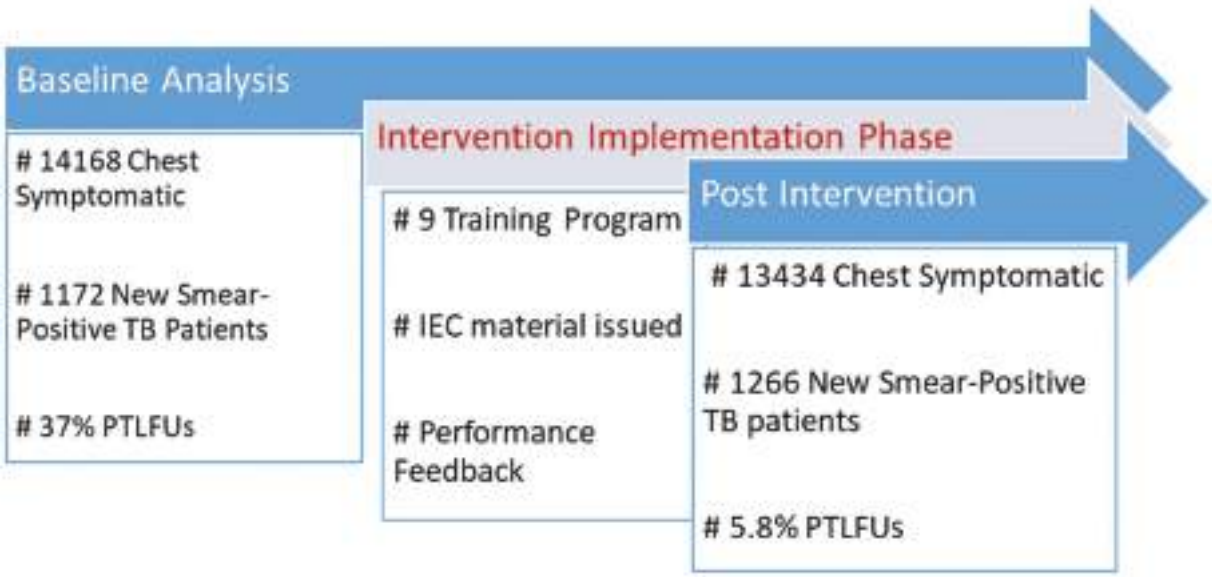


Fig. 3: **Impact on Intervention in Reducing PTLFU cases in Chennai**



Scalability: Our study intervention tools can be used in metropolitan cites in India which are have high-volume of urban registration gap like Bangalore, Mumbai, Ahmedabad, Delhi, Hyderabad, Kolkata, Surat.

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Sustainability: For sustainability of these positive work changes among Chennai RNTCP staff, the Chennai DTO recruits/allocates skilled staffs for providing training for monitoring and evaluation.

Conclusion: The health system components of RNTCP, must be improved for ending PTLFU rates in India. The periodical training program for RNTCP staff and periodical monitoring and evaluation helps to motivate RNTCP staffs on ending PTLFU in RNTCP.

Funding / Acknowledgements: This implementation research study was supported by The Global Fund to fight AIDS, Tuberculosis and Malaria. The Global Fund is an International financing organization, implemented through the Central TB Division, Directorate General of Health Services, Government of India and Indian Council of Medical Research (ICMR) New Delhi and National Institute for Research in Tuberculosis, Chennai.

SB-10: A study on psycho social issues facing MDR patients & design appropriate intervention strategies to promote drug adherence

Principal Investigator	:	Dr. E Thiruvalluvan (email: thiruvalluvane@nirt.res.in)
Source of Funding	:	Intramural project
Study Period	:	2015-2017

Background: Globally in 2016 about 490,000 people were estimated to have become ill with MDR-TB. Both psycho-social well-being and treatment outcome in MDR-TB are not satisfactory compared to drug sensitive TB. Treatment of MDR-TB is characterised by rigorous treatment regimen for long duration, higher incidence of adverse side effects, lower cure rate and high treatment costs. Phase I of this study clearly documented that majority of MDR-TB patients experienced substantial enacted stigma, leading to break-down of family relationships and isolation within the family. Participants were made to feel disgraced and discriminated by the health care workers. Patients and their family members feared about the loss of their economic stability because of the disease which often generates a great deal of stress. These findings highlight the need to apply an integrated invervention model that includes counseling and active health education, in addition to incorporating successful experiences of generic models of service delivery. The findings were compiled and re-grouped under the broad areas viz., psychological challenge, socio-economic challenge, and medication challenge.

Objectives:

(i) To understand the psychosocial issues facing MDR-TB patients (Depression, stigma, social support)

(ii) To gain insight on the factors that influence treatment adherence and quality of life and

(iii) To explore the feasibility and acceptability (effectiveness) of intervention strategies to promote adherence suitable for MDR-TB patients

Methods: Based on the findings of phase I, an intervention strategy was devised that consisted of motivational interviewing module, counselling module and nutritional support.

1. Motivational interviewing intervention focussed on 4 main topics viz.,

Session 1: MDR – TB Management/ Challenges/ Nutrition,
Session 2: Psycho-social issues, intervention,
Session 3: Alcohol/smoking/ suicide thought and
Session 4: Rehabilitation.
Study participants visited on 5 occasions time during the study period i.e., 0,6th, 12th, 18th and 24th month over *a* two year period (Table 21). During the sessions the following research tools were administered:
i. socio-demographic measurement,
ii. Scales to measure anxiety (CESD),
iii. Quality of life (WHO QOL BREF),
iv. Alcohol and drug abuse (AUDIT)
v. Smoking (Fagerstorm)

Table 21: Intervention schedule

Activity /Time point	0 month			6th month			12th month			18th month			24th month		
Assessment/ Motivational Interviewing	√			√			√			√			√		
Counselling	1	2	3	4	7	8	9	10	13	14	15	16	19	20	21
MDR – TB Management/ Challenges/ Nutrition	√			√			√			√			√		
Psychological issues intervention		√			√			√			√			√	
Alcohol/smoking/ suicide thought Agenda		√			√			√			√			√	
Rehabilitation			√			√			√			√			√

2) Individual Counselling sessions numbering 15 were held. During the study period various psycho social issues were addressed.

3) Nutritional support was offered to all participants but 2 participants declined to accept nutritional support. Nutritional flour packet weighing ½ kg was offered to participants every month.

Results: Altogether 35 participants were enrolled for the study (Table 22) One third of participants were women. One participant was lost to follow-up and the other one died. Finally, 33 participants were included for analysis. The mean age of participants was 38 years (SD+13). One third of participants were educated up to high school level. More than half the participants were married. Before diagnosis of MDR–TB, only 3 participants were unemployed but after diagnosis, unemployment rate increased to 69.7%. Sixty three percent of participants were tenants, living in line houses.

Table 22: Profile of patients

	Total	Male	Female
No.of participants enrolled	35	24	11
Lost	1	1	0
Died	1	1	0
No. of eligible participants	33	22	11
Issues faced	9	6	3
Discrimination by house owner	2	1	1
NTM	6	5	1

Findings revealed that majority of participants were unaware of 'MDR-TB'. Most of the TB patients have not disclosed their TB status, even to their family members. Ninety percent of participants found government health facility as the primary source for treatment and equal percentage of participants were aware of two years of treatment period.

As for dependence on tobacco and alcohol, 9 participants had habit of smoking and 12 participants had history of drinking. However, AUDIT scale suggested one participant with drinking problem and Fagerstorm smoking scale suggested 6 participants as very low

dependence and 3 participants with medium dependence.

Motivational interviewing intervention was received by all 33 participants at 5 different time points and administered depression assessment scale and quality of life scale.

At the start of treatment for MDR-TB, 81.8% participants experienced depression which came down to 21.2% at the end of treatment. Likewise 57.6% participants experienced poor quality of life score at the start of treatment which at the end of treatment was 6.1%. Dissatisfaction with own health came down to 15.2% from 72.7% at the start of treatment (Table 23). Quality of life scores in all 4 domains and depression level scores were statistically significant.

Conclusion: This study has shown that offering motivational interviewing, counselling and nutritional supplement had great impact not only on the quality of life of MDR-TB patients but also contributed to required adherence and resultant good treatment outcome. Therefore researchers suggest that services of trained, professional MDR-TB counsellors may be tapped at the initiation of treatment itself and structured counselling module may be made available preferably in local language, to the counsellors. This will contribute not only for treatment adherence but also to ensure better quality of life.

Table 23: Comparison of depression and quality of life scales at baseline and end of treatment

Comparison between baseline and 24th month:

Depression and QOL at baseline	Baseline	24th month	p-value
CESD depression scale			0.006*
No depression	6 (18.2)	14 (42.4)	
Depressed	27 (81.8)	7 (21.2)	
WHO-QOL scale			
Dissatisfied with own health	24 (72.7)	5 (15.2)	0.008*
Physical health (Mean ± SD)	11 ± 4	16 ± 3	<0.001&
Psychological (Mean ± SD)	12 ± 3	17± 3	<0.001&
Social relationships (Mean ± SD)	11 ± 4	15 ± 2	0.022&
Environment (Mean ± SD)	13 ± 3	15 ± 3	0.002&
Poor QOL	19 (57.6)	2 (6.1)	<0.001*

*McNemar Test
&Paired t-test

STUDIES IN PROGRESS:

SB-1: To estimate the burden of TB among tribal population and develop an innovative health system model to strengthen TB control in the tribal areas

Principal Investigator	:	Dr. Beena E. Thomas (email: beenathomas@nirt.res.in)
Source of Funding	:	ICMR
Project period	:	2015-2019

National Institute for Research in Tuberculosis (ICMR) is the coordination site for this multicentre ICMR task force study.

Background: There is limited knowledge with few area specific studies on the burden of TB among the tribal population. A Meta-analysis of these studies projected a pooled estimate of 703/100000 with wide variation among tribal groups. Hence, this study was proposed to estimate the burden of tuberculosis situation among the tribal population in the country in terms of prevalence of PTB, risk factors for TB and the health seeking behavior of chest symptomatics. It is hoped that the findings of this study will help towards developing a health system model towards effective interventions.

Objectives:

(i) To estimate the burden of TB amongst tribal groups in various states of the country

(ii) To find out the health seeking behavior patterns of persons having symptoms suggestive of TB and

(iii) To develop a tribal health system model with feasible interventions to improve case finding and compliance for TB treatment through a community based approach

Summary and Progress: The study has been conducted in 6 states in 48 villages.

The main problems identified through the situational analysis and the qualitative components were:

(1) inaccessibility of health facilities

(2) non-availability of staff in the health facilities

(3) poor awareness on TB

(4) issues related to transportation

(5) delays in health seeking and treatment initiation

(5) risk factors identified such as indoor air pollution, alcohol dependence, smoking, malnutrition

(6) poor hospital conditions

(7) over-dependence on ASHAs

The study has helped to estimate the burden of TB with a reported prevalence of 328 per lakh population. The study findings have also helped to understand the health seeking behavior patterns among the tribal population.

Some of the interim interventions that have been done in these sites based on the problems identified include increasing awareness of TB among the tribals, ensuring follow-up care of the chest symptomatics who reported sputum negative, ensuring initiation of TB treatment for those found positive, diagnosis of MDR of those culture positive, LPA and spolygotyping of the culture positives.

Study in progress: The findings of this study are being fed into the current intervention project funded through the global fund to ensure need based interventions.

The second phase of this project covering 11 states has been initiated and is ongoing.

SB-2: Fostering resilience to psychosocial and HIV risk in Indian MSM

Principal Investigators	:	Beena E Thomas (India PI) (email: beenathomas@nirt.res.in)
	:	Dr. Conall O'Cleirigh (US PI)
Source of Funding	:	National Institute of Mental Health, USA
Study Period	:	2015 – 2020

Background: India is the world's third largest HIV epidemic, and given the population size, one of the largest is populations of men who have sex with men (MSM) in the world. MSM in India have an estimated seroprevalence of 14.7%. HIV prevention efforts for MSM in India are limited to condom distribution and HIV education, with no large scale efficacy trials of interventions and therefore no sufficient evidenced-based interventions in this population.

This proposal is the outcome of successful community-based Indo-US research collaboration between MGH and NIRT. The two NGOs dedicated to HIV prevention among MSM are Sahodaran (Chennai) and The

Humsafar Trust (Mumbai). We have, in every phase of the development of this proposal engaged the community in developing the research ideas, forming the program of research, designing / refining the studies, and disseminating the results to plan further.

Objectives:

(i) To test the efficacy of the self-acceptance-based intervention in comparison to HIV / voluntary counselling & testing (VCT) on co-primary outcomes: sexually transmitted infection (STI) incidence and reduced episodes of unprotected anal sex among HIV-infected and uninfected MSM in Chennai and Mumbai India

Methods: This is a two-arm randomized controlled trial comparing a self-acceptance based HIV risk reduction intervention with VCT among MSM in Chennai and Mumbai, India. The self-acceptance based HIV risk reduction intervention consists of 4 group sessions and 6 individual sessions and HIV and STI testing (at baseline and 12-months). The STIs would include for 3 STIs: Chlamydia, gonorrhea and syphilis. If the participant test positive for HIV/STI, they are referred for care and treatment as required. Participants in both arms will be followed for one year, with behavioral and psychosocial assessments conducted at baseline, 4, 8, and 12 months

Summary and progress: During this period of reporting, both the sites (Humsafar in Mumbai and NIRT, Chennai) have recruited 415/608 participants for the study.

The intervention sessions have been very useful for the participants and the feedback of the sessions have been rewarding. This study will help towards sexual risk reduction and HIV control through a community participatory psychosocial intervention approach.

During this period of reporting (April 2017 - March 2018), 16 batches were recruited (Intervention – 139 & control 137) in the Chennai site with a total of 276 participants. And in Humsafar Trust, Mumbai, 292 participants (Intervention – 146 & Control – 146) have been recruited during this period.

The study is ongoing.

SB-3: Community driven health committee – It's feasibility and effectiveness in addressing gender issues in public health with special reference to TB

Principal Investigator	:	Dr.E Thiruvalluvan (email: thiruvalluvane@nirt.res.in)
Source of Funding	:	DHR
Study Period	:	2015 – 2018

Background: As gender based disparity/discrimination is well documented across the nations, gender specific actions are needed because of the fact that of over a billion people worldwide who live in absolute poverty, 70% are women. In order to address the issues concerned with any targeted sections of population, voices of the concerned must be heard. Hence, an intervention research was undertaken in PHCs to understand the feasibility of bringing women as a forum to make existing public health system gender sensitive.

Objectives:

(i) To understand the gender barriers in TB control strategy

(ii) To study the feasibility and effectiveness of community based health committees and its contribution to accessibility, improved quality and gender sensitive TB health care services and

(iii) To develop guides and tools to minimize gender disparities in TB programme

Methods: This intervention study adopting mixed method design using qualitative and quantitative methods was conducted in 2 phases following a sequential approach in 2 districts namely Tiruvallur district in north and Madurai district in south Tamil Nadu.

Results / Study progress: In the situational analysis phase, focus group discussion (FGD), in-depth interviews, key informant interviews and personal interaction were used to understand the status of women and health seeking behavior in the intervention settings and to identify members for a community forum. A total of 378 individuals were interviewed and also participated in FGD. 70% (266) of participants had expressed willingness to be a part of community forum. Among those who expressed willingness, 59 participants were shortlisted initially but finally only

31 could continue to take part in community forum.

The shortlisted members organized regular monthly meetings in PHC to discuss various issues both general and gender specific in the PHC and arrive at a series of resolutions. In order to broaden their understanding on health issues and skill up gradation, the Community forum members were trained followed by refresher training and skill building sessions. Also stakeholders meeting with health authorities were organized to promote interactions between them.

Feasibility and effectiveness of this study was evaluated on three aspects viz.,

(i) care receivers satisfaction,

(ii) facility improvement in the Primary Health Centre and

(iii) support offered to TB case finding and case holding at the PHC.

Establishing and counting on the community forum members proved to be possible as forum members could attend 60 monthly meetings. Secondly, in the forum meetings, the members could identify a range of gender sensitive issues, discuss and resolve them. Some of the needs that were raised in the meetings and addressed in public health system were

1. Separate toilet facility,
2. Separate injection room and
3. Drinking water.

Activities of community forum could also resulted in increased care receiver's satisfaction level in areas such as;

1. Satisfaction at registration
2. Satisfaction at injection room
3. Satisfaction about basic facility
4. Satisfaction about working hours
5. Satisfaction about health / disease / treatment related queries

Therefore, it is suggested that community forum essentially consisting of community women can be incorporated in public health system. In order to facilitate this trained professional social workers with skills in human relations, communication skills and sensitive to needs of women may be appointed at each PHC. Trained professional social workers can facilitate coordination between Health Care providers and community forum members apart from mobilizing forum members and create a link with the public health system. Certain quantum of funds may be allocated to each PHC to conduct meetings at regular interval

and offered some sort of compensation to members at least to meet expenses on the day of meeting. A specific place or room may be earmarked in the PHC for the community forum to meet.

The researchers strongly recommend that RNTCP may initiate women led community forum in every treatment unit that will ensure achievement of the honourable Prime Minister's National Strategic Plan for Tuberculosis (TB) Elimination 2017-25.

Further, in order to devise further strategies, larger studies may be taken up at regional level /across states in India.

SB-5: Strengthening implementation and operational research under the RNTCP in India

Principal Investigator	:	Dr. Beena E Thomas (email: beenathomas@nirt.res.in)
Source of Funding	:	Global fund through Central TB Division
Study Period	:	2016 – 2018

This is an innovative project aimed at strengthening implementation and operational research focusing on the priority areas as defined by the RNTCP in India. There was "Call for Proposals "which required researchers to submit their proposals as teams. These teams needed to include clinicians, social scientists, epidemiologist, statistician or an economist. Responding to this call, 68 concept proposals were received. These proposals were screened by a team of steering committee members who are experts from various disciplines. A total of 15 proposals were shortlisted, and were called for the Protocol Development workshop convened by the NIRT, Chennai, India from May 30 to June 03, 2016. A total of 69 participants, SAC (13), mentors (11), Principal Investigators and research scholars (43) from various academic and research institutions across the country including the representatives from WHO / Research and Training in Tropical Diseases (TDR), Geneva (02) have attended the workshop. Among the 15 proposals, 13 were reviewed in detail

by the SAC and 10 were developed into full proposals and recommended for funding from GFATM through the Central TB Division and ICMR. Additionally, the committee has recommended a project titled “Improving treatment adherence among tuberculosis patients through evening DOTS in Chennai, India” for funding from GFATM. All the selected projects were given specific names by their investigators for further correspondence (Table 24).

Table 24: Details of projects

S.no	Title of the study	Name of the project	Location	
			Districts	State
1	Engaging public sector AYUSH practitioners to increase referral of presumptive TB cases for early tuberculosis case detection in Shimla and Kangra districts of Himachal Pradesh, India	Himachal leopard	Shimla and Kangra	Himachal Pradesh
2	Improving treatment adherence among tuberculosis patients through evening DOTS in Chennai, India	E-DOTS	Chennai	Tamil Nadu
3	Provision of DOTS by a family member to prevent treatment default in tribal and hard to reach areas in the state of Chhattisgarh, India	Indravati – Family DOTS	Kondagaon	Chhattisgarh
4	Systematic screening for presumptive pediatric TB patients and gastric aspirate specimen collection in primary and secondary health care facilities in Rajnandgaon, Chhattisgarh, India	Mahanadi	Rajnandgaon	Chhattisgarh
5	A multi-component health system strengthening intervention to reduce pre-treatment loss to follow-up of smear positive tuberculosis patients in Chennai A quasi-experimental study	Marina - PTLFU	Chennai	Tamil Nadu
6	Identifying costs contributing to catastrophic expenditure amongst TB patients registered under RNTCP in two metro cities in India	Neelaganga	Chennai	Tamil Nadu
			New Delhi	New Delhi
7	Strengthening Tuberculosis and HIV detection and management through intensified case finding in Central Jail , Aizawl, Mizoram	Nuaben	Aizawl	Mizoram
8	Evaluation of Programmatic management of drug resistant TB using revised tools in three states of northern India.	Gulab - PMDT	-	New Delhi Punjab Jammu and Kashmir
9	Developing an integrated strategy to improve utilization of TB services among injecting drug users in Mizoram	Redcap - IDU	-	Mizoram
10	Identifying barriers and sustainable solutions to low	Rudramma	-	Telangana state

	RNTCP paediatric TB case notification in Telangana state			
11	Identifying and addressing the factors contributing to pre-treatment loss to follow-up of tuberculosis patients referred for treatment from medical colleges in Pondicherry, India	PIMS - PTLFU	-	Pondicherry

All the above studies have been implemented in the respective settings and they are being monitored for their activities. Interim reports from all PIs have been received for the period till Jan 2018 and it has been reported to ICMR. Preparation of the final progress report is in progress.

SB-9: Targeted intervention to expand and strengthen TB control in tribal populations under the RNTCP, India (TIE-TB Project)

Principle Investigator	:	Dr. Beena Thomas (email: beenathomas@nirt.res.in)
\\Source of Funding	:	Global Fund through ICMR and CTD
Study Period	:	July 2016-June 2018

Background: ICMR in collaboration with Central TB Division has undertaken this project to cover defined hard to reach, tribal areas spread over the central and western parts of India. In this multicentre study, NIRT has been given the responsibility of implementation in Gujarat in 4 tribal dominant districts. This project focuses on interventions of structured community engagement, involvement of traditional healers and targeted usage of Mobile vans equipped with Digital X-ray and sputum microscopy services in an effort to improve access to TB care services and improve the health seeking behaviour of the tribal populations.

Objectives and Proposed Interventions:

1.1 Strengthen access to RNTCP services in the tribal population
Deployment of Mobile TB Diagnostic Vans (MTDV) (Equipped with Digital X-ray and Sputum Microscopy services) to Geographically Remote Places.

1.2 Involving the community to bridge the gap between the health systems and the community in providing RNTCP services through Community Health Volunteers (CHV) to improve and promote early case detection and treatment adherence in the tribal population.

1.3 Improve awareness on TB and RNTCP services through community based advocacy, communication and social mobilization activities – Involving traditional healers and local community groups into the RNTCP ambit.

Project Progress status:

The project has three distinct phases: (1) Situational analysis; (2) Implementation of the intervention and (3) End line survey. The project has been initiated at four Tribal districts identified by the RNTCP at Gujarat. i.e. The Dangs, Dahod, Narmada and Vyara (Tapi).

Community Health volunteers have been recruited in all the villages and will serve as the community liaison for the intervention part of this study.

The role of community volunteers will be primarily on identification and referral of chest symptomatics in their own villages. CHVs will be involved in the process of referral to health facility for diagnosis and rolling out various IEC activities. Table 25 shows the number of CHVs recruited and trained during the reporting period.

Table 25: Details of CHVs engaged in the study

District	Number of volunteers recruited
Dahod	360
Dangs	300
Vyara	315
Narmada	45
Total	1020

Details of MTDCVs used in 4 districts are shown in Table 26.

Table 26: Distance travelled by seven MTDVs employed in 4 districts

No of MTDV	District	Taluka	Intervention Remote Villages	Distance travelled (in KMs)	Initiation of MTDV visits
4	Dahod	8	313	9919	09-08-2017 09-08-2017 07-09-2017 11-09-2017
1	Dangs	3	311	3838	11-08-2017
1	Narmada	5	169	3068	18-08-2017
1	Tapi	5	177	6227	14-08-2017
7	4	26	958	23,052	

The details of chest symptomatics identified, X-ray positive, sputum positive and both X-ray and sputum positive cases identified through MTDV are shown in Table 27.

Table 27: MTDV intervention TB case identification

MTDV output progress (09.08.2017 to 31.01.2018)							
District	No of Villages	Population	No. of presumptive cases	Sputum tested	Chest X ray done	X ray positive	Both X ray and Sputum positive
Dahod	185	551759	6904 (1.25%)	6904 (100%)	6889 (100%)	572 (8.3%)	165 (2.4%)
Tapi	106	234170	833 (0.36%)	651 (78%)	831 (100%)	44 (5.3%)	21 (2.5%)
Narmada	78	79960	795 (1.0%)	377 (47%)	468 (59%)	57 (12.2%)	9 (1.1%)
Dangs	96	73924	808 (1.1%)	800 (99%)	800 (99%)	61 (7.6%)	12 (1.5%)
Total	465	939813	9340 (1.0%)	8732 (93%)	8988 (96%)	734 (8.2%)	207 (2.2%)

The study is ongoing.

SB-11: Evaluating 99DOTS novel strategy for monitoring adherence to TB medication

Principle Investigator	:	Dr. Beena Thomas (email: beenathomas@nirt.res.in)
Source of Funding	:	Bill & Melinda Gates Foundation
Study Period	:	July 2017- Dec 2018

Background: India's RNTCP is currently transitioning away from thrice-weekly TB therapy and towards provision of daily therapy for all TB patients. In this context, new patient-centered strategies for monitoring TB medication adherence are urgently needed. 99DOTS is a cell phone based strategy to report the adherence of TB medication.

While this novel monitoring strategy is promising, no formal research has been conducted to evaluate their impact on TB patients and healthcare providers in a real-world setting. Therefore the study aims to evaluate the following questions: (1) the accuracy of 99DOTS for assessing adherence (that is, the extent to which the 99DOTS adherence record correlates with actual pill ingestion);

(2) the acceptability and feasibility of 99DOTS and the medication event reminder monitor (MERM) to both patients and providers. We enrol patients into the study and conduct unannounced home visits to collect 600 eligible urine samples to find out the accuracy of 99DOTS. To evaluate the acceptability and feasibility of 99DOTS we conduct in-depth interviews with 60 patient and 30 health care providers.

Objectives:

(i) To evaluate the accuracy of 99DOTS for monitoring adherence to daily TB therapy among drug-susceptible TB patients in the RNTCP and

(ii) To evaluate the acceptability and feasibility of 99DOTS for both patients and healthcare providers

We proposed to complete 600 home visits to evaluate the accuracy of 99DOTS. To evaluate the acceptability and feasibility of 99DOTS, we proposed to conduct 60 in-depth interviews with the TB patients and 30 interviews with the health care providers.

Methods: We enrolled TB patients on treatment through the Government program in Chennai (HIV co-infected patients) and Mumbai (HIV un-infected patients). We conducted unannounced home visits to collect urine sample for

isoniazid test (IsoScreen). We compared the patient's medication adherence record as recorded by 99DOTS to the urine isoniazid test result to assess 99DOTS' accuracy of 99DOTS.

Progress: To evaluate the accuracy of 99DOTS (Aim 1), 495 TB patients were enrolled in our study and 328 home visits has successfully been completed and the urine sample for INH test has been collected. To evaluate the acceptability and feasibility of 99DOTS (Aim 2) we have conducted interviews for 60 patients and 30 health care providers. Out of 90 interviews (60 + 30), 50 translations have been completed so far and 40 translations are yet to be completed.

SB-12: Evaluating the acceptability and accuracy of the MERM for monitoring medication adherence in MDR-TB patients

Principle Investigator	:	Dr. Beena Thomas (email: beenathomas@nirt.res.in)
Source of Funding	:	Bill & Melinda Gates Foundation
Study Period	:	July 2017- Dec 2018

Background: MDR-TB patients have very poor treatment outcomes, with nearly half of all patients dying, failing therapy, or becoming loss to follow-up before the end of treatment.1,2 Therefore, there is an urgent need for better adherence monitoring that can assist in early detection of patient non-adherence (i.e., real-time monitoring) or provide structured adherence information that can assist providers in counselling patients during follow-up visits (even if not in real time).

The MERM is an electronic pillbox that uses cellular connections to transmit data regarding whether and when the pillbox was opened, also presuming that the opening of the pillbox corresponds with the medication being "in-hand" and likely ingested. The medication event reminder-monitor (MERM) has features that may facilitate its use for monitoring

TB medication adherence for MDR-TB patients.

While this novel monitoring strategy is promising, no formal research has been conducted to evaluate their impact on TB patients and healthcare providers in a real-world setting. Preliminary research is needed to evaluate the acceptability and accuracy of MERM. Department of Social and Behavioral Research, NIRT, will be responsible for coordinating this research agenda, which will help inform decision-making regarding whether there should be further research on this adherence monitoring technology.

Aim of the study:

(i) To evaluate the acceptability and accuracy of medication event reminder monitor (MERM) we proposed to conduct 40 interviews with the MDRTB patients in Chennai and 25 interviews patients in Mumbai

Methods: We enrolled MDR-TB patients who are using (MERM) box in Chennai and Mumbai to assess the MERM's failure rate, patient acceptance of the device, and barriers to use.

Evaluation of the accuracy of the MERM for monitoring adherence is peformed by doing pill counts and administering an adherence questionnaire during unannounced home visits with these patients and comparing these findings to the medication adherence record as captured by the MERM.

Progress: For MERM (Aim 3), out of 65 MDR-TB patients, 58 have been enrolled and in-depth interviews for these patients have been completed. So far, 22 interviews have been translated.

SB-13: Utilization of school students as Ambassadors in TB sensitization in Chennai city

Principal Investigator	:	Mrs. Priscilla Rebecca (email: Priscilla.r@nirt.res.in)
Source of funding	:	The United Nations Office for Project Services
Study Period	:	2016-2018

Background: One of the important Stop TB strategies is TB education and empowerment of communities to take appropriate TB care. There are evidences from several studies and health programs carried out in many developing countries showing that involving school children in health education is effective and successful. With this background the current intervention aims to improve the TB specific health literacy in an urban poor community by engaging school students.

Objectives:

(a) To promote health literacy among school students about TB and equip them as TB advocates in the community

(b) To develop an intervention model to engage school students as TB advocates to impart TB health literacy in an urban poor community and

(c) To study the feasibility of this intervention by the students in the community

Methods: The study is being carried out in three phases. First phase is the formative phase done to equip the students through intensive TB intervention training sessions. Prior to he intervention training base line assessments is done. During this phase student TB ambassadors will be selected who will be actively involved in the TB sensitization activities among the student community with in their respective schools which is the second phase of the study. Finally in the third phase an end line assessment will be done to ascertain the level of rise in the TB literacy and the impact and feasibility of the interventions.

Study progress: The study was initiated after obtaining permission from the authorities and the schools were selected from the Chennai Corporation Zones. A total of 57 schools were line listed. Baseline data collection was completed in 10 schools that covered 420 students. Intervention tools for

primary and secondary interventions were finalized. It included children friendly animation movie on TB for 8 minutes. Other intervention tools were designed which included several IEC materials such as book marks, caps, pens, badges and stickers with TB messages printed on them. Primary intervention was completed in 24 out of 57 schools covering approximately 8108 students. A total of 240 student ambassadors were selected from the schools. These student ambassadors have been actively involved in conducting TB awareness programs in their respective schools for other students. Overall, 3775 students have been covered through the student ambassador programs. In parallel, end line surveys are also being done in the schools where the interventions have been completed. The study is on-going.

SB-14: Social network analysis as a tool to improve active case finding at community level in Chennai, south India

Principal Investigator	:	Dr.N. Karikalan (email: karikalan.n@nirt.res.in)
Source of Funding	:	The United Nations Office for Project Services
Study Period	:	2017-2019

Background: Early case detection and treatment is important for controlling TB. Delayed TB diagnosis can increase the transmission of infection, hasten disease progression and increase the risk of mortality among populations. Active case finding (ACF) is being prioritized as a strategy under the RNTCP in India to address the delay in diagnosis. ACF is resource intensive and holds considerable challenges since TB is an air borne disease and the susceptible individuals or subgroups cannot be singled out easily. Alternate and novel strategies (e.g., Social Network Analysis) for implementing ACF at the community level are needed.

Social network analysis (SNA) is the study of social structure which connects individuals. Social network has three sub components, which are:

i) The network relationships;

ii) Network structures and

iii) The network functions

The characteristics of social network members with whom any TB patient interact greatly influence the TB transmission within the network. Social networks and the geographical places of networking contextualize the TB transmission in any community. There is a need to systematically understand the social network of TB patients which could provide insights on underlying social structure and the key social network members who influence the TB transmission within the network members and the key geographical locations which facilitate the transmission. In this study, we aim to test the feasibility of SNA as a strategy to explore TB transmission patterns which otherwise could have missed from being detected by routine contact investigation.

Objectives:

(i) To identify key social network members facilitating TB transmission in

North Chennai for complementing active case finding efforts

(ii) To identify importance of places of social congregation of TB cases and their social network contacts which facilitate TB transmission in North Chennai for complementing active case finding efforts and

(iii) To identify TB case and contact dyads and other social network relationship patterns in program settings of North Chennai.

Method: This is a mixed method exploratory study involving both quantitative and qualitative study methods. The study sample for qualitative interview is 15-20 and for quantitative study it is 300 TB patients. The study setting is TUs in North Chennai Corporation.

Progress of the study: As per the proposed project plan, collection and compilation of RNTCP secondary data in North Chennai TUs for the past five years (from 2013- to 2017) has been done. The retrospective analysis of the same to identify streets and areas with high density of TB patients is under progress. The qualitative social network data collection from TB patients through In-depth interviews has been completed. The transcription, translation and analysis of the data are under process. Ego-centric social network survey in North Chennai TUs has been initiated and 33 surveys have been completed so far. Hot spots were identified, where the social network members of our index cases converged frequently and where there was possibility to identify additional TB cases/symptomatics. GIS locations for these spots are yet to be obtained.

SB-15: Patients' perception on quality of care in TB care settings in Chennai

Principal Investigator	:	Mr. P. Murugesan (murugesan.p@nirt.res.in)
Source of Funding	:	The United Nations Office for Project Services
Study Period	:	2017-2019

Background: Quality of Care plays a vital role in the status of TB control, which influences early and timely diagnosis, treatment adherence, and treatment compliance. Quality of TB care in general context and in India, is conventionally assessed in the context of treatment outcome measures like mortality and microbiologic cure and has not focused on patients' expectations and preferences such as satisfaction with care. The patient centric quality is crucial in influencing clinical and treatment outcomes of the patients. Therefore, there is a need to understand the patients' perception on quality of TB care.

Objectives:

(i) To explore and understand patients' perception on the quality of care in TB care settings

(ii) To identify the reasons for choice of providers, reasons for shift of providers and

(iii) To develop a patient specific quality of care tool in TB care settings

Methods: This is a sequential exploratory mixed method study that includes both qualitative and quantitative design following a sequential approach implemented in a phased manner. The study is conducted within the randomly selected 15 zones of Chennai. Probability proportion to size sampling method is used in enrolling the participants for the study. TB patients above 18 years and those registered under the RNTCP and Public – Private Mix are included in the study.

Study progress: The study was initiated in January 2018. So far, 35 in-depth interviews have been completed, out of which 23 participants are continuing TB treatment in the same sector (either public/private) and the remaining 12 are those participants who have shifted from public to private health facility and vice versa. Totally 5 focused group discussions covering 35 patients have been completed till date. Transcriptions and translation of in-depth interviews are going on.

The study is ongoing.

LABORATORY STUDIES

Department of Bacteriology

STUDIES COMPLETED:

B-6: Molecular drug resistance characterization of extensively drug-resistant strains of *M. tuberculosis* from south India

Principal Investigator : Dr. S Siva Kumar
(e.mail:shanmugamsiva@nirt.res.in)
Source of funding : ICMR-Extramural
Study period : 2014-2017

Background: The ever-increasing burden of drug resistance is a serious concern in developing countries, particularly for patients with *M. tuberculosis* infection. *M. tuberculosis* uses various mechanisms to evade killing by therapeutic drugs, including mutations in genes that code for drug target proteins. TB control and prevention programs are based on early diagnosis followed by rapid identification of drug resistance. The development of rapid molecular methods, which can be performed within 1 or 2 days, is important for the timely detection.

Aims:

(i) To determine the molecular drug resistance pattern for 1st and 2nd line anti-mycobacterial drugs and compare it with the phenotypic results and

(ii) To identify mutations in drug target genes of extensively drug resistant (XDR) strains of *M. tuberculosis* prevalent in the south Indian population

Methods: Seventy five clinical strains of *M.tuberculosis* (25 XDR, 25 MDR & 25 all sensitive [pan-sens]) were grown on solid LJ Medium or Liquid MGIT. They were coded, sub cultured to obtain heavy growth and DNA was extracted. Spoligotyping and WGS were performed on 19 isolates. Pyro sequencing was performed on 29 isolates (16 XDR, 6 MDR and 7 pan-sensitive strains) to detect mutations in the following genes: *rpoB, katG, embB, rrs, gyrA, gyrB, rpsL, fabG1, ahpC, thyA, embA, embC, pncA, rrl* and the promoter regions of *inhA, ahpC, embA-B, fabG1, rpsL, thyA* and *pncA.* Additional SNPs/genes involved in resistance also were looked at if the phenotypic and genotypic resistances did not correlate.

Results: We analysed data from WGS for 19 XDR/MDR isolates Fig. 4

presents the drug specific mutations frequency detected in the XDR-TB strains. Analyzed data on XDR-TB strains showed 3 out of 7 OFX resistant isolates (*gyr*A A90V) were likely treatable with MFX. Among the 8 XDR isolates which were phenotypically kanamycin resistant, 5 had mutation *(rrs* 1401G) that conferred resistance to kanamycin, amikacin & capreomycin, the other 3 had mutation (eis-12 C/T promoter) that conferred low level resistance to kanamycin alone and hence offered the choice of other aminoglycoside for treatment.

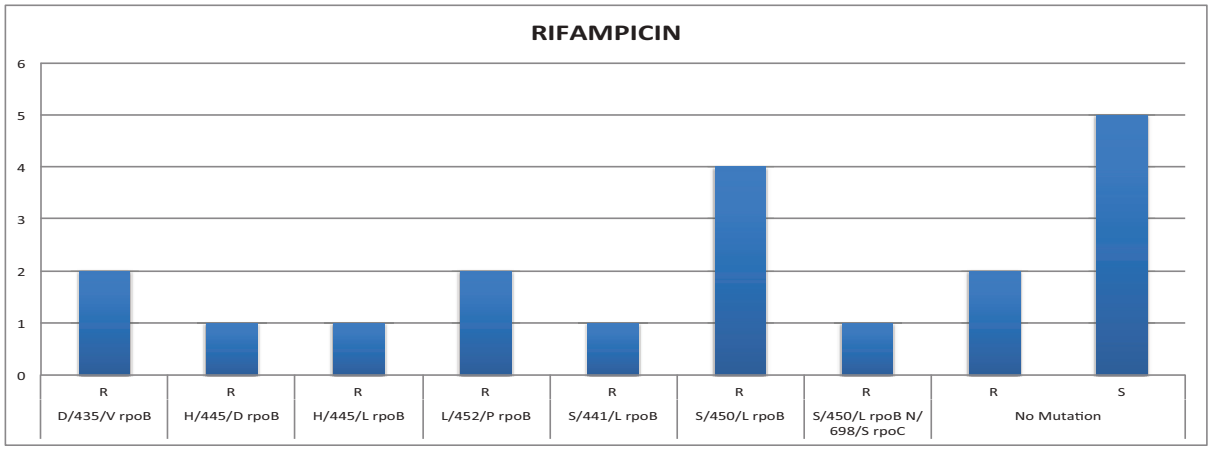
WGS analysis on XDR isolates revealed that, one isolate harbored a novel mutation in RplC and two isolates had one novel *rrl* mutation which are targets for the drug linezolid.

The drugs oxazolidinone AZD5847 and sutezolid (PNU-100480), which are currently in clinical development, are believed to share the same resistance mechanism. This clearly suggests that WGS can be a choice of test in the case of XDR and pre XDR patients to assist in individualized treatment regimen. Data suggests that *alr* mutations likely confer D-cycloserine (DCS) resistance, although allelic exchange experiments are required to formally prove this (particularly for R373L, which coincided with a deletion in *ald* and, consequently, may not be sufficient to confer resistance on its own). Although the relationship between MICs and IC50s can be complex, the observation that MICs increased by only 4 to 16-fold versus at least 25-fold increase for IC50s supports the notion that DCS inhibits multiple targets, as noted earlier. This study should be complemented with extensive MIC testing of phylogenetically diverse pan susceptible MTBC strains to define the epidemiological cutoff value, given that it is unclear based on which evidence the current WHO critical concentration on LJ has been set. Moreover, further MIC testing of likely DCS-resistant strains is needed to investigate whether the Sensititre system is less reliable at detecting DCS resistance than are LJ and MGIT. Finally, the impact of *alr* mutations on resistance on terizidone remains to be investigated. Phenotypic testing is currently standardized to correlate the results for all the reserved drugs including delamanid and bedaquiline.

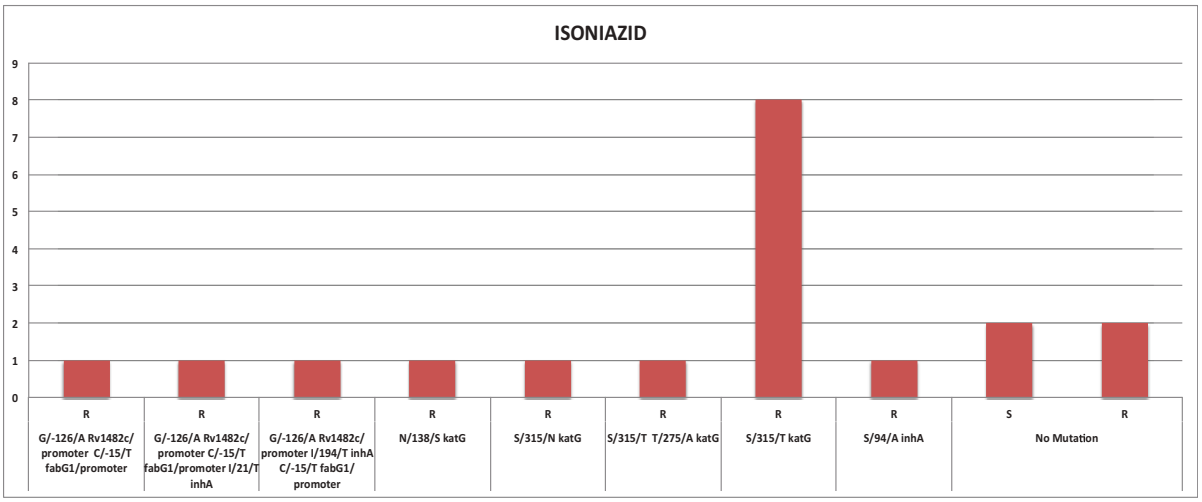
Applied value of the project: The project work planned in this study will lead to identification of more promising markers for improving both sensitivity and specificity of detection of resistance and cross-resistance to 1st line and 2nd line anti-mycobacterial drugs for the XDR/MDR strains. This study will pave the way to future molecular diagnostics which will need to include more mutations and genes in order to accurately and sensitively detect resistance and cross-resistance to 1st and 2nd line of ATT drugs.

Fig. 4: Prevalent mutations in the *M.tuberculosis* strains tested

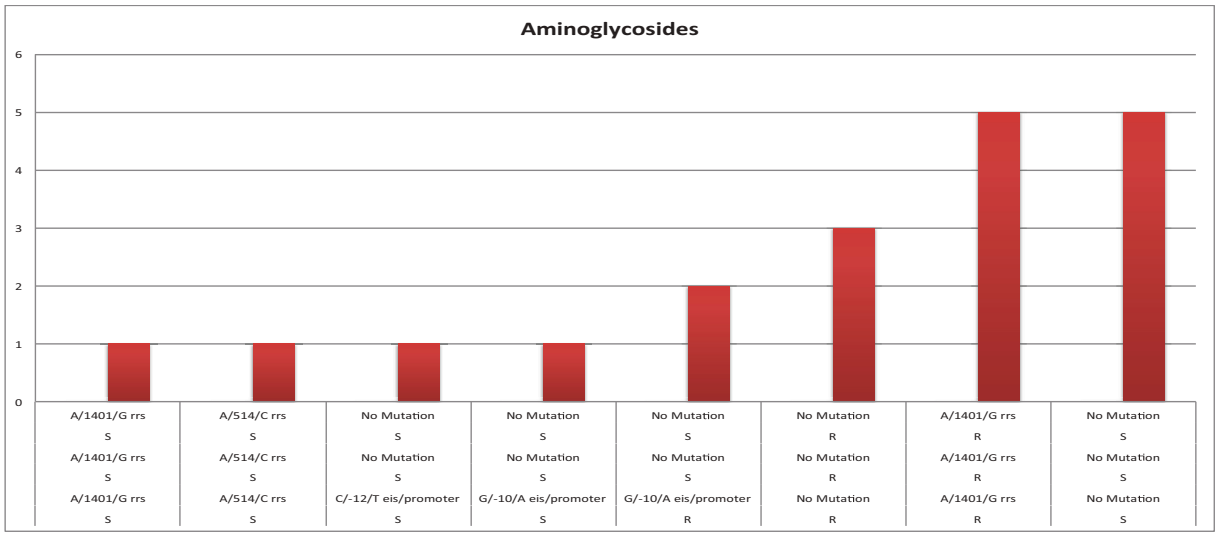
A: Isoniazid



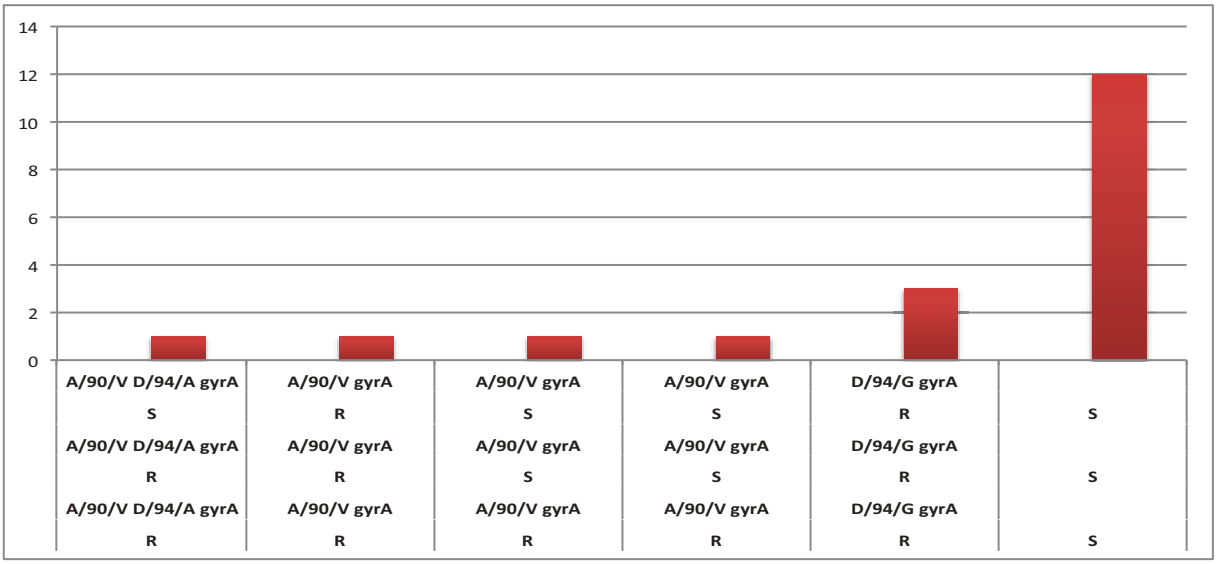
B: Rifampicin



C: Aminoglycosides



D: Fluoroquinolones



B-2: Multi-centric validation of an indigenous test for diagnosis of PTB from sputum sample

Principal Investigator	:	Dr. N. S. Gomathi (email: gomathisharma@nirt.res.in)
Source of funding	:	Department of Biotechnology – Extramural
Study Period	:	2014-17

Background: The Truenat MTB™ is a chip-based nucleic acid amplification test for detection of *M. tuberculosis* from sputum samples. The manufacturers claim that this technology is meant for decentralized, "point-of-care" use, but there are no published data on their feasibility in microscopy centers.

Aim:

(i) To validate the indigenously developed Truenat MTB test against the laboratory reference standards – culture and GeneXpert

Sites: The kit was validated in four sites namely, AIIMS - New Delhi, NITRD - New Delhi, JALMA - Agra and NIRT - Chennai.

Methodology: Two sputum samples were collected from eligible presumptive PTB patients attending the TB clinics under the RNTCP after obtaining informed consent. A total of 3541 patients from were included in the study. From each sample direct smear and Truenat were performed directly. About 0.5ml of sputum was removed from each sample, pooled and a single Xpert test was performed. Remaining sample was decontaminated by the NaLC-NaOH method and from the pellet, culture by LJ and MGIT960 were done. All assays were performed as per manufacturer's / standard protocol. The candidate assay Truenat was done only after blinding the samples.

Results: Site-wise distribution of the patient intake is given in Table 28. Among the 3541 patients included, 818 were smear positive and 2723 were smear negative.

Table 28: Site-wise distribution of available results

AIIMS	747
JALMA	550
NITRD	1144
NIRT	1100
Total	3541

Results from the candidate and gold standard tests are given in Table 29.

Table 29: Distribution of results from candidate and gold standard tests

Test	Positive	Negative	Error/In,	Missing	Total
Truenat	1590 (44.9%)	1707 (48.2%)	244 (6.9%)	0 (0.0%)	3,541
Xpert	1055 (29.8%)	1904 (53.8%)	32 (0.9%)	550 (15.5%)	3,541
Smear	999 (28.2%)	2542 (71.8%)	0 (0.0%)	0 (0.0%)	3,541
MGIT	1113 (31.4%)	2317 (65.4%)	111 (3.1%)	0 (0.0%)	3,541
LJ	878 (24.8%)	2455 (69.3%)	208 (5.9%)	0 (0.0%)	3,541
Culture	1183 (33.4%)	2320 (65.5%)	38 (1.1%)	0 (0.0%)	3,541

Using available results, performance of Truenat MTB assay was compared with comprehensive and individual gold standard tests. Comparison of Truenat with the comprehensive gold standard results are presented in Table 30.

Table 30: Performance of Truenat MTB assay with gold standard tests

			TRUENAT (n=2685)	
Smear	**Culture**	**XPERT**	**Pos.**	**Neg.**
NEG	**NEG**	**NEG**	**277 (18.7%)**	**1208 (81.3%)**
NEG	NEG	POS	111 (66.9%)	55 (33.1%)
NEG	POS	NEG	55 (56.1%)	43 (43.9%)
NEG	POS	POS	129 (86.6%)	20 (13.4%)
POS	NEG	NEG	8 (44.4%)	10 (55.6%)
POS	NEG	POS	58 (92.1%)	5 (7.9%)
POS	POS	NEG	81 (84.4%)	15 (15.6%)
POS	**POS**	**POS**	**575 (94.3%)**	**35 (5.7%)**
		Total	**1294 (48.2%)**	**1391 (51.8%)**

In comparison with the combination of phenotypic (smear and culture) and genotypic (Xpert tests), Truenat yielded an agreement of 94.3% and 81.3% in identifying positives and negatives respectively.

Summary of the performance of Truenat in comparison with the gold standards for detection of MTB is given in Table 31. Truenat yielded a sensitivity of 91.1%, 87.8% and 88.5% in comparison with smear, culture and Xpert respectively while specificity was 69.5%, 72.5% and 75.5% respectively. The assay showed moderate agreement with smear and culture, and it was concordant with the molecular test Xpert.

Table 31: Performance of Truenat in comparison with the gold standard tests for detection of MTB

Characteristic	Smear	Culture	Xpert
Sensitivity	91.1 %	87.8%	88.5%
Specificity	69.5 %	72.5%	75.5%
Observed Agreement	75.8 %	77.8 %	80.3%
Kappa value	0.508	0.551	0.602
Strength of Agreement	Moderate	Moderate	Good

Performance of the two molecular tests – Truenat and Xpert were compared with the existing reference standards – smear and culture to better understand the usefulness and applicability of the new candidate test if adopted in the program. The results are presented in Table 32.

Table 32: Comparison of performances of the molecular tests

	Smear		Culture	
	Xpert	**Truenat**	**Xpert**	**Truenat**
Sensitivity	85.5%	91.1%	79.6%	87.8%
Specificity	83.0%	69.5 %	86.7	72.5%
Obs. Agreement	84.0%	75.8 %	84.0%	77.8 %

Conclusion: Truenat MTB test appears to be better in identifying true positives in comparison with smear and culture. Lower specificity may be due to its capacity to identify positives even with lower bacterial load. However, specificity of the kit needs to be ascertained using samples from non disease specific patients.

B-4: Surveillance of PZA drug resistance among new sputum smear positive patients

Principal Investigator	:	Dr.N.S. Gomathi (email: gomathisharma@nirt.res.in)
Source of funding	:	USAID through MDP/ICMR intramural
Study Period	:	2014-2017

Background: PZA is a drug given to new smear positive patients under Category I. Limited information is available on the prevalence of resistance to PZA globally and none from India. Surveillance studies on PZA resistance are crucial for making policy decisions on inclusion of the drug in treatment regimens for management of TB.

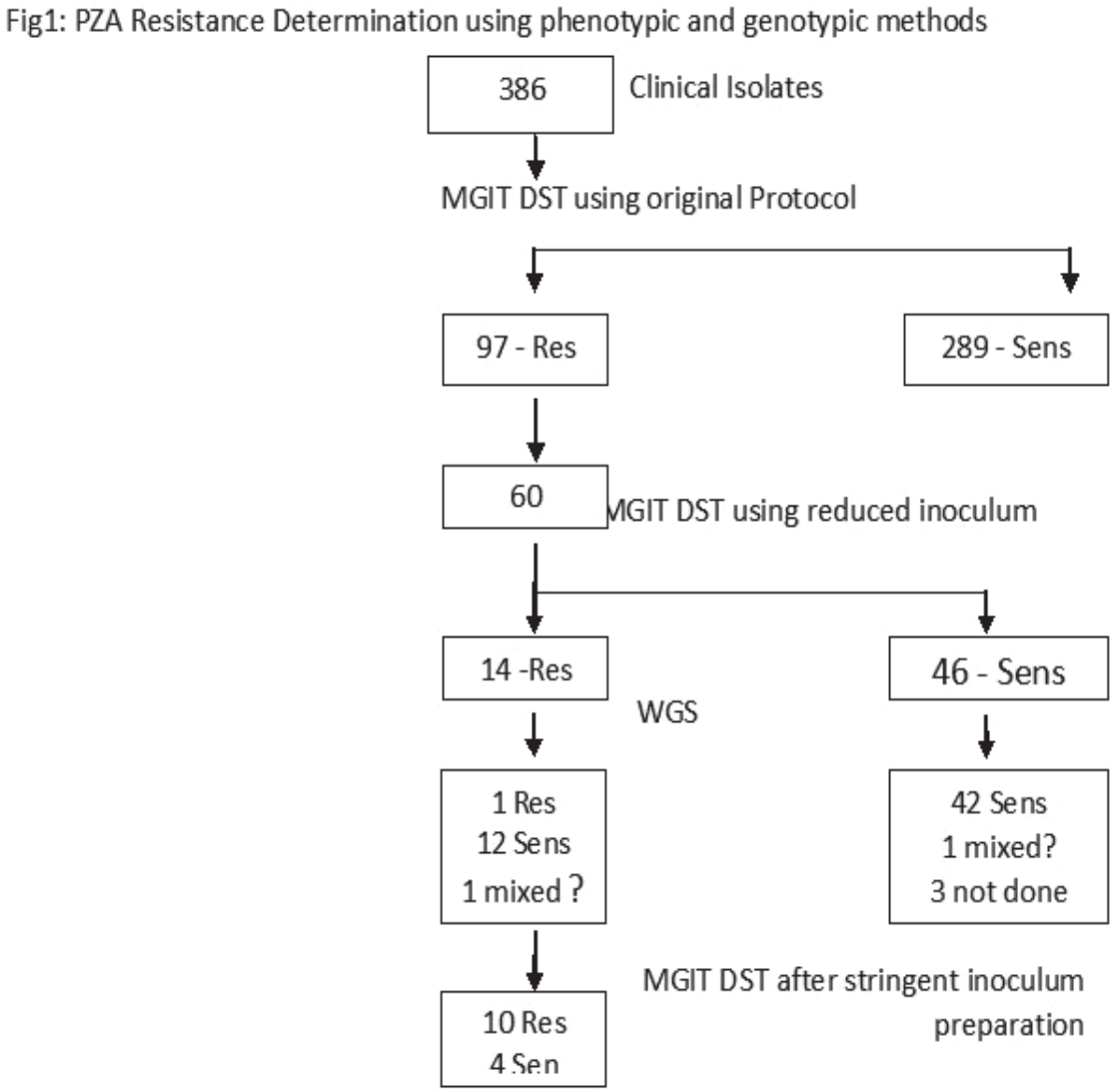
Aim:

(i) To estimate the prevalence of PZA drug resistance among new sputum smear positive (NSP) cases of *M. tuberculosis* using BACTEC MGIT 960 system

Method: Five hundred clinical isolates stored at -80^{o}C from new smear positive patients from the Tamil Nadu Drug Resistance Surveillance were selected based on availability and sub-cultured on to BBL MGIT 7ml tubes for the study. DST to PZA was set up in BACTEC MGIT960 at a concentration of 100 g/ml as per the manufacturer's protocol. Of the 386 cultures that yielded interpretable results, 97 (25.12%) were found to be resistant. Retesting using a modified methodology on 60 of the 97 cultures identified 14 cultures as resistant. Whole Genome sequencing of the strains to identify mutations in the *pnc*A gene was done on 57 available cultures. WGS identified one isolate as PZA resistant, 12 as PZA susceptible and 1 as mixed (of the 14 resistant by MGIT) and 42 as PZA susceptible and 1 as mixed (of the 43 suceptible by MGIT). DST was repeated for the 14 resistant cultures using stringent inoculum preparation where-in a fresh subculture of the isolate was let to stand for 24 hours, the 3 ml of the top portion of the culture was removed and subjected to rigorous vortexing with sterile beads for 5 minutes. The suspension was allowed to stand for 20 minutes and 2 ml of the top portion was used as neat inoculum. Ten of the cultures (including the mixed culture and the WGS resistant culture) were found to be PZA resistant while 4 were

identified as PZA susceptible. Summary of the findings is presented in Fig.5.

Fig. 5: PZA resistance determination using phenotypic and genotypic methods



Conclusion: The study identified one out of 386 clinical isolates of *M. tuberculosis* from new smear positive patients as resistant to PZA by the genotypic method while the phenotypic method yielded varying results when the method of inoculum preparation varied, thus indicating the crucial nature of the inoculum preparation while using a phenotypic method. While WGS appears to be more reliable in identifying PZA resistance, more studies correlating the results with clinical outcomes are required.

B-10: Operational feasibility and performance of Truenat MTB RiF assays in field settings under the RNTCP

Principal Investigator	:	Dr. Srikanth Tripathy (email:: srikanthtripathy@gmail.com)
Source of funding	:	Ministry of H and Family Welfare / ICMR- ITRC
Study Period	:	2017-2018

Background: Truenat MTB is an indigenous molecular test developed by MolBio Diagnostics Pvt Ltd Goa, for rapid detection of MTB or DRTB using real-time micro PCR. Automated, battery-operated devices are used for extraction of DNA (Trueprep Auto device) amplification (Truenat MTB chip) and reading the presence of specific genomic sequences (TruelabUnoDx real-time PCR analyser) using patients' sputum samples. Any resistance to rifampicin (RR) is detected by doing a second RTPCR as a reflex test using the same DNA that identifies selected rifampicin resistance associated mutations.

Following earlier evaluations in India, the current multi-centric field study is being planned to demonstrate the performance of Truenat MTB Rif in routine programmatic conditions. In addition, end-user input will be obtained through use of questionnaires.

Aim:

(i) To assess the operational feasibility and performance of Truenat MTB Rif assays in field settings under the RNTCP

Method: Ten states were selected using Probability proportional to size (PPS) sampling - size being number of DMCs in each state. From each state 5 districts were selected and from each district 2 DMCs were selected. The 2 DMCs were selected based on annual volume of slides in selected DMCs/Districts as per 2016 data. Sputum samples from all consecutive presumptive PTB patients attending the DMCs over a period of 1 month will be included. At the DMC, the LT performed Truenat assay and smear in addition to filling up a feedback questionnaire every day. Samples were then shifted to the CBNAAT site where Xpert was performed. Preliminary analysis of the data has been completed.

Results:

Operational feasibility of Truenat MTB assay in Field conditions:

Data obtained from the feedback questionnaires was analysed and the following inferences were drawn on:

1. Packaging – Good and sufficient as no damages during transit, all documents in place
2. User friendly – The kit was easy to install, easy and quick to start up
3. Data entry – Instrument screens had clear display, key board easy to operate for input, editing; instructions and icons easy to understand
4. Indication of charge or battery level in the instrument to be improved
5. DNA extraction – Easy, good and complete
6. PCR – No error in reading barcodes, no loading and unloading failure, run time matching with expected time, run display to improve, clear result display, easy to print
7. Connectivity – easy to connect and transfer results to server
8. Instrument robustness – light weight and easy to handle, no temperature build up, no run stop during error, no reboot required, easy to surface clean, battery back-up as given, minimal need for instrument
9. Need for change of instrument during the study period was in 11% out of the 125 sites
10. Need for software updation in the 125 machines installed for the study was 9%
11. Training and training material – sufficient
12. Recommendation by LTs – 97%

Performance:

As per available data, a total of 15625 sputum samples were tested and the positivity rates among the three tests viz. Smear, Truenat and Xpert were 7%, 18.9% and 15.5% respectively (Table 33). Performance characteristics of Truenat in comparison with Smear and Xpert as per preliminary analysis are given in Table 34.

Table 33: Positivity among the three tests

	Total	Available	Positive	Negative	Error
Smear	15625	12737	1669 (10.7%)	11061 (70.8%)	7 (0%)
TRUENAT	15625	15187	2946 (18.9%)	11249 (72.0%)	992 (6.3%)
XPERT	15625	13221	2423 (15.5%)	10537 (67.4%)	261 (1.7%)

Table 34: Performance of Truenat in comparison with smear and Xpert

	Smear	Xpert
Sensitivity	95.5 %	81.7 %
Specificity	92.7 %	94.6 %
Agreement	93.0 %	92.0 %
Positive Predictive Value	66.7 %	77.6 %
Negative Predictive Value	99.2 %	95.7 %

Conclusion: Operational feasibility of Truenat MTB Rif assay in field settings has been demonstrated. In terms of performance, the assay has shown additional positivity of 8.2% and 3.4% in comparison with smear and Xpert respectively. The assay has shown an agreement of 93% and 92% in comparison with Truenat and Xpert respectively for detection of MTB. A detailed analysis of the data will establish the performance of the molecular test for adaptation in the Program.

B-11: Accelerating access to quality TB diagnosis for pediatric cases in 9 major cities in India

Principal Investigator	:	Dr. K. R. Uma Devi (email: umadevi.r@nirt,res,in)
Collaborators	:	Dr. Neeraj Raizada, FIND, Delhi, Hyderabad, Kolkata
Source of Funding	:	USAID/FIND
Study period	:	April 2014 – 2017

Pediatric TB diagnosis is complicated due to the fact that it mimics other common childhood diseases as well as due to the inability of children to expectorate sputum. Therefore it poses a lot of limitation in the diagnosis of childhood TB. To address this gap, recently in the global guidance document released by WHO, it has been recommended that Xpert MTB/RIF may be used rather than microscopy and culture as the initial diagnostic test in all children presumed to have TB. Xpert MTB/RIF is a cartridge based automated nucleic acid amplification test for TB and RMP resistant-TB case detection. It can be used for detection in pediatric specimen types such as gastric lavage, BAL, induced sputum, lymph node aspirates, etc.

At NIRT, the project was launched on 17th April, 2014, by Dr. Jagdish Prasad, Director General Health Services, Ministry of Health and Family Welfare, Govt. of India at NIRT, Chennai with support from USAID. This project represented concerted efforts of RNTCP, USAID, NIRT and FIND, for a possible solution to the pediatric diagnostic gap under RNTCP.

The aim was to increase the notification of pediatric TB to RNTCP from public and private sector institutions by improving the quality of

TB diagnostic services in children. The target populations in this project were pediatric TB suspects (pulmonary and extra pulmonary) in 4 major cities, namely, Delhi, Hyderabad, Chennai and Kolkata. These labs were to provide accurate evidence based diagnosis in line with internationally accepted standards of TB care. This project offered this diagnostic solution with no cost to patient or provider both in the private and public sector. Any pediatrician both in the public and private sector in these 4 cities were

requested to send specimens for testing to these laboratories. The tests were done on the same day and the results were communicated to the referring provider electronically and at the same time notified to RNTCP under Nikshay.

At NIRT, so far 4333 samples have been processed within 12 months (April 2016 to March 2017). The details are listed in the table given Table 35.

Table 35: Details of samples tested at NIRT

Type of specimen	Total No. of samples	Sensitive	Resistant
Pulmonary	1798	52	6
Extrapulmonary	2535	142	6
Total	4333	194	12

We have screened 7199 suspects, out of which pulmonary samples were 3610; of these 98 (2.7%) were positive for MTB and 5 (0.1%) Rif Resistant. The extra pulmonary samples were 3580, of which 244 (6.8%) were positive for MTB and 9 (0.2%) were Rif Resistant.

B-12: Development of a novel method to improve sensitivity of TB diagnosis by culture using Resuscitation Promoting Factor

Principal Investigator	:	V. N. Azger Dusthackeer (email: azger@nirt.res.in)
Source of funding	:	ICMR (Intramural)
Study period	:	2014-2017

Primary objective: To tap the potential of resuscitation promoting factor (RPF) proteins both from the culture filtrate supernatant of *M. tuberculosis* H37Rv cells and the recombinant protein to enhance the sensitivity/ detection limit in the sputum samples of patients with various stages of active PTB

Objectives:

(i) To increase culture yield for diagnosing TB in smear –ve, culture –ve, PTB sputum samples by treating them with RPF from H37Rv and

(ii) To reduce the time to detection in smear +ve, culture +ve sputum samples by treating the samples with RPF and

(iii) To determine if rRPF protein can improve the detection of cultivable bacteria from sputum in comparison with the RPF from culture filtrate in smear +ve, culture -ve sputa

Methodology: A:

- Stored Petroff processed sputum deposits were used.
- Plated onto Middlebrook 7H10 agar plate after treating with culture filtrate of $H_{37}Rv$ and plain 7H9 liquid media for determining the CFU.
- Growth rate measured by observing the turbidity.

Thirty three specimens yielded colonies altogether, whereas culture filtrate supernatants resulted in viable colonies at much earlier time points than the liquid and solid based conventional methods in 14 specimens among them.

Methodology: B:

- Stored Petroff processed sputum deposit used.
- Plated onto Middlebrook 7H10 agar plate after treating with culture filtrate of $H_{37}Rv$ and plain 7H9 liquid media (control) for determining the CFU.
- Growth rate measured by viable colony count and turbidity measurement

Result and Discussion: Presence of tubercle bacilli under various metabolic stages in the clinical specimens from patients suffering from TB was determined in the presence of the CF in 7H9 medium. The first part of the work dealt with sputum specimens which were smear and culture negative, but the patients were diagnosed of PTB based on other clinical findings. This is the first report demonstrating the effect of CF in comparison with 7H9 over the *M. tuberculosis* present in sputum specimen which was negative by the existing method of culture using solid media. Majority of the specimens which were declared negative by the conventional method resulted in the presence of non-cultivable (NC) (based on OD) and growth of viable bacilli. This

was more prominent in the presence of CF. Still majority of them were in NC forms, but 25% of them grew in the presence of CF in 7H11 agar medium. When we were able to use some of these patients end of treatment specimen which was declared negative by the conventional method, still 21 of them resulted in the presence of non-replicating persisters. Out of these, two grew in the presence of CF which clearly demonstrated its significance in their capability of reactivating these populations which is very significant in clarifying the true nature of the specimen.

The efficiency of CF seen in some of the culture positive specimen did not always result in cultivable cells, which otherwise grew in LJ media, but presence of AFB were documented and increase in their optical density were noted in all these specimens. This undermined the role played by CF, but could be due to the longer storage of the sputum deposit under frozen state before being used.

The muralytic nature of CF enhances the growth rate of the bacilli which was demonstrated in this work and it was significantly more in comparison with 7H9 and hence CF could bring about early growth of *M. tuberculosis* in the specimens. Bavesh Kana had reported this with recombinant protein of RPF in cultures.

Use of a crude CF from H37Rv in reactivating the metabolically inactive *M. tuberculosis* was compared with the cocktail of the recombinant RPF protein in picomole concentration in smear positive culture negative specimen. For unknown reasons rRPF did not reactivate some of the cultures in comparison to CF and clearly CF was more superior in its role of reactivating the cells.

STUDIES IN PROGRESS:

B-13: NIRT Bacteriology Automation System

Co-ordinator	:	Dr. R. Priya
Source of funding	:	ICMR
Duration	:	2015 – 2019

Background: Laboratory investigation is the prime means of diagnosis in modern medical practice. The process includes (a) registering of patient (b) sample collection (c) stages of investigation (d) result entry (e) result validation and (f) final reporting. The Laboratory Information System (LIS) provides a framework for the systematic completion of these activities. Considering the importance of automating the above processes, NIRT engaged a software company (M/s ETPL, Trivandrum), through a public tendering process to specially develop and implement a business process re-engineering of the existing LIS to one using Wi-fi enabled laptop computers and bar codes. The requirement specification for this automation project was drawn up in 2013 and implementation was started in mid 2015. The system was made operational in the last quarter of 2015 and is fully operational now.

The existing workflow using sample-specific barcodes is illustrated in Fig. 6 and is functional in all the sections of the Department by the use of barcode readers and laptops provided in all the sections. Interlinking all the sections is the backbone of the network. A CAT6 cable link interlinking all the systems to a Server class DELL machine was set up in the computer centre. This system runs 24x7 and contains the Data base management system. It also provides reporting facility that may be used by the clinicians and statisticians to get any required report from the system. The administrative functions are designed to be co-ordinated and controlled by the Head of the Department with a laptop. Various MIS (Management Information System) reports are available in HOD's laptop.

Status: Phase I of the NBAS facility did not include crucial sections such as MGIT, LPA and Xpert as they were not part of clinical trials. Inclusion of these tests in clinical studies currently has

identified the gaps and necessitated the need for expanding the system to make it comprehensive. While validation of the Phase I system is being initiated (by STQC, Thiruvanmiyur, Chennai), Phase II automation system will be initiated in near future and tender process is being carried out.

Fig. 6: Existing workflow in Bacteriology Automation Lab

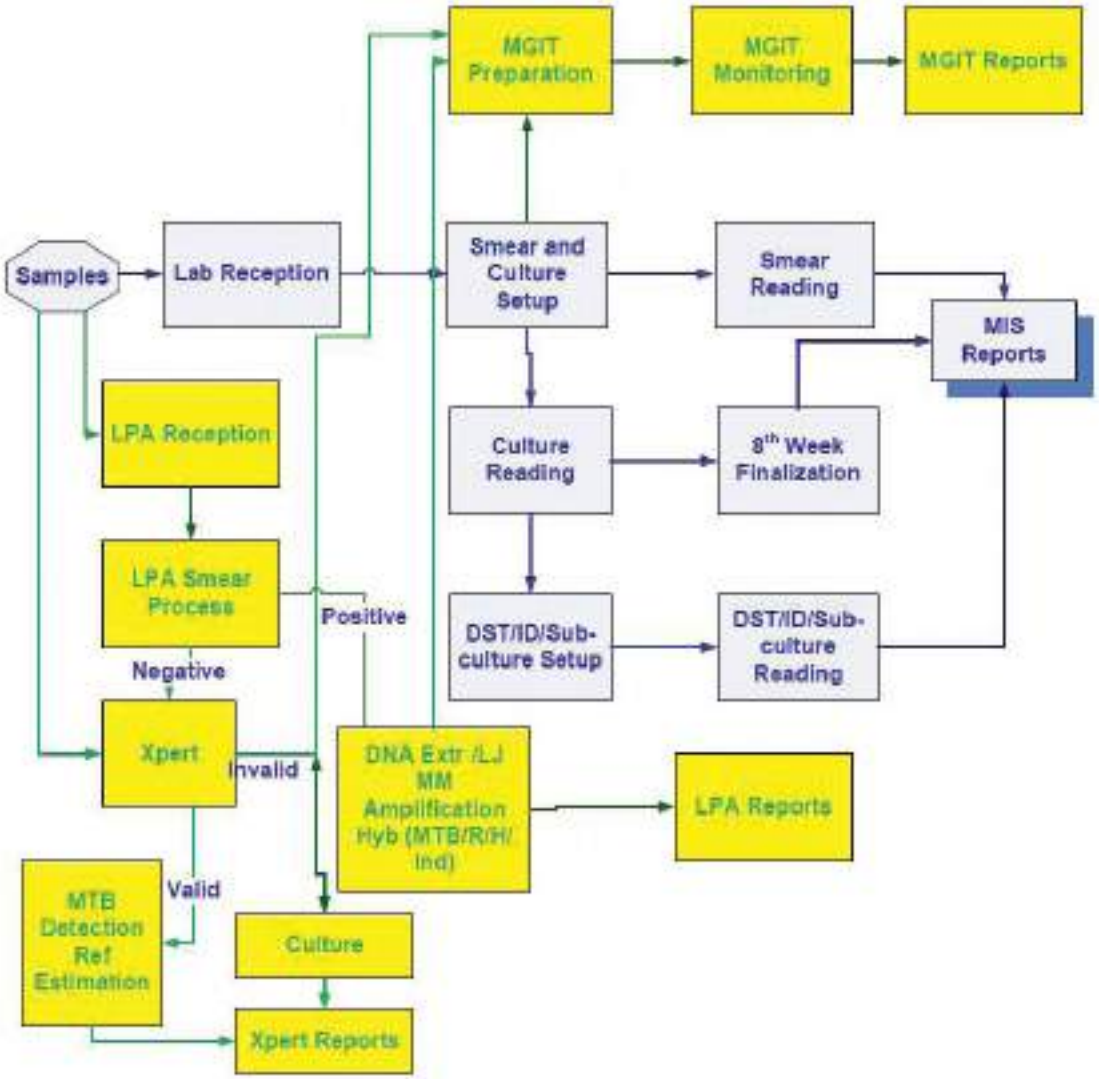


Fig 1 : Existing Workflow in Bacteriology Automation Lab

Workflow in the bacteriology lab in NIRT. The boxes in blue are part of the currently running automation system (Phase I). The boxes in yellow are proposed to be included in Phase II automation system.

Dept. of Biochemistry & Clinical Pharmacology

COMPLETED STUDIES:

BCP-3: Dose related pharmacokinetics of rifabutin during concomitant ritonavir administration in HIV - infected TB patients: a multicentric study

Principal Investigator	:	Dr. Geetha Ramachandran (email: geethar@nirt.res.in)
Source of funding	:	NACO
Study period	:	2016- 2017

Background: TB is the most common opportunistic infection for a significant proportion of HIV-infected patients who are eligible for ART. It is therefore a common scenario that HIV-infected patients eligible for ART also have concomitant TB and require treatment for both infections. Current recommendations are to treat patients with HIV & TB with a regimen including a rifamycin for the full course of ATT. The rifamycin class of compounds is known to be potent inducers of the hepatic CYP450 enzyme system, which is responsible for the metabolism of several drugs. The rifamycins vary in their potential as CYP450 inducers, with RMP being most potent, rifapentine intermediate, and rifabutin (RBT) being much less active. RMP markedly lowers the serum levels of protease-inhibitors (PIs) and NNRTIs by inducing the activity of cytochrome P450 CYP3A. It may result in suboptimal antiretroviral activity and, therefore, subsequent acquired drug resistance. RBT has been shown to be as effective against TB as RMP, and has little, if any, effect on the serum concentrations of PIs, which are metabolized through the CYP3A system. The combination of RBT (if available) with PI-based ART is the preferred form of therapy for patients unable to take NNRTI-based ART. However, ritonavir (RTV), being a *CYP3A4* inhibitor markedly increases serum concentrations and toxicity of RBT (9). The dose of RBT during RTV co-administration remains a matter of debate.

Aim:

(i) To study the pharmacokinetics of RBT at two dosing levels (150 mg daily & 300 mg thrice weekly) during concomitant RTV administration in HIV-infected TB patients

Methods: This was a multi-centric pharmacokinetic study conducted at

four sites in India between April 2016 and July 2017. The sites were:

(i) Government Hospital for Thoracic Medicine, Chennai,

(ii) School of Tropical Medicine, Kolkata,

(iii) BJ Medical College, Pune and

(iv) Government Rajaji Hospital, Madurai.

HIV-infected patients with TB were recruited from these sites if they were adults in the age range of 18 to 60 years, receiving standard ATT with RBT-containing regimens for minimum two weeks and ART containing RTV for minimum two weeks before enrolment. The study was approved by the Institutional Ethics Committees of all the study sites, and written informed consent was obtained from all the patients.

On the day of the study, serial blood samples (2ml) were collected at pre-dosing and at 1, 2, 4, 6, 8, 12 and 24 hours after drug administration in heparinised vacutainer tubes under direct supervision. Plasma was separated and stored at -20^{o}C and transported to NIRT for RBT estimation. TB treatment outcomes (cured/treatment completed, failure, death or default) at end of ATT were noted from the TB register/treatment card. Occurrence of adverse events were also noted from the patients' records.

Plasma RBT was estimated by HPLC according to the method developed and validated at NIRT. Based on the plasma RBT concentrations obtained at different time points, pharmaco-kinetic parameters, such as, peak concentration (C_{max}), trough concentration (C_{min}), and time to attain C_{max} (T_{max}), area under the concentration-time curve (AUC_{0-24}) and half-life ($t_{1/2}$) were calculated based on non-compartmental analysis using Phoenix ™ WinNonlin 6.4 ® software (Certara LP).

Data were analysed using STATA 15.0 (StataCorp, College Station, Texas, USA). Chi-square-Test was used to evaluate the association between variables. Proportion of patients having C_{max} within the therapeutic ranges (0.45 - 0.9µg/ml) were calculated. Multivariate linear regression analysis by stepwise method was carried out to identify factors that influenced C_{max} and AUC_{0-24} of drugs. A $p \leq 0.05$ was considered statistically significant.

Results: A total of 45 patients were recruited to the study, 36 and 9 patients respectively were receiving 300mg thrice weekly and 150mg daily RBT doses. The pharmacokinetics of

RBT at both dosing levels are shown in Table 36. The median C_{max} of RBT were 0.75ug/ml and 0.58ug/ml for 300mg thrice-weekly and 150mg daily doses. Both these values were within the therapeutic range of RBT. The time at which C_{max} (T_{max}) was attained was 4 hours at both doses.

There were 4 of 9 (44%) and 8 of 36 (22%) patients respectively having C_{max} below the therapeutic range (<0.45µg/ml) for 150mg daily and 300mg thrice weekly RBT doses. A higher proportion of patients (15/36; 42%) receiving 300mg thrice weekly dose had C_{max} above the therapeutic range (0.9µg/ml) compared to those who received 150mg daily dose (1/9; 11%). However, these differences were not statistically significant.

Considering a value of 0.06µg/ml as the minimal inhibitory concentration (MIC) of RBT, a higher proportion of patients receiving 300mg thrice weekly dose had C_{min} below the MIC (16/36; 44%) compared those who received 150mg daily dose (2/9; 22%); the associations were not statistically significant.

Multivariate linear regression analysis by stepwise method showed significant influence of CD4 cell counts on C_{max} ($p = 0.049$) and gender on AUC_{0-24} ($p = 0.042$) of RBT.

Of the 45 patients recruited in this study, TB treatment outcomes were available for 34 patients; 27 and 7 respectively in the 300mg thrice weekly and 150mg daily arms of treatment. One patient was still on treatment in the 150mg daily arm. The remaining 10 patients had migrated or were lost during follow-up. Of those patients whose outcomes were known, 22 (81%) and 5 (71%) patients receiving 300mg thrice weekly and 150mg daily respectively had favourable outcome; difference was not statistically significant. No serious adverse events that required stopping RBT was recorded in any of the patients.

Conclusions: This is probably the first and only study from India reporting on the pharmacokinetics of RBT at two dosing levels, namely 300mg thrice weekly and 150mg daily during RTV co-administration in HIV-infected patients with TB. From the pharmacokinetic standpoint, both doses yielded similar RBT plasma concentrations, were well tolerated and outcomes were similar. Thus, RBT can be administered at a dose of 300mg thrice weekly when ATT is intermittent and at 150mg daily dose

when ATT is daily during RTV co-administration in HIV-infected patients with TB. Although the study was limited by a small sample size, we included patients from southern, western and eastern India and is representative of the entire country.

There is a need to undertake pharmacokinetic studies of RBT in children. It is important to identify a safe and effective dose of RBT for young children in need of concomitant treatment with PIs.

Table 36: Pharmacokinetics of rifabutin at two doses

Variables	150mg (n = 9)	300mg (n = 36)
C_{min} (µg/ml)	0.14 (0.07 - 0.18)	0.08 (0 - 0.13)
C_{max} (µg/ml)	0.58 (0.26 - 0.81)	0.75 (0.52 - 1.23)
T_{max} (h)	4 (2 - 4)	4 (2 - 6)
AUC_{0-24} (µg/ml.h)	6.32 (3.91 - 10.33)	9.27 (7.00 - 12.34)
$T_{1/2}$ (h)	20.11 (10.23 - 23.21)	13.54 (11.24 - 16.24)

C_{min} - Trough Concentration; C_{max} - Peak Concentration; T_{max} - Time at which peak concentration is attained; AUC - Exposure; $T_{1/2}$ - half-life

BCP-6: Pharmacokinetics of second-line anti-TB drugs in children with MDR-TB

Study investigators (NIRT, Chennai) :		Dr Geetha Ramachandran; Dr AK Hemanth Kumar (email: geethar@nirt.res.in; hemanthkumarak@nirt.res.in)
Study investigators (SN Medical College, Agra)	:	Dr Rakesh Bhatia; Dr. Rajeshwar Dayal; Dr. Dipti Agarwal
Souce of funding	:	Intramural
Study period	:	2017 - 18

Background: DR-TB is a continuing threat and is also an issue in children. A survey by the RNTCP in India found that 9% of children with TB were already resistant to rifampicin, which is an important first-line anti-TB drug, before they started treatment. DR-TB has microbial, clinical, and programmatic implications.

According to RNTCP guidelines, children with MDR-TB are treated for 24 months with a daily regimen consisting of 6 months of kanamycin, levofloxacin (LFX), ethionamide (ETH), cycloserine (CS), pyrazinamide (PZA) and ethambutol (EMB) for 6 months followed by LFX, ETH, CS and EMB for the remaining 18 months (3).

There is paucity of pharmacokinetic data of anti-TB drugs in children with MDR-TB. A review of literature showed that most studies were from South Africa (12 - 14). Not much is known about the pharmacokinetics of second-line drugs in children with MDR-TB in India.

Aim:

(i) To study the pharmacokinetics of LFX, PZA, ETH and CS in children with MDR TB being treated according to RNTCP guidelines

Methods: A prospective, observational study was undertaken in children aged 5 to 18 years who were attending the paediatrics department/TB chest department at the Sarojini Naidu Medical College, Agra, North India between April to August 2017. All the children were bacteriologically confirmed to have MDR-TB based on drug susceptibility tests. Diagnosis and treatment were in accordance with the RNTCP guidelines. All the children received drugs from the RNTCP, as tablets which were available as LFX 250mg & 500mg, PZA 500mg & 750mg, ETH 250mg and CS 250mg. The drug doses were according to the weight bands as shown in Table 37.

Consecutive children who were HIV sero-negative, willing for hospitali-sation and blood draws were included

in the study. Children who were too sick or moribund were not included. The parent/guardian of the child gave informed written consent and children aged seven and above gave assent. The study commenced after obtaining Institutional Ethics Committee approval.

On the study day, serial blood samples at pre-dosing and at 2, 4, 6 and 8 hours after directly observed drug administration were collected in heparinised vacutainer tubes. The blood samples were centrifuged immediately, plasma separated and stored at -20^{o}C. Plasma samples were transported to the NIRT, Chennai, south India in dry ice for drug estimations.

Plasma concentrations of LFX, ETH, CS and PZA were estimated by HPLC at NIRT according to validated methods.

Pharmacokinetic variables such as peak concentration (C_{max}), time at which C_{max} was attained (T_{max}), area under the concentration-time curve (AUC_{0-8}), half-life ($t_{1/2}$), and Clearance (CL) were calculated based on non-compartmental analysis using Phoenix ™ WinNonlin 6.4 ® software (Certara LP). Area under the concentration-time curve (AUC_{0-8}) was calculated using the linear trapezoidal rule.

The nutritional assessment was carried out by calculating z scores for weight and height using the EPI-NUT component of the EPI-INFO 2002 software package.

Data were analysed using STATA 15.0 (StataCorp, College Station, Texas, USA).

Results: A total of 25 children were included in the study. A high proportion of children (92%) had BMI below 18.5kg/sq.m. Females constituted 64% of the study population. There were 5 and 20 children aged below and above 12 years respectively. There were 3 children with extrapulmonary TB.

The median (range) values of the pharmacokinetic variables of drugs and proportion of children having C_{max} within/above/below the therapeutic range of drugs are shown in Table 38. Peak concentrations were attained at 4 hours for all the drugs. The C_{max} of PZA was significantly lower in underweight children compared to those with normal weight (8.7µg/ml vs 31.2µg/ml; p = 0.029), females had significantly higher C_{max} of LFX compared to males (11.5µg/ml vs 7.3µg/ml; p = 0.017) and children below 12 years of age had significantly higher AUC_{0-8} of ETH (17.5µg/ml.h vs 9.4µg/ml.h; p = 0.030). None of the

other differences were statistically significant.

Multiple linear regression analysis by stepwise method was carried out to identify factors (age, gender, WAZ, HAZ and mg/kg drug dose) that significantly influenced C_{max} and AUC_{0-8} of LFX, PZA, ETH and CS. The factors that emerged significant were gender on C_{max} of ETH and age on C_{max} and AUC_{0-8} of CS. Male gender was likely to decrease C_{max} of ETH by 4.92µg/ml and increase in age of one year was likely to reduce C_{max} of CS by 1.12µg/ml and AUC_{0-8} by 6.37µg/ml.h.

Conclusions: Wide variations in drug concentrations were observed, probably because tablets had to be broken and administered to children to achieve the required dose in a particular weight band. The peak concentration of PZA was lower in underweight children than those with normal weight. Females had higher peak concentration of LFX than males. The pharmacokinetic data from India appeared to be different from that reported in South African children, probably due to differences in drug doses.

Although a systematic assessment of occurrence of adverse events was not undertaken, no serious adverse events that required hospitalisation were observed. All children were continuing treatment.

This study conducted in a small number of children has generated preliminary pharmacokinetic data from India. Additional studies on the safety and pharmacokinetics of second-line anti-TB drugs in children with MDR-TB and their impact on treatment outcomes and occurrence of adverse events should be considered.

Table 37: Weight band recommendations followed in the RNTCP

S.No.	Drugs	16-25 kg	26-45 kg	46-70 kg
1	Kanamycin	500 mg	500 mg	750 mg
2	Levofloxacin	250 mg	750 mg	1000 mg
3	Ethionamide	375 mg	500 mg	750 mg
4	Ethambutol	400 mg	800 mg	1200 mg
5	Pyrazinamide	500 mg	1250 mg	1500 mg
6	Cycloserine	250 mg	500mg	750mg
7	Pyridoxine	50 mg	100mg	100mg

Table 38: Pharmacokinetics of drugs & therapeutic ranges

Variables	Levofloxacin (n=24)	Pyrazinamide (n=22)	Cycloserine (n=25)	Ethionamide (n=25)
C_{max} (µg/ml)	8.9 (2.9 - 29.0)	21.4 (2.2 - 99.4)	31.8 (10.6 - 63.0)	2.4 (1.1 - 5.0)
T_{max} (hours)	4 (0 - 6)	4 (2 - 6)	4 (2 - 6)	4 (2 - 6)
$AUC_{(0-8)}$ (µg/ml.h)	43.1 (14.4 - 172.0)	80.2 (16.0 - 501.4)	192.1 (68.7 - 440.8)	11.6 (5.2 - 24.0)
CL (ml/min)	0.15 (0.02 - 0.35)	0.59 (0.04 - 2.56)	0.65 (0.18 - 3.02)	0.03 (0.01 - 0.10)
Half life (hours)	7.9 (0.9 - 24.0)	17.2 (1.3 - 235.2)	10.5 (2.1 - 36.2)	3.37 (1.0 - 24.8)
Within therapeutic range*	9 (37%)	7 (32%)	11 (44%)	18 (72%)
Below therapeutic range*	10 (42%)	10 (45%)	4 (16%)	6 (24%)
Above therapeutic range*	5 (21%)	5 (23%)	10 (40%)	1 (4%)
Therapeutic range (µg/ml)	8 - 13	20 - 60	20 - 35	2 - 5

Values were presented as Median (Range); *n(%);

Cmax - peak concentration; Tmax - time at which peak concentration is attained; AUC - exposure; CL – Clearance

BCP- 7: Factors influencing TB treatment outcome in PTB patients on thrice weekly regimens in India: a prospective cohort study

Principal Investigator	:	Dr. Geetha Ramachandran (email: geethar@nirt.res.in)
Source of funding	:	DBT
Study period	:	2016- 2018

Background: In the RNTCP in India, patients were treated for TB with thrice-weekly regimens. Favourable treatment outcomes were reported to be achieved in about 85% of sputum smear positive patients receiving standard short-course chemotherapy with INH, RMP, PZA and EMB. Administration of medications through directly observed therapy (DOT) ensured patient adherence and is currently recommended in the RNTCP in India. Despite these established standards, there is paucity of information on the possible mechanisms to explain treatment failures, relapses, and acquired drug resistance in programmatic settings. Understanding anti-TB drug concentrations is critical to inform on drug dosing.

Aim:

(i) To study plasma concentrations of RMP, INH and PZA and their relationship to treatment outcomes in adult PTB patients

Methods: A cohort of newly diagnosed pulmonary TB patients (>14 years) (n = 394), receiving thrice-weekly ATT in the RNTCP were recruited at Chennai and Pune. At months 1 and 5, 2-hour post-dose concentrations of RMP, INH and PZA were determined by high performance liquid chromatography, after directly observed drug administration. The effect of drug concentrations on unfavourable TB treatment outcomes (death, failure, recurrence) was assessed using random effects logistic regression.

Results: Sub-therapeutic RMP, INH and PZA were observed in 86% & 74%, 29% & 22% and 12% of patients at months 1 and 5 respectively. Sub-therapeutic cut-offs of drugs were taken as RMP < 8µg/ml; INH < 3µg/ml; PZA < 20µg/ml. Females had higher INH and PZA than males (p = 0.013 and 0.008 for INH and PZA respectively), and those with diabetes mellitus had lower PZA levels than those without diabetes (p = 0.012). RMP levels were significantly lower in HIV co-infected than HIV uninfected (1.6 vs 4.6µg/ml; p = 0.015).

Favourable outcome was observed in 85% patients, RMP being significantly lower in unfavourable than favourable responders (2.5 vs 4.7µg/ml; p = 0.037). RMP concentration was associated with unfavourable treatment outcome (aOR, 0.89; 95% CI 0.80 - 0.98) (Table 39).

Conclusions: This study undertaken in adult TB patients treated in the RNTCP in Chennai and Pune under programmatic settings showed sub-therapeutic RMP in a high proportion of patients on intermittent ATT. Sub-therapeutic RMP was identified as a risk factor for unfavourable outcome. Currently used dosages of anti-TB drugs are not based on careful pharmacokinetic studies and a hot topic in TB research is optimization of treatment with available drugs. A study on drug levels in patients on daily ATT and treatment outcome is in progress.

Table 39: Factors associated with outcome

Factors		OR (95% CI)	P value	aOR (95% CI)	P value
Age					
	< 35	1.00		1.00	
	≥ 35	0.76 (0.51 - 1.12)	0.163	0.78 (0.36 - 1.69)	0.527
Smoker					
	No	1.00		1.00	
	Yes	2.00 (1.34 - 2.97)	0.001	1.82 (0.84 - 3.95)	0.128
Alcoholic					
	No	1.00		1.00	
	Yes	1.75 (1.18 - 2.59)	0.005	1.25 (0.56 - 2.79)	0.582
Diabetes					
	No	1.00		1.00	
	Yes	0.68 (0.44 - 1.04)	0.075	0.80 (0.39 - 1.66)	0.548
Body mass index					
	< 18.5	1.83 (1.18 - 2.83)	0.006	0.99 (0.47 - 2.07)	0.972
	≥ 18.5	1.00		1.00	
Baseline Smear					
	Negative	1.00		1.00	
	Positive	2.45 (1.48 - 4.07)	0.001	1.92 (0.70 - 5.30)	0.206
Baseline INH resistance					
	Sensitive	1.00		1.00	
	Resistant	1.53 (0.72 - 3.25)	0.269	1.53 (0.58 - 3.99)	0.389
Drug Concentrations					
	RMP	0.96 (0.90 - 1.02)	0.154	0.89 (0.80 - 0.98)	0.019
	INH	0.94 (0.89 - 0.99)	0.020	0.96 (0.89 - 1.04)	0.362
	PZA	1.00 (0.99 - 1.02)	0.653	-	

Random effects logistic regression was used.
Gender, HIV were excluded in the multiple logistic regression due to collinearity.
PZA was excluded due to less number of values.

BCP-5: Effects of co-administration of Unani Pharmacopoeial formulation (UPF; *Qurs Tabasheer Sarthani* (QTS) & *Arq Hara Bhara* (AHB) with Anti TB (CAT-I) drugs in adult Wistar albino rats

Study Investigators:

(i) NIRT, Chennai:

Principal Investigators : Dr..A.K. Hemanth Kumar (email:hemanthkumarak@nirt.res.in)

(ii) Regional Research Institute of Unani Medicine, Chennai

Principal Investigators : Dr..N. Zaheer Ahmed
Co-Investigators : Dr.M. Abdul Kareem; Dr. Athar Parvez Ansari

Source of funding : Central Council for Research in Unani Medicine (CCRUM), New Delhi
Study period : 2016 - 18

Background: *Qurs Tabasheer Sarthani* (QTS) & *Arq Hara Bhara* (AHB) are commonly prescribed drugs for the treatment of TB-like conditions in humans. The ingredients of these Unani pharmacopeial formulations are shown to have hepato-protective and anti-mycobacterial properties. Intervention with these classical formulations in combination with anti-TB drugs (CAT-1) may help in improving the quality of life and reducing hepato-toxic side effects caused by ATT.

Aims:

(i) To evaluate the effect of co-administration of Unani Pharmacopoeial Formulation (UPF; *Qurs Tabasheer Sarthani* (QTS) and *Arq Hara Bhara* (AHB) with anti-TB (CAT-I) drugs in Wistar albino rats

Methods: The animal experiments were carried out at the Regional Research Institute of Unani Medicine, Srinagar, J&K. The experimental design is given in Table 40. Before the initiation of the study, body weight of the animals were recorded. The baseline biochemical data were determined in the blood collected from the ocular plexus or tail vein. On 15th, 61st and 181st day, blood samples were collected from the animals for haematological and biochemical investigations. After the blood collection, the animals were sacrificed; a portion of liver and kidney tissues were subjected to histopathological examinations. The

extent of liver toxicity was examined by analyzing the activity of liver enzymes and interpreting the cell morphology. **Conclusions:** There were no significant differences between groups in liver toxicity, haematological and biochemical parameters. Further studies in human subjects are being planned.

Table 40: Experimental design

Groups		Sub-groups
Group-I	Control	Group-I a (8 animals; 14 days) Group-I b (8 animals; 60 days) Group-I c (10 animals; 180 days)
Group-II	Unani Pharmacopoieal Formulation (UPF)	Group-II a (8 animals; 14 days) Group-II b (8 animals; 60 days) Group-II c (10 animals; 180 days)
Group-III	Category I Regimen (CAT-I)	Group-III a (8 animals; 14 days) Group-III b (8 animals; 60 days) Group-III c (10 animals; 180 days)
Group-IV	CAT-I + UPF	Group-IV a (8 animals; 14 days) Group-IV b (8 animals; 60 days) Group-IV c (10 animals; 180 days)

STUDIES IN PROGRESS:

BCP – 4: Pharmacokinetics of second-line anti-TB drugs in MDR-TB patients

Principal Investigator	:	Dr AK Hemanth Kumar (email :hemanthkumarak@nirt.res.in)
Source of funding	:	Intramural
Study period	:	2016-2019

Introduction: MDR-TB has become a significant public health problem in a number of countries and an obstacle to effective TB control. Under the RNTCP in India, MDR-TB patients are treated with six drugs including an aminoglycoside and a fluoroquinolone for a period of 24 months under DOTS plus. The intensive phase of treatment is for 6 months with kanamycin, levofloxacin, ethionamide, cycloserine, PZA and EMB daily and the continuation phase of treatment is for 18 months with levofloxacin, ethionamide, cycloserine and EMB daily. Limited information is available on the pharmacokinetics of the drugs used to treat MDR-TB patients and factors that are likely to impact drug levels.

Aims:

(i) To study the pharmacokinetics of anti-TB drugs in MDR-TB patients who are receiving treatment with the DOTS plus regimen in the RNTCP in India and to relate drug levels with time to culture conversion

Methods: This is a prospective, cohort study conducted in adult MDR-TB patients treated at the Government Hospital of Thoracic Medicine, Tambaram, Chennai. Patients who are found suitable for the study are explained about the study procedures and informed written consent obtained before commencement of the study. On the study day, serial blood samples are collected at pre-dosing and at 1, 2, 4, 6, 8 and 12 hours after supervised drug administration. Plasma drug concentrations are estimated according to validated methods by HPLC. Based on the plasma concentration of drugs obtained at different time points, defined pharmacokinetic variables will be calculated.

Bacteriological tests: Sputum samples are collected from all the patients at baseline and every month up to six months of start of treatment. Baseline

samples will be subjected to culture by solid media and DST by MGIT. Subsequent samples will be subjected to culture only.

Sample size: The sample size has been calculated as 471 patients who will be recruited over a period of three years.

As on 18th May, 2018, 246 patients have been recruited. The study is in progress.

BCP-8: Pharmacokinetic drug-drug interactions between first line anti-TB and anti-diabetic drugs

Principal Investigator	:	Dr.A.K. Hemanth Kumar (email: hemanthkumarak@nirt.res.in)
Research Scholar	:	Ms. Rebecca Mary
Source of funding	:	Intramural
Study period	:	2017 - 2020

Background: Diabetes mellitus (DM) is a risk factor for TB with prevalence rates among TB patients ranging from 10 to 30%. Cohort studies and a recent meta-analysis provide further convincing evidence that TB is more common in patients with diabetes. Type 2 diabetes mellitus seems to be associated with a less favourable response to TB treatment. A study from Chennai observed delayed sputum conversion and high failure rates in new smear positive PTB patients with DM. A systematic review of multiple studies conducted globally highlighted that the coexistence of DM and TB was associated with increased rates of TB treatment failures and deaths. A retrospective cohort study showed that type 2 diabetes mellitus was a risk factor for death in TB patients.

Most forms of TB are treated with 6-month Category I treatment, which consists of RMP, INH, PZA and EMB thrice-weekly for two months, followed by RMP and INH thrice-weekly for four months. Treatment of DM is possible with oral hypoglycemic agents that help

to reduce blood sugar levels. Current therapeutic agents available for DM include biguanides, sulfonylureas and related compounds, thiazolidinediones, α-glucosidase inhibitors and insulin.

Patients with TB and DM will be treated with both classes of drugs; there are likely to be drug-drug interactions between the drugs used to treat TB and DM. Limited information is available on the pharmacokinetic drug – drug interactions between first-line anti-TB and anti-diabetic drugs.

Aims:

(I) To study the effect of anti-diabetic drugs (biguanides and sulphonlyurea) on the pharmacokinetics of first line anti-TB drugs;

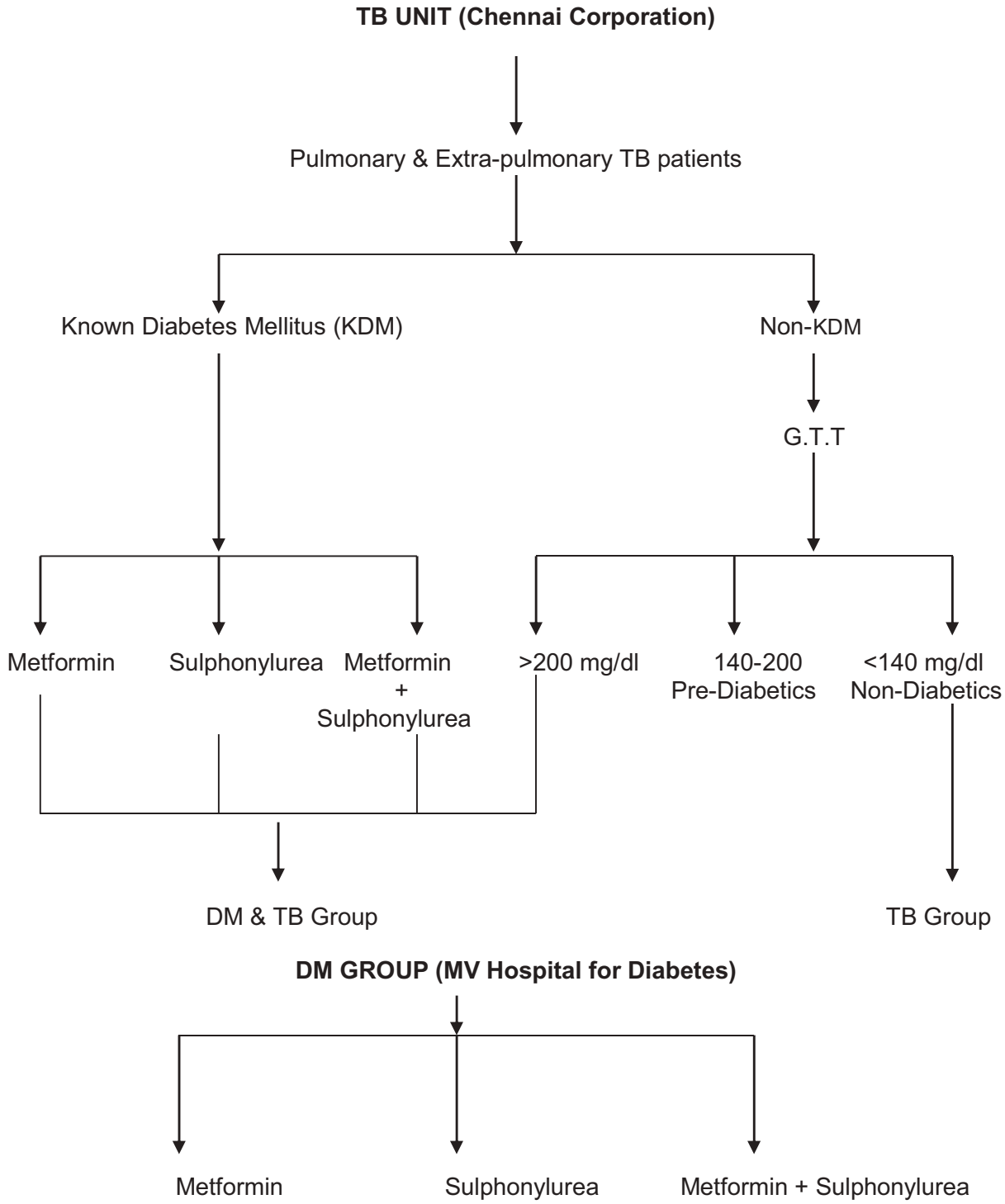
(ii) To study the impact of first line anti-TB drugs on the pharmacokinetics of these anti-diabetic drugs.

Methods: This prospective, obser-vational pharmacokinetic study is being conducted in three groups of patients, namely, TB, DM &TB and DM. Patients fulfilling the study criteria are being recruited from the RNTCP treatment centres. Those TB patients who are known diabetic (KDM) and are receiving anti-diabetic treatment (biguanides, namely Metformin or sulphonlyurea or both) along with first-line anti-TB drugs will be treated as DM & TB patients. Those TB patients whose diabetic status is not known will be tested for Glucose Tolerance (GTT). Fasting and 2 hour post glucose (75 gms) samples will be tested for glucose. Blood glucose <140 mg/dl will be treated as Non-diabetic TB and those TB patients with blood sugar after oral glucose >200 mg/dl will be treated as DM & TB patients. The study design is illustrated in the flow chart (Fig. 7).

The study will be conducted while the TB patients are in the intensive phase of ATT. On the day of the study, serial blood samples will be collected at pre-dosing and at 1, 2, 4, 6 and 8 hours after drug administration. All drug estimations (RMP, INH, PZA, Metformin and Sulphonyl urea) will be undertaken at NIRT by HPLC according to validated methods.

A pilot study has been initiated recently. The required sample size is 12 in the TB group and 36 each in DM and DM &TB groups respectively. The study is in progress.

Fig. 7: Schematic representation of patient requirement



BCP-9: A study on the effectiveness of food supplementation on treatment outcomes and nutritional status of adults with PTB on a retreatment regimen

Co-Investigator	:	Dr N Saravanan (email: Saravanan.n@nirt.res.in)
Source of funding	:	Johnson & Johnson Private limited
Study peiod	:	

Background: Several reports suggest that undernutrition is an important risk factor for TB. Once TB infection is established, undernutrition becomes the cause of the morbidity. Undernutrition is shown to be associated with mortality and poor treatment outcomes in active TB patients, while it is also a risk factor for the contacts to develop active TB.

Aim:

(i) To determine vitamin D concentrations during ATT in adult patients with PTB on a re-treatment regimen

Methods: This study is conducted in patients attending RNTCP centers in Vellore district, Tamil Nadu. Various biochemical indices including Vitamin D concentrations are being estimated at different time points during TB treatment. Vitamin D concentrations are estimated by ELISA. The study is in progress.

BCP-10: Estimation of the efficacy of novel RMP analogues

Principal Investigator	:	Dr. Geetha Ramachandran
ICMR - Post-doctoral fellow	:	Dr. P. Saravanan
Source of funding	:	ICMR - PDF fellowship
Study period	:	2016 - 2018

Background: Development of drug resistance against the conventional antibiotics is a matter of great concern that requires immediate attention**.** Since MDR- and XDR-TB have become serious problems worldwide, there is an urgent need to discover new anti-TB compounds. In the present study we have employed synthetic chemistry applications to explore a potentially efficient drug against TB and devise alternative strategies to improve the therapeutic application of the same.

Aim:

(i) To synthesise novel RMP analogues and determine its activity among isolates of *M. tuberculosis* strains

Methods: Novel analogues of RMP were synthesised by covalent attachment of different bio-active molecules into The RMP core. The synthesised products were characterised by 1D and 2D NMR, FT-IR and MALDI-TOF MS methods. All the synthesized novel RMP analogues are being screened for anti-TB activity by Luciferase reporter phage (LRP) assay. The promising molecules are further tested and confirmed by enumerating colony-forming unit (CFU) by broth dilution method. Wild type *M. tuberculosis* strains are used for this study. Further experiments are in progress.

DEPT. OF HIV

COMPLETED STUDIES:

HIVL-9: Analysis of the patterns of HIV DRMs in first line ART failures enrolled under NACO's ART Program in a representative population from south India

Principal Investigator : Dr. Luke Elizabeth Hanna
(email: hanna@nirt.res.in)
Source of funding : Intramural
Study Period : 2016-2017

Background: In 2010, WHO issued its revised guidelines for antiretroviral therapy that recommended countries to phase out stavudine (d4T) because of its association with metabolic toxicity and complications. India also phased out stavudine (d4T) gradually and substituted d4T with tenofovir (TDF) in first-line therapy. The first-line antiretroviral therapy regimen in India comprises of two nucleoside/nucleotide analogue reverse transcriptase inhibitors (NRTI) and a non-nucleoside reverse transcriptase inhibitor (NNRTI). In late 2014, the programme adopted a TDF, lamivudine, and efavirenz-based fixed drug combination as the preferred regimen to initiate antiretroviral therapy. Alongside these revisions and implementation of the revised recommendations, comparative surveillance studies for HIV drug resistance are critical to monitor the outcomes of the revised program performance. In this study, we determined and compared the HIVDR outcomes with respect to drug resistance acquired during the pre and post d4T era.

Objective:

(i) To evaluate acquired drug resistance after implementation of the revised treatment guidelines and to compare it with that seen during the pre-d4T era and

(ii) To examine the impact of the drug resistance mutations (DRM) identified on recycling options for subsequent second line regimen

Methods: The study included 95 HIV-1 infected individuals on first line ART and experiencing virological failure. This included 43 individuals who received a stavudine (d4T)-based first line regimen (Group A) and 52 individuals who were initiated on a non-stavudine (TDF)-

based regimen (Group B) in combination with a cytidine analogue lamivudine (3TC) and an NNRTI (Nevirapine [or Efavirenz). The HIV-1 pol gene was sequenced using an in-house protocol and mutations were analysed using the HIV-1 Stanford algorithm.

Results: Out of the 95 individuals, 78.4% (n=76) were males with median HIV-1 viral load of 118,000 copies/mL (inter quartile range/IQR - 57800-364177 copies/mL). Overall, DRMs to anti-retroviral drugs were seen in more than 90% of virological failures in both the groups. The prevalence of major NRTI and NNRTI DRMs seen in Group A were 88.37% and 93%, and in Group B were 86.53% and 98.07% respectively. Proportion of sequences with Thymidine analog mutations (TAMs) were 51.1% and 23.1% in Groups A and B respectively.

Conclusion: Although the frequency of major DRMs was similar in both the groups, the prevalence of TAMs was significantly lower in those receiving a TDF-based regimen as compared to those on a stavudine-based regimen, suggesting a better scope for recycling of NRTIs in those on a AZT/TDF-based first-line regimen.

HIVL-10: Molecular characterization of the envelope of vertically transmitted HIV-1 strains from infants with HIV infection

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Research Scholar	:	Mr. Ashok kumar
Collaborator	:	Dr. Ujjwal Neogi, Karolinska Institute, Sweden
Source of funding	:	Intramural
Study period	:	2015-2017

Background: Adequate information on the precise molecular and biological composition of the viral strains that establish HIV infection in the human host will open up ways for designing effective means of immunization against HIV infection.

Objectives:

(i) To identify and characterize the unique biological features of transmitted/founder HIV-1 strains that endow them with the unique capability of establishing infection in recently infected children

Methods: A total of 250 patient-derived gp120 envelope glycoproteins were cloned into pMN-K7-Luc-IRESs-NefΔgp120 to obtain chimeric viruses from eight infants who had recently become infected with HIV through mother-to-child transmission and two adults who acquired infection through the heterosexual route and were in the chronic stage of disease. Of the 250 clones tested, 65 chimeric viruses were found to be infectious. The chimeric viruses were analysed for biological properties like co receptor usage, per particle infectivity, drug sensitivity, susceptibility to broadly neutralizing antibodies, as well as molecular features of the envelope gene.

Results: Based on the genotypic and phenotypic characteristics of the viral clones, we identified 10 transmitted founder (TF) viruses from the eight infants. The TF viruses were characterized by shorter V1/V2 regions, a reduced number of potential N-linked glycosylation sites, and a higher infectivity titre as compared to the virus variants obtained from adults during chronic infection. An interesting observation was CXCR6 co-receptor usage, in addition to CCR5 use, by 13 of the 65 chimeric viruses analyzed. The sensitivity of the TF variants to maraviroc and a standard panel of neutralizing monoclonal antibodies (VRC01, PG09, PG16, and PGT121)

was found to be much lower than that of the virus variants from adults in the chronic stage of infection.

Conclusion: Some of the unique features of TF viruses identified in this study had shorter V1V2 regions, reduced number of potential N-linked glycosylation sites and higher infectivity titer as compared to chronic virus variants. The sensitivity of the TF variants to Maraviroc and a standard panel of neutralizing monoclonal antibodies were found to be much lower than that of chronic viruses.

HIVL-11: Characterization of neutralization specificity of a unique HIV-2 sample exhibiting potent intra and inter type cross neutralization potential

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Research scholar	:	Ms. Vidya Vijayan
Source of funding	:	Intramural
Study Period	:	2017-2018

Background: HIV-2 infection is associated with better prognosis, slower disease progression and transmission, longer latency period and a reduced mortality rate as compared to HIV-1 infection. The pathogenic difference between the two viruses has been an intriguing topic of research. Understanding HIV-2 as a model of a naturally less pathogenic infection could bring to light important insights into the best fitted immune responses that ultimately lead to better control of HIV infection.

In a previous study, we identified a unique HIV-2 infected individual with broad and potent cross-neutralization potential against both HIV-1 and HIV-2 strains. Preliminary findings suggested the presence of glycan-targeting antibodies in this sample.

Objective:

(i) To characterize the neutralization specificity of the sample from the unique HIV-2 infected individual.

Methods: To identify the glycan binding potential of the plasma sample, standard neutralization assay was performed with the wild type HIV-1 DU156 pseudovirus and its single mutant. For further confirmation of the glycan-dependent nature of the neutralization response, the sample was also tested in a neutralization assay with HIV-1 JRFL wild type virus and its single and double mutant forms widely used for such studies. Briefly, TZM-bl cells were infected with the pesudoviruses in the presence or absence of plasma. After 48 hours, 100 µl of BriteLite substrate was added to the wells. 100µl of supernatant was transferred to a solid opaque plate and luminescence was measured as relative light units (RLU) using the Vector 3 luminometer. Healthy human plasma was used as the negative control and each experiment was repeated on two independent occasions.

Results: Neutralization assays with wild type tier-2 HIV-1 subtype C virus DU156, and its mutant DU156N160K is widely employed for the identification of V1/V2 glycan specific NAbs (PG9/16) among HIV infected individuals. This class of antibodies requires the presence of an N-linked glycan at position 160 in the V1/V2 region for neutralization of HIV. A significant decrease in neutralization sensitivity to the order of 3- fold or more in ID_{50} value for the mutant DU156 is generally considered to be indicative of the presence of PG9/PG16–like antibodies in the sample.

This sample exhibited strong neutralization activity against DU156WT with a 1.6-fold decrease in neutralization sensitivity of the mutant DU156N160K pseudovirus, indicating the possible presence of PG9/PG16-like antibodies. Previous studies have shown that the presence of glutamic acid (E) at position 168 in the HIV-1 subtype B JR-FL WT strain makes it resistant to neutralization by PG9/PG16 -like bNAbs. In the single mutant, JR-FL E168K, E is replaced with lysine (K) making it sensitive to neutralization by this class of bNAbs. We found that the BCN sample neutralized the single mutant pseudovirus better than the JR-FL WT virus with a significant reduction in neutralization of the double mutants JR-FL E168K N156K0 and JR-FL E168K N160K, as compared to the single mutant.

The plasma was also tested for the presence of V3 glycan dependent class of antibodies, belonging to the PGT series using a neutralization assay with HIV-1 DU156 and DU156N332A. A 1.5 fold decrease in neutralizing activity was observed with the single mutant as compared to the wild type virus. This was further confirmed by repeating the neutralization assay with HIV-1 JR-FL WT and JR-FL N332A mutant.

Conclusion: Our study identified a unique HIV-2 sample with cross-type neutralizing property. Preliminary studies indicate the presence of glycan-dependent antibodies in this sample.

HIVL-14: Study on HIV-1 Drug resistance patterns in ART experienced children with virological failure

Principal Investigator : Dr. Padmapriyadarsini
(email: padmapriyadarsinic@nirt.res.in)

Source of funding : Intramural
Study Period : 2016-18

Background: There is very limited data on HIV drug resistance (HIVDR) in treatment experienced paediatric populations in India. This study aimed to assess the prevalence and patterns of acquired drug resistance mutations among this population.

Objective:

(i) To determine the frequency of occurrence and type of HIV drug resistance mutations in children experiencing virological failure at the end of 12 months of antiretroviral therapy

Methods: A retrospective study conducted in HIV-1 positive paediatric individuals receiving antiretroviral therapy was undertaken. HIV-1 pol gene sequences covering the full protease region and partial RT gene were successfully amplified and sequenced from base line and follow-up in 46 children with virological failure at the end of 12 months of antiretroviral therapy.

Findings: Three hundred and seventy-eight antiretroviral therapy -naïve children living with HIV were enrolled into the study. Of these, 74% were started on nevirapine-based and 24% on efavirenz-based antiretroviral therapy. Virological failure was seen in 29% (94/378) of children. At the time of virological failure, multiple NNRTI-associated mutations were observed in 80% of the children; K103N and Y181C were the major NNRTI DRMs. M184V was seen in 79% of children. Baseline drug resistance among the virological failures was only 6%.

Conclusion: Unlike other studies, our cohort had a low rate (6%) of baseline NNRTI mutations. Among those who developed virological failure, M184V, K103N and Y181C were the most common DRMs observed as in other pediatric HIV studies.

HIVL-15: HIV drug resistance genotyping work between National JALMA Institute for Leprosy and other Mycobacterial diseases and NIRT

Principal Investigators	:	Dr. S. P. Tripathy (email: srikanthtripathy@gmail.com) Mr. Sushant Barick (JALMA), Mr. K. Ramesh (NIRT)
Source of funding	:	Intramural
Study Period	:	2016-17

Background: The study determined the prevalence and pattern of HIV drug resistance in Agra, Northern India, since there is very limited data from this part of India on HIV drug resistance.

Methods: The study population comprised of 3 groups of HIV positive individuals: those on ART for 1-2 years, those on ART for 3-4 and those on ART for 5-6 years. The study subjects were recruited from the ART Center at the Sarojini Naidu Medical College, Agra. Blood samples were obtained in the form of dried blood spots during the period December 2009 to November 2016. The HIV-1 polymerase gene (complete protease and part of the

reverse transcriptase gene) were amplified by nested PCR and sequenced using Sanger sequencing method.

Results: The median duration of ART in the three groups were 23.95 months, 42.65 months and 67.74 months respectively. There was no NRTI mutation detected in 35% (7/20), 15% (3/20) and 0% of individuals in the 3 groups. Similarly, there was no NNRTI mutation detected in 20% (4/20), 5% (1/20) and 0% of individuals in the three groups. The major NRTI drug resistance mutation was M184V and NNRTI drug resistance mutation was K103N. The number of polymorphisms in the drug resistance sites was found to be directly related to the duration of disease and period of treatment.

Conclusion: The study determined the prevalence of HIV drug resistance mutations in a representative population from Northern India and correlated it with the duration of treatment.

STUDIES IN PROGRESS:

HIVL-4: Cohort for TB Research by the Indo-US Medical Partnership (C-TRIUMPH) study

Principal Investigator	:	Dr. Padmapriyadarsini (email: padmapriyadarsinic@nirt.res.in)
Source of funding	:	DBT/NIH
Study Period	:	2013-2018

Background: This study aims to investigate host and microbial factors associated with TB treatment outcomes in Indian adults and children (in an active TB cohort), investigate the host and microbial factors associated with progression from latent infection to active TB disease in adults and children (in the Household Contacts cohort) and explore the host and microbial factors associated with TB transmission (Household Contacts and Control Cohorts). In addition, the study involves collection and storage of different types of biological samples from the above cohorts, mainly for biomarker research. During the reporting year, various kinds of biological samples were obtained and stored from 77 and 11 subjects belonging to cohorts A and B at baseline, and 281 and 217 subjects during follow-up for cohort A and B respectively (Tables 41-43).

Table 41: No. of C-TRIUMPh study samples received and processed during the period April 2017 to March 2018

	Cohort A	Cohort B	Total
No. of Baseline Samples received	77	11	88
No. of Follow up samples received	281	217	498
Total No. of samples received and Processed	358	228	586

Table 42: No. of samples processed for QGIT (Cohort-B) in C-TRIUMPh study during the period April 2017 to March 2018

Visit	Samples Received	Samples Tested
Entry Level	11	11
4th month	27	27
12th month	47	47
24th month	44	44
Total samples	139	139

Table 43: No. of C-TRIUMPh study samples (aliquots) stored during the period April 2017 to March 2018

Cohort	PBMC	PLASMA	QGIT (PLASMA)	PAXGENE	DNA
A	1087	4400	0	550	97
B	347	1352	1269	169	12
Total	1434	5752	1269	719	109

HIVL-12: Molecular mechanisms of HIV pathogenesis in T- cells and endothelial cells

Principal Investigator : Dr. A. R. Anand (email: anand.a@nirt.res.in)
Source of funding : DBT/Extramural
Study Period : 2015-2020

Background: HIV hijacks several components of the cellular machinery to enter its target cells and to replicate and disseminate in the body. However, several aspects of host-cell machinery that regulate HIV replication in its main target cells, the CD4+ T-cells, have not been thoroughly explored. Furthermore, recent studies indicate that the survival in HIV patients remains decreased

particularly due to excess deaths from non-infectious illness including atherosclerosis and other cardio-vascular diseases. However, the mechanisms involved in endothelial dysfunction leading to cardio-vascular disease in HIV-infected individuals are not well-studied.

Aim:

(i) To identify and delineate specific host-cell signalling networks that contribute to HIV-1 pathogenesis in immune cells and to elucidate the various factors that contribute to endothelial dysfunction in HIV infection

Methodology: We are using primary human umbilical vein endothelial cells (HUVEC) as a model, stimulated with various components of HIV, the whole virus and microbial translocation components to study endothelial dysfunction. Simultaneously, T-cell signalling molecules that regulate HIV replication are being studied using T-cell lines, MT-4 and Jurkat T-cells on infection with lab adapted strains of HIV.

Results: We are exploring synergies between HIV proteins (Gp120 and Tat) and LPS using primary HUVEC cells. Our studies indicate that that LPS and HIV gp120 show synergistic activity in production of cytokines such as IL-8 and IL-6. Evaluation of LPS and HIV gp120 on adhesion molecule expression such as VCAM-1 and ICAM-1 also show differences when treated in combination in comparison to individual treatment. Other endothelial functional effects such as endothelial permeability by FITC-dextran migration, monocyte transendothelial migration assays and measurement of transendothelial electrical resistance are being evaluated. We are also exploring the importance of actin cytoskeletal reorganization, especially the role of the actin-binding protein, leukocyte-specific protein-1 (LSP1) in HIV replication. To study the role of LSP1, the expression of LSP1 in various T-cell lines (MT4 and Jurkat T-cells) has been determined. The importance of LSP1 in HIV replication has been demonstrated by using LSP1-knock down using specific siRNA in T-cell lines. The signalling pathway used by LSP1 to regulate HIV replication is being deciphered.

These study findings shed new light on the mechanisms of HIV pathogenesis in T-cells and endothelial cells.

HIVL- 13: Role of microenvironment in the pathogenesis of HCV and HCV/HIV-associated hepatocellular carcinoma

Principal Investigator : Dr. A. R. Anand (email: anand.a@nirt.res.in)
Source of funding : DBT/Extramural
Study Period : 2015-2019

Background: Hepatitis C virus (HCV)-associated liver fibrosis and hepatocellular carcinoma (HCC) is a significant public health concern worldwide, including India. Despite the significant adverse clinical consequences of HIV/HCV coinfection, the underlying molecular mechanisms by which HIV infection impacts HCV disease progression and development of HCC are not known.

Aim:

(i) To characterize the profibrotic and proinflammatory environment in HCV-infected hepatocytes in the presence and absence of HIV

Methodology: We are using a multi-disciplinary approach to analyze HIV/HCV-induced changes in hepatocytes. Firstly, HCV JFH1 constructs are being used to generate viral particles using in-vitro transcription followed by transfection of permissive hepatocyte cell lines. Further, we are characterizing the profibrogenic /pro-inflammatory mediators as well as EMT markers expressed in the cells and/or in the conditioned medium (CM) using real-time qPCR, western blotting (WB) and ELISA.

Results: We have standardized *in vitro* culture of infectious HCV using the HCV JFH-1 constructs using i transcription and transfection of permissive hepatocyte (Huh 7.5) cells. We observed up-regulation of pro-fibrogenic cytokine, TGF-β1 in the HCV infected Huh 7.5 cells compared to uninfected cells. TGF-β levels in the cells were estimated by determining mRNA expression by RT-PCR. We are presently standardizing the expression of active TGF-β1 protein levels by ELISA. At the same time, we are evaluating the expression of other pro-fibrogenic cytokines such as CTGF. We have also standardized the HCV/HIV co-infection model, using the HIV protein, gp120, that has been shown to induce bystander effects during HCV/HIV co-infection.

Simultaneously, we are also evaluating the bidirectional cross-talk between HCV-infected hepatocytes and hepatic stellate cells in the presence/absence of HIV gp120. We are using the hepatic stellate cell line LX-2, as a model. Our studies show that HSCs express minimal CD4 and CCR5 levels, but express significant CXCR4 levels. We have shown that HIV gp120 induces the expression of adhesion molecules such as ICAM-1 and VCAM-1 as well as profibrogenic cytokines such as TGF-beta in hepatic stellate cells.

The study findings will help in gaining insights into the mechanism of liver fibrosis and development of HCC during HCV monoinfection and HIV/HCV co-infection.

HIVL-16: Study of SNPs and non-synonymous amino acid substitutions in the genome of viral isolates obtained from individuals recently infected with HIV-1

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email:hanna@nirt.res.in)
Research Scholar	:	Mr. Ashok kumar
Source of funding	:	Intramural
Study Period	:	2017-2019

Background: Among the repertoire of transmitted viral variants, the viral isolate(s) that establishes successful infection has not been well characterized yet. An insight into the salient features of the transmitted variants would contribute to the design of an effective vaccine against HIV.

Objective:

(i) To investigate the non-synonymous amino acid selected by HIV-1 variants for establishment of infection and fitness/survival against host immune pressure

Methods: Plasma samples were obtained from 8 children who recently acquired HIV-1 infection from the mother through the vertical route, and 2

adults with chronic HIV-1 infection who acquired the infection through horizontal transfer. The near full-length HIV-1 genome was amplified as two fragments (F1Gag-Vpu and F2Vif-3LTR) and sequenced using the Illumina HiSeq2500 platform. The sequence reads were processed and analysed using a pre-established bioinformatics pipeline.

Results: The percentage of SNPs in the env and pol region were identified to be 30% and 24% in viral isolates obtained from chronically infected individuals, while in those obtained from recently infected subjects it was 52% and 12% respectively. Non-synonymous amino acid substitution rate for env and pol was significantly higher in recent infection with 68% and 25% as compared to 56% and 12% in chronic infection. The number of SNPs and non-synonymous amino acid substitution was very less in rest of the genes in HIV-1 genome. Interestingly, we found some major and minor drug resistance mutations in two of the individuals who acquired HIV-1 infection through mother-to-child transmission. Further analysis is ongoing.

HIVL-17: Evaluation of mucosal immune responses to HIV infection in sero discordant couples

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email:hanna@nirt.res.in)
Research Scholar	:	Mr. M. P. Sivasankaran
Source of funding	:	Department of Health research-ICMR -HRD
Study Period	:	2017-2020

Background: Mucosal surfaces serve as a major port of entry for HIV, and the resident immune cells in mucosa alarm the entry of foreign pathogen to immune system. Understanding the role of the mucosal microenvironment in HIV transmission could give important clues that can be exploited for developing an

effective vaccine against HIV. The goal of this study is to measure critical mucosal immune responses in HIV-exposed sero negative partners among discordant couples and controls (HIV infected women) and to identify the immune responses that should be stimulated at the genital tract mucosa for effective sterilization of HIV infection.

Objectives:

(i) To determine the role of innate immune factors, cytokines and anti-viral factors that favor control of early infection in the mucosal surface

(ii) To identify relevant immune cells and their subtypes involved in the control of HIV-1 during the transmission event in the mucosal tissue by characterizing the phenotype and function of cells in the mucosal tissue and

(iii) To characterize the role of co-receptors in HIV infection at the mucosal surface

Methods: At least two cytobrush specimens are obtained from each individual. The specimens are transported on ice to the laboratory and processed within 3 hours of collection. Mononuclear cells are obtained by density gradient centrifugation, washed and resuspended in complete RPMI medium containing 100U/ml penicillin and 100µg/ml streptomycin. Cervicovaginal lavage (CVL) is also collected by gently washing the cervicovaginal area with 10 ml of sterile normal saline (pH, 7.2). Following CVL collection, samples are immediately frozen at -80°C.

Total and HIV-1 (gag) specific binding antibody levels in mucosal washings are estimated using commercially available ELISA kits. Levels of cytokines and chemokines that serve to modulate viral replication and to recruit target cells to sites of infection, are determined in mucosal washings using a multiplex bead assay that simultaneously quantifies 22 human cytokines and chemokines, including IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p70), IL-13, IL-15, IL-17, IL-1α, IFN-γ, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor alpha (TNF-α), CCL11/eotaxin, CCL2/monocyte chemo attractant protein 1, CCL3/MIP-1α, CXCL10/IP-10, and CCL5/RANTES. Perforin and granzyme B production by stimulated lymphocytes are determined using commercial ELISA. Cells obtained from

the mucosal samples are quantified by flow cytometry using monoclonal antibodies against phenotypic markers including CD3, CD4, CD8, CD14, CD19, CD25. Their maturational stage is assessed using monoclonal antibodies to CD45RA, CD27 and CCR7. CCR5 and CXCR4 expression on various cell types are determined. FOX P3 mRNA levels are quantified by real-time PCR.

The study is ongoing.

HIVL-18: Differences in neutrophil responses during the course of anti-TB treatment - a pilot study

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Post-Doctoral Fellow	:	Dr. Nancy Hilda J
Source of funding	:	ICMR
Study Period	:	2017-2019

Background: The pathogenesis of TB is the product of the interaction between bacterial virulence and host resistance, which are two distinct and independent variables. A well defined explanation of how our immune system aids in the elimination of bacilli and/ or how the bacilli evades the immune system of the host is the demanding need of the hour. Among the cells of the immune system, polymorphonuclear cells or neutrophils are a type of leukocytes, referred as the "first line defense of the innate immune system" especially against bacterial infections. Neutrophils continuously patrol the vasculature, monitoring for signals of bacterial infection or inflammation. These leukocytes act as first innate immune cells in containment and clearance of infectious particles. The present study aims to investigate the role of neutrophils in TB disease and changes in neutrophil response during anti-TB treatment.

Objectives:

(i) To assess the cytokine release by neutrophils before, during and after the treatment of TB

(ii) To study the differences in expression levels of cell surface receptors of neutrophils during the course of TB infection

(iii) To understand the genetic modulations in neutrophils as a result of TB infection

(iv) To elucidate neutrophil dependent responses generated by other immune cells during infection and

(v) To study Neutrophil Extra cellular Traps formed during TB infection

Methods: Human neutrophils from 15 healthy volunteers and 15 newly diagnosed PTB patients are separated from peripheral whole blood using density gradient centrifugation and enriched using dextran sedimentation. Purified cells are stimulated with TLR ligands - LPS, FSL-1 and Pam_3csk_4 and cultured overnight in RPMI medium in a humidified 5% CO_2 incubator. The cell free culture supernatants are harvested and frozen at -80°C for measuring the concentration of the cytokines like IL-8, MIP-1α, MCP-1β, IL-1β, TNF-α, CXCL5 and IFN-γ using commercial ELISA kits. Expression of cell surface markers like TLR2, TLR4, CD64, CD16, CD18, CD11B and Granzyme B are evaluated using flow cytometry.

Progress of the study: Base line sample collection and testing have been completed. Follow-up is ongoing.

HIVL- 19: Regional prospective observational research in TB (RePORT), India – Common protocol

Principal Investigator	:	Dr. Padmapriyadarsini (email: padmapriyadarsinic@nirt.res.in)
Source of funding	:	DBT/NIH
Study Period	:	2017-2021

Background: The RePORT India Consortium is a bi-lateral, multi-organizational collaborative effort that was established under the Indo-U.S. Vaccine Action Program to address the threat of tuberculosis. The RePORT India Consortium comprises of 5 clinical research units located in various parts of the country, each recruiting cohorts of TB patients and their household contacts, to undertake studies to advance regional TB science and at the same time strengthen TB research capacity and infrastructure in India, and serve as an entity to foster research collaboration within India and internationally with the aim of carrying out a wide range of basic and clinical research that can lead to clinically important biomarkers, vaccines, drugs and diagnostics.

As part of this project, various kinds of biological specimens are collected by each of the participating sites using common protocols and standard operating procedures and stored in a centralized biorepository for future cutting edge research (Table 44). The NIRT has been appointed to house the Indian owned and managed TB biorepository which contains well-characterised specimens from TB cohorts as well as house hold contact cohorts.

Table 44: Details of samples stored in the biorepository

SITE NAME/ SIDE ID	PBMC	PLASMA	QGIT (PLASMA)	PAXGENE	DNA	URINE	SPUTUM	SALIVA	MTB ISOLATES
MVDRC (103)	250	1086	0	143	560	544	476	79	80
JIPMER (102)	468	1743	503	571	524	771	402	452	100
BJMC (106)	1016	2033	0	144	730	972	163	0	0
BMMRC (107)	10	0	0	0	0	0	0	0	0
NIRT (105)	544	2138	1200	324	796	1063	345	402	80
CMC (101)	11	0	0	0	0	0	0	0	0

HIVL-20: Establishment of an in-country PBMC cryopreservation PT program

Principal Investigator : Dr. Luke Elizabeth Hanna (email:hanna@nirt.res.in)
Source of funding : CRDF/NIH
Period : 2017-2018

Background: The use of cryopreserved peripheral blood mononuclear cells (PBMCs) for immunologic assays has increased in recent years. The utility of cryopreserved PBMCs is dependent on their viability and functional ability. The NIRT PBMC Cryopreservation proficiency testing (PT) program measures viability and viable recovery of the processed PBMC samples at the participating laboratories in the RePORT India studies.

Study Progress: The PT program has been established at NIRT and is operating successfully. Table 45 lists the on-going activities of the PBMC EQA program at NIRT.

Table 45: PBMC EQA related activities during the period April 2017 - March 2018

SITE NAME	SITE ID	NO. OF PBMC SAMPLES RECEIVED & THAWED
CMC (VELLORE)	101	20
JIPMER (PONDICHERRY)	102	12
MVDRC (CHENNAI)	103	34
NIRT (CHENNAI)	105	8
BJGMC (PUNE)	106	10
BMMRC (HYDERABAD)	107	12
	TOTAL	**96**

Department of Immunology

COMPLETED STUDIES:

I-1: Biophysical and biochemical characterization of two DNA binding proteins (Rv3716c and Rv3405c) from *M. tuberculosis*

Principal Investigators	:	Dr. Alamelu Raja; Dr.Ramalingam B (email: ramalingam.b@nirt,res,in)
Research Scholar	:	Akilandeswari Gopalan
Source of Funding	:	DST Inspire Fellowship.
Study Period	:	2012-2018

Background: Though there are number of drugs available for TB treatment, most of them are not designed based on the specificity of drugs to the target. Drug targets obtained from comprehensive *in silico* analysis and characterization of the same may aid in proper validation of the use of the protein as a drug target. The target antigens can be segregated according to its function namely cellular metabolism, virulence, detoxification, adaptation, cell and cell wall processes, regulatory proteins etc. In this respect, DNA binding proteins, categorized under regulatory proteins, play a major role in growth, survival, and pathogenesis of the bacilli. Thus, the study was initiated with exploring two such DNA binding proteins: Rv3716c- found to be associated with LTB diagnosis and Rv3405c- a candidate drug target from *in silico* predictions.

Aim:

(i) Characterization of two DNA binding proteins (Rv3716c and Rv3405c) from *M. tuberculosis*

Objectives:

(i) To over express, purify and characterize Rv3716c, a nucleoid associated protein, by biophysical and biochemical methods

(ii) To elucidate the structure of recombinant Rv3716c protein by X-Ray diffraction studies

(iii) To clone, over-express and purify Rv3405c, a tetracycline family of transcription factor in *E.coli* expression system and

(iv) To functionally characterize recombinant Rv3405c by biophysical, biochemical and bioinformatic methods

Results:

*Determination of the structure of Mtb*Rv3716c: Calculation of Mathew's coefficient suggested the presence of only one protomer of rRv3716c in the crystal asymmetric unit (VM was ~2.31

$Å^3$ Da^{-1}) with a solvent content of 46.8% (Fig.8). The structure of *Mtb*Rv3716c was determined at 1.9 Å resolution by the molecular replacement (MR) method using the program PHASER of the CCP4 suite. A template of a hypothetical DNA binding protein (PDB code 1YBX) sharing 41% sequence identity with the rRv3716c was used to obtain the phase information. The model obtained by PHASER was subjected to initial rigid body refinement using the program REFMAC5 of the CCP4 suite. The final model had R_{work} and R_{free} values of 17.68 % and 21.52 % respectively. Electron density was continuous for residues 22-92 and most of the main chain as well as the side chain atoms in this stretch could be fitted into the density. The asymmetric unit contains 107 water molecules, one Cd^{2+} ion and three ethylene glycol (EDO) molecules. The model has an average temperature factor of 29.23 $Å^2$. The refined structure had good geometry as indicated by the PROCHECK server with most residues falling in the fully allowed region of the Ramachandran plot and no residue in the disallowed region. The bond lengths and angles were in close agreement with the anticipated values.

DNA binding studies of rRv3716c: Experiments were done to examine the ability of rRv3716c to bind one of the sequences surrounding erpAB operator 2 region of *B. burgdorferi*. This region was selected because Ebfc protein has been reported to specifically bind to this sequence and cause bending of DNA. Earlier studies have also demonstrated that Ebfc forms higher order structures such as tetramers and octamers leading to bridging of DNA. YbaB of *E. coli* as well as *H. influenza* were also found to bind to this DNA sequence. Thus the probe (GTTAAAAT GTAACAGCT GAATGTAAC) spanning the palindromic region was constructed, procured from a commercial source, annealed, ends were labelled with γP^{32} and used as the probe in gel retardation assays. The observed mobility shift in the gel confirms that the rRv3716c has DNA binding activity (Fig. 9). Thus, rRv3716c binds DNA but the complex is not prone to aggregation. Further investigations are needed to determine the oligonucleotide specificity of the protein and the effect of binding on the structure of DNA.

Fig.8: Cartoon representation of rRv3716c protein

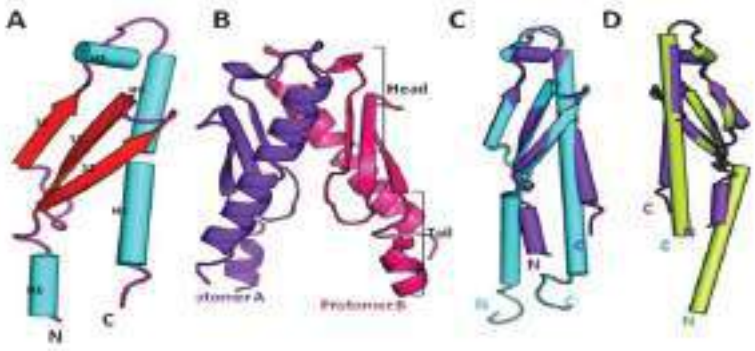
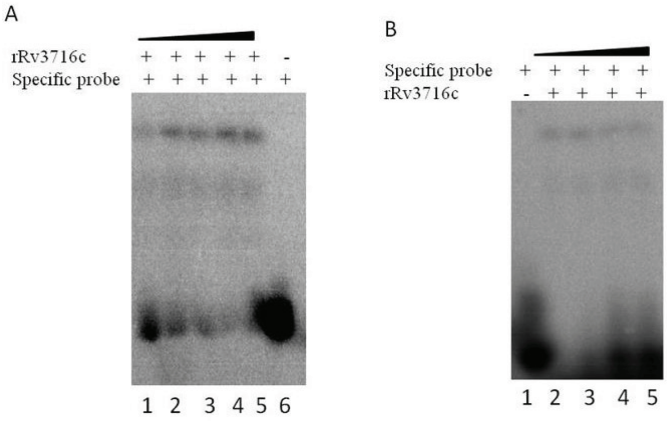


Fig.9: EMSA studies on rRv3716c protein



β-galactosidase reporter assay: In order to study the physiological function of the protein, a LacZ based reporter assay was performed using both wild type and mutant proteins. The 101bp PCR product containing the inverted repeat corresponding to upstream sequence of Rv3406 to which Rv3405c is proposed to bind was cloned upstream to LacZ gene into the promoter-less pMP220 vector (pMP220-PMR) (Fig.10a). Rv3405c gene was cloned into the compatible pBAD24 vector (Fig.10b). *E. coli* Top10 cells were co-transformed with the empty reporter vector (pMP220) or pMP220 with upstream region of Rv3406 and the compatible pBAD24 vector cloned with either wild type Rv3405c or triple mutant construct. The expression of the protein was checked before performing the reporter assay. At the same time, colony PCR was performed to check the co-transformation.

Fig. 10a & b: Confirmation of cloning of promoter (101bp) in pMP220 by PCR and ……..

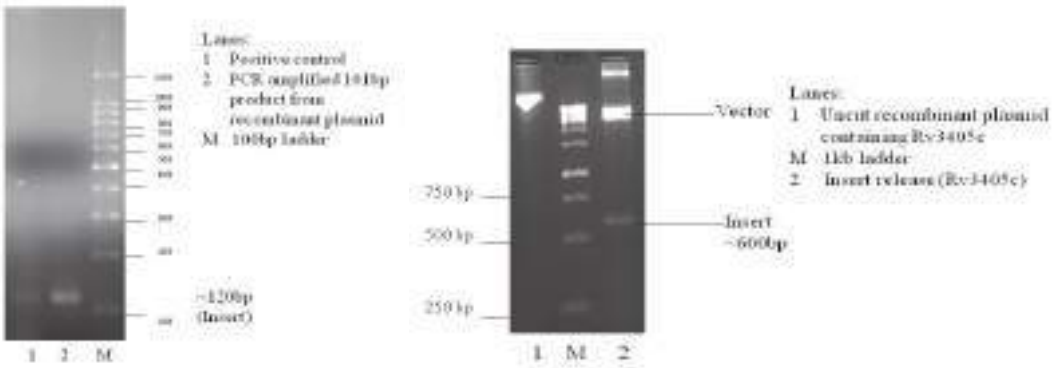
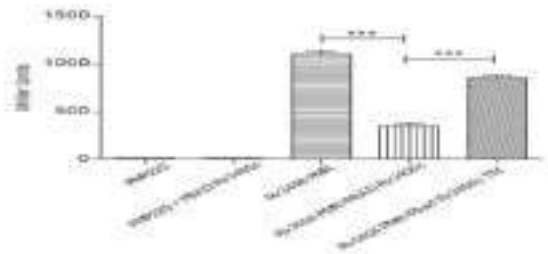


Fig. 11: β-galactosidase assay in *E.coli* showing that rRv3405c is a TetR repressor



The co-transformed cells were grown and induced with of 0.2% arabinose followed by Miller's assay. The activities of β-galactosidase was expressed in terms of Miller units and plotted for each condition (Fig. 11). The assay revealed that when rRv3405c was expressed upon induction with arabinose, there was reduction in the reporter gene *lacZ* expression while in the case of the triple mutant construct of rRv3405c, the *lacZ* expression was restored.

Summary and conclusions: Targeting antigens from Mtb play an important role in drug discovery and management of treatment therapy of tuberculosis disease. In this study, we have characterized two DNA binding proteins namely, Rv3716c and Rv3405c, based on previous immunological characterization and *in silico* drug targeting analysis respectively.

(i) Rv3716c was found to be a member of Ybab family DNA binding proteins

which along with RecR cotranscription, aid in replication recovery related DNA repair

(ii) Structural characterization of recombinant Rv3716c protein showed that the protein exists as a dimer with tweezers or V-shaped architecture.

(iii) EMSA study indeed proved that the Rv3716c protein has double stranded DNA binding ability but it does not bind to single stranded DNA.

(iv) The second protein, encoded by Rv3405c is annotated to be a tetR family of transcription factor involved in the repression of the adjacent gene (Rv3406) activity.

(v) The physiological function of the protein, the repressor activity, the characteristic of a TFTR protein, was confirmed through β-galactosidase reporter assay in *E.coli*.

I-4: Promoter polymorphisms of IL-10 (-592 A/C) and TGF-β1 (-509 C/T) in TB and HIV patients of south Indian population

Principal Investigators: Dr.P.Selvaraj; Dr. Ramalingam B.
(email: ramalingam.b@nirt,res,in)

Source of Funding : DBT / Intramural, NIRT.
Study Period : 2016-2017

Background: Genetic factors play an important role in the development of disease susceptibility or protection. Cytokine gene polymorphisms are reported to be associated with altered levels of cytokine production that can impact disease progression in HIV and TB.

Aim:

(i) To find out whether allele and genotype frequencies of promoter IL-10 and TGF-β1 associated with susceptibility or resistance to TB and HIV in south Indian population

Methodology: Isolated genomic DNA by simple salting out procedure used for genotyping. Both the promoter polymorphism genotyping have been carried out by PCR followed by RFLP method.

Study subjects:

The study population consisted of:

Healthy controls – 122

TB – 122

HIV – 100

IL-10 genotyping: The PCR product digested with the restriction enzyme RsaI at 37°C for 16hrs. The homozygous genotype "AA" containing the restriction site yielded 2 bands of 236 bp and 176 bp size. The homozygous genotype "CC" which lacked the restriction site yielded a single band of 412 bp size. The heterozygous genotype "CA" yielded 3 bands of 412 bp, 236 bp and 176 bp size.

TGF-β genotyping: The PCR 808bp product was digested with the restriction enzyme Bsu36I at 37°C for 16 hrs. The homozygous genotype "CC" containing the restriction site yielded 2 bands of 617 bp and 191 bp sizes. The homozygous genotype "TT" which lacks the restriction site yielded a single band of 808 bp size. The heterozygous genotype "CT" yielded 3 bands of 412 bp, 236 bp and 176 bp sizes.

Analysis: The allele and genotype frequencies were determined by using SNP stats software. Pearson χ^2 test was used to find out whether the genotype frequencies are in Hardy–Weinberg equilibrium. The 2x2 table, p-value with Yates correction and odds ratio with 95% confidence intervals were analysed using epi info version 6.04. For genotypic associations, p values with OR adjusted for gender and age were calculated by logistic regression under co-dominant, dominant, recessive and over-dominant models using the online SNPstats program. The best fitting model of association was determined using the Akaike information criterion (AIC) and Bayesian information criterion (BIC) provided by the software. The model with lowest AIC and BIC values was considered as the best fitting model. Based on this, dominant model and over dominant model was selected for IL-10 and TGF-β polymorphisms. A p value ≤0.05 was considered statistically significant.

Results:

IL-10 Gene Polymorphism in HIV and TB

IL-10 -592 promoter polymorphism, allele 'C' generally distributed as a major allele, whereas allele 'A' was found as minor allele frequency in this population. In HCs 'C' allele was present among 63% population while it was present in 58% and 52% in TB and HIV subjects respectively. The minor allele 'A' was present in 37%, 42% and 48% in HCs, TB and HIV.

Among the “CC”, “CA” and “AA” genotypes, the heterozygous “CA” genotype was found in higher frequency in all study groups followed by “CC” and “AA” genotype. In HCs and TB patients, under dominant model (CC vs C/A+AA) no significant association was found with any of the genotypes [OR: 1.37(0.79-2.36)] (Table 46). When the genotypes were compared under dominant model between HCs and HIV patients, ‘A’ allele either ‘CA’ or ‘AA’ combinations significantly associated with susceptibility to HIV (OR: 1.88(1.05-3.35); p=0.030) (Table 46).

TGF-β Gene Polymorphism in HIV and TB

TGF-β -592 promoter polymorphism allele ‘C’ was found to be the major allele, whereas allele ‘T’ was the minor allele. In all study groups, no significant association was found in allele frequencies (Table 47). Genotypes “CC” and “TT” were higher in HCs (40% and 20%) compared to TB patients (35.3% and 18.6%), whereas heterozygous “CT” genotype was higher in TB patients (46.1%) compared to HCs (40%). Similarly, a higher frequency of “CC” and “TT” genotype was observed in HCs compared to HIV patients (CC: 24%; TT:11%). For the “CT” genotype, frequencies of 40% in HCs and 30% in HIV were observed. But, overall genotype comparison between HCs vs HIV [OR: 1.47(0.76-2.85)] and HCs vs TB [OR: 1.24(0.68-2.27)], no significant association was found with TGF-β promoter polymorphism (Table 47).

Table 46: The promoter IL-10 -592 allele and genotype frequencies in healthy controls (HCs), HIV and PTB patients

		Allele frequencies				Genotype frequencies		Odds ratio (95% CI)	p-value
SNP	**Allele**	**HCs**	**HIV**	**p-value**	**Genotype**	**HCs n=122**	**HIV n=100**		
IL-10 -592 (C/T)	C	0.63	0.52		CC	0.41 (50)	0.27 (27)		
	A	0.37	0.48	**0.030**	CA	0.44 (54)	0.51 (51)	1.88 (1.05-3.35)#	**0.030#**
					AA	0.15 (18)	0.22 (22)		
	Allele	**HCs**	**PTB**			**HCs n=122**	**PTB n=122**		
	C	0.63	0.58		CC	0.41 (50)	0.33 (40)		NS
	A	0.37	0.42		CA	0.44 (54)	0.50 (61)	1.37 (0.79-2.36)#	
					AA	0.15 (18)	0.17 (21)		

In HCs, HIV and PTB column, numbers in parenthesis denote number of individuals positive for that genotype.

 # - Based on dominant model, odds ratio and p-value mentioned**.**
NS – Not significant, p=0.260

Table 47: The promoter TGF-β -509 allele and genotype frequencies in HCs, HIV and PTB patients

SNP	Allele	Allele frequencies HCs	Allele frequencies HIV	Genotype	Genotype frequencies HCs n=122	Genotype frequencies HIV n=100	Odds ratio (95% CI)
TGF-β -509 (C/T)	C	0.60	0.60	CC	0.40 (40)	0.37 (24)	
	AT	0.40	0.40	CT	0.40 (40)	0.46 (30)	1.47 (0.76-2.85)#
				TT	0.20 (20)	0.17 (11)	
	Allele	**HCs**	**PTB**		**HCs n=100**	**PTB n=102**	
	C	0.60	0.58	CC	0.40 (40)	0.353 (36)	
	T	0.40	0.42	CT	0.40 (40)	0.461 (47)	1.24 (0.68-2.27)#
				TT	0.20 (20)	0.186 (19)	

In HCs, HIV and PTB column, numbers in parenthesis denotes number of individuals positive for that genotype.

- Based on overdominant model, odds ratio value mentioned.
p-value is not significant. Under overdominant model, HCs vs HIV; p=0.250 and HCs vs PTB; p=0.48.

Results:

The genotype frequencies were stratified and analyzed based on sex, with age adjusted to determine whether there were any differences among male and female individuals between HCs and HIV as well as HCs and TB. The results revealed no significant difference between males and females in IL- 10 or TGF-β promoter polymorphisms. However, in IL-10 promoter polymorphism, females were found to be more at risk with HIV in 'AA' genotype followed by 'CA' genotype compared to males [AA, female OR: 2.53 (0.86-7.41) vs male OR: 2.06 (0.64-6.65)]; CA, female OR:

1.81 (0.82-4.01); male OR: 1.64 (0.63-4.29)]. But in TB, 'AA' genotype were found to be at risk in females [OR: 2.21(0.65-7.49)] compared to males [OR: 1.03(0.37-2.85)]. In TGF-β polymorphism, 'TT' genotypes were found to be at risk to TB in males [OR: 1.48(0.51-4.24)] compared to females [OR: 0.50(0.09-2.84)], but the differences were not statistically significant.

Conclusion: This study suggests an association of IL-10 -592 'A' allele with either "AA" and "CA" genotype combinations to HIV susceptibility under dominant model in the south Indian population. In contrast, a lack of association was observed for the TGF-β promoter -509C/T polymorphism with HIV as well as TB. Moreover, a trend towards higher risk to HIV was found in females compared to males in IL-10 - 592 "AA" genotype. Further studies with larger sample sizes may be useful to confirm this finding and understand the role of these cytokines in progression of HIV and TB.

STUDIES IN PROGRESS:

I-3: Prevalence of TB (*M. tuberculosis* and *M. bovis*) in cattle and animal handlers in Chennai region

Principal Investigator	:	Dr.P.Kannan (email: kannanp@nirt.res.in)
Collaborators	:	Dr. Dhinakar Raj; Dr.Maroudham Tamil Nadu Veterinary and Animal Sciences University, Chennai
Source of funding	:	Intramural
Study period	:	2015-2018

Background: In most of the developing countries including India the burden of zoonotic TB is not estimated or under estimated. India ranks first in buffalo and second in cattle population in the world, where bovine TB is not controlled at all. It has been postulated that zoonotic TB represents significant risk in areas where humans and animals share common environment. Agricultural workers may acquire the disease by inhaling cough spray from infected cattle and develop typical PTB and such patients may transmit the infection to cattle and humans. Like Animal-Human transmission, Human – Animal transmission occurs at locations of active animal-human interaction. This is posing a formidable challenge in controlling and eradicating mycobacterial diseases.

Objective:

(i) To estimate the prevalence of zoonotic and reverse zoonotic transmission of *M.bovis* and *M.tuberculosis* in animal handlers and cattle

Methods: We have screened four government farms and one private farm for TB in animal handlers and animals.

Screening of animal handlers: All the animal handlers in the three cattle farms were interviewed for symptoms of TB (cough for 2 weeks, fever, night sweat, weight loss) and also screened by chest X-ray. Two sputum (spot and early morning) samples were collected from those with symptoms of tuberculosis and abnormal chest X-ray for bacteriological examination.

Screening of animals: All the cattle housed in the three farms were screened by using comparative intradermal tuberculin test. The comparative intradermal tuberculin test is used to differentiate between animals infected with *M.bovis* and those responding to bovine tuberculin

as a result of exposure to other mycobacteria. This sensitization can be attributed to the antigenic cross reactivity among mycobacterial species and related genera. The test involves the intradermal injection of bovine tuberculin and avian tuberculin into different sites, usually on the same side of the neck, and measuring the response 3 days later. In the interpretation of the intradermal comparative test, a reaction is usually considered to be positive if the increase in skin thickness at the bovine tuberculin site of injection is more than 4mm.

Spoligotyping: Direct locus- DRa (0.2 µmol/µl) and DRb (0.2µmol/µl) primers were used for spoligotyping. The spacers between the direct repeats in the target region were amplified by using two 18-nucleotide primers (primer 5'-CCAAGA GGGGAC GGAAAC-3' and biotinylated primer 5'-GGTTTTGGGTCTGACGAC-3'). The PCR products were then hybridized to a Biodyne C membrane (Isogen Bioscience, Maarsen, The Nether-lands). This membrane contains immobilized synthetic oligomeric spacer sequences derived from the direct-repeat region of *M. tuberculosis* H37Rv and *M. bovis* BCG. Hybridized DNA was detected by using an enhanced chemiluminesence kit (Biobasic, Israel), with exposure to X-ray film producing a pattern or profile reminiscent of a bar code. The hybridization pattern was analyzed using SPOTCLUST (http:// tbinsight.cs.rpi.edu/run_spotclust.html) database as per the standard procedure.

Results: A total of 606 animal handlers were screened, of whom 71 were symptomatic and 6 were positive for TB. A total of 470 cattle were screened by comparative tuberculin skin testing of which 40 animals were positive for TB. Four animals were culled in one government farm and extensive TB lesions were noticed in various organs (Fig. 12). Tissue samples were collected for isolation of the bacteria. Spoligotyping of 4 isolates from animal origin and 6 isolates from animal handlers were performed (Table 48). Spoligotyping was also performed using DNA samples from Milk and Nasal swab of animal origin (Table 49). The results indicated the presence of same strains in ruminants and human in the same geographical region during the period under study. This suggested the existence of an active 'spill-over' mechanism of *M. tuberculosis* infection in bovine animals.

The study is ongoing.

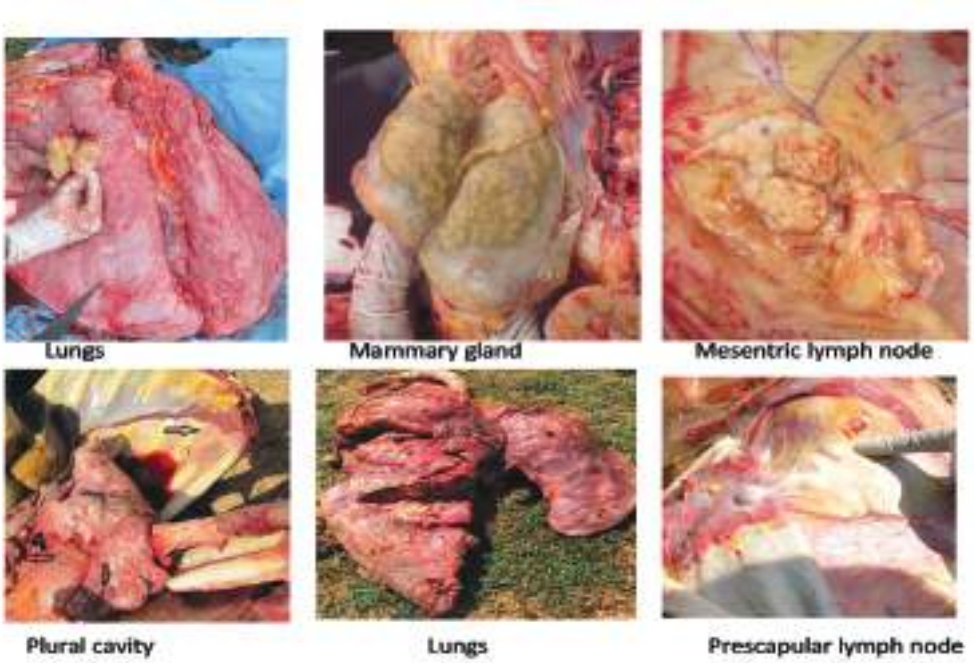
Table 48: Spoligotyping patterns and drug susceptibility profile of the isolates obtained from both animal and animal handlers

Laboratory identification No.	Source	Sample	Animal Farms	Spoligotyping Identification			MGIT DST SIRE
				Octal Code	SpolDB3 based Lineage	Binary patterns	
AH 03	Animal Handler	Sputum	Organised Farm 1	477777777413071	EAI3-IND	1001111111111111111111111111 1000010110001111	SSSS
AH 29	Animal Handler	Sputum	Organised Farm1	467777777413071	EAI3	1001101111111111111111111111 1000010110001111	SSSS
AH 69	Animal Handler	Sputum	Organised Farm 2	467777777413071	EAI3	1001101111111111111111111111 1000010110001111	SSSS
AH 85	Animal Handler	Sputum	Organised Farm 2	477777777413071	EAI3-IND	1001111111111111111111111111 1000010110001111	SSSS
AH 90	Animal Handler	Sputum	Organised Farm 2	477777777413071	EAI3-IND	1001111111111111111111111111 1000010110001111	SSSS
AH 91	Animal Handler	Sputum	Organised Farm 2	473776077411771	EAI2	1001110111111111110000111111 1000010011111111	SSSS
KH 126	Cattle	Lung, Udder	Organised Farm 2	477777777413171	EAI 5	1001111111111111111111111111 1000010110011111	SSSS
KH 127	Cattle	Lung	Organised Farm 2	477777777413071	EAI3-IND		SSSS
KH 128	Cattle	Lung, Spleen	Organised Farm 2	477777777413171	EAI5	1001111111111111111111111111 1000010110011111	SSSS
KH 143	Cattle	Lung, Udder	Organised Farm 1	477347777413161	EAI3	1001111110111001111111111111 1000010110011101	SRRS

Table 49: Spoligotyping patterns revealed from nasal swab and milk from live cattle

S.No	Cattle farm	Source	Octal code	SpolDB3 based lineage
1	Unorganized Farm 3	Nasal swab	777777777777771	Manu1
2	Unorganized Farm 3	Nasal swab	777777777733771	Manu1
3	Unorganized Farm 3	Nasal swab, Milk	777777777773671	Orphan
4	Unorganized Farm 3	Nasal swab, Milk	474377777413771	EAI5
5	Organized Farm 2	Nasal Swab	477777777413031	EAI5
6	Organized Farm 2	Milk	474377777413771	EAI5
7	Organized Farm 2	Nasal Swab	777777777773771	Manu1
8	Organized Farm 2	Milk	473776077411771	EAI2
9	Organized Farm 2	Nasal Swab	477777777413071	EAI3- IND
10	Organized Farm 2	Nasal swab, Milk	477347777413161	EAI3
11	Organized Farm 2	Nasal Swab	757756377773771	Orphan
12	Organized Farm 2	Nasal Swab	777777777777771	U
13	Organized Farm 2	Nasal Swab	777777777777671	U

Fig. 12: Post-mortem identification of tubercular lesions in the organs of the culled animals



I-5: Crohn's disease in India: A multicenter study from a country where Intestinal TB as well as Johne's disease is endemic

Principal Investigator	:	Dr.P.Kannan (email: kannanp@nirt.res.in)
Collaborator	:	Dr. B.S. Ramakrishna SRM institute for Medical Sciences (SIMS), Chennai
Funding Agency	:	ICMR- DHR
Study period	:	2016-2019

Background: Inflammatory bowel diseases (IBD), including the spectrum of ulcerative colitis (UC) Crohn's disease (CD), and indeterminate colitis (IC) are chronic, progressive and relapsing inflammatory disorders of unknown etiology that may cause disability over time. IBD represents a life-long disorder that may occur at any time from early childhood to late adulthood. UC is characterized by ulcerations and inflammation of the large bowel mucosa. CD is a trans-mural inflammatory disorder that may involve various sites of the gastrointestinal tract: in 40%-70% of cases the terminal ileum is involved.

M. avium subspecies *paratuberculosis* (MAP) has been postulated as a pathogenic agent for granulomatous ulceroconstrictive disease of ileocaecal region particularly CD in human and it causes Johne's disease (JD), a granulomatous enterocolitis of domestic and wild ruminants and causes heavy economic losses to the dairy industry. The zoonotic potential of the organism has been considered because MAP has been frequently implicated in Crohn's disease .CD was considered almost non-existent in India until 1986. However, during last decade, the disease has been reported more frequently from different parts of India. Association of MAP with CD in India has been most recently reported in a preliminary study that showed presence of MAP in CD patients, and MAP antibody in farm workers and affected and healthy population of north India. This multi-centric study focuses on relationship of MAP infection and ulceroconstrictive diseases of ileocaecal and colonic region in humans.

Objective:

(i) To study the association of *M. avium* subspecies *paratuberculosis* with Crohn's disease

Methods: We have received a total of 62 patient's samples (blinded) from SRM Institutes for Medical Sciences

(SIMS), Chennai. Blood, colonic biopsy and serum samples were received for the detection of MAP and Intestinal TB.

Blood: Buffy coat separation was done by gradient centrifugation and cells were inoculated into MGIT tubes containing Mycobactin J for MAP isolation. The DNA was extracted from PBMCs using Qiagen DNA extraction kit and PCR for presence of MAP DNA.

Tissue: Samples were homogenized and processed by NALC-NAOH method for decontamination (1:1). The concentrated sediment from each sample was cultured on 2 MGIT tubes (OADC, PANTA and 2ug Mycobactin J) to rule out *M. tuberculosis* and incubated at 37^0C. Briefly the sediment was transferred to the tube containing 4.5 ml of MGIT medium (Becton Dickinson) supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC), PANTA (40 U of polymyxin B per ml, 4 µg of amphotericin B per ml, 16 µg of nalidixic acid per ml, 4 µg of trimethoprim per ml, 4µg of azlocillin per ml [final concentrations]), and 2 µg of mycobactin J (Allied Monitor, Fayette, Mo.) per ml. The cultures were incubated for 8 weeks and examined weekly for evidence of growth. For MAP, the cultures were incubated for 1 year with IS 900 PCR performed every month, as the MGIT 960 may not give a positive signal.0.5 ml culture were removed aseptically for testing by the IS*900*-specific nested PCR.

Serum: Serum was separated from 2ml of blood for ELISA for MAP antigens.

Results: Out of 62 samples, 6 samples were culture positive for *M. tuberculosis.* Four samples were positive for MAP by qPCR.

The study is ongoing.

I-6: Characterization of mycobacterial intermediary metabolic enzymes as novel drug targets by comparative omics

Principal Investigator	:	Dr. K. R. Uma Devi (umadevi.r@nirt.res.in).
Research scholar	:	Mr.C. Yuvaraj
Source of funding	:	Intramural / Inspire Fellowship
Study period	:	2014-2019

Background: Current treatment regimens for MDR and XDR-TB are toxic, lengthy, and usually associated with low clinical success rates. The treatment outlook has been improving, as witnessed by the recent approvals of Bedaquiline (BDQ) and Delamanid (DEL) and multiple other drugs under development. There is however a need for finding a better companion drug that can be administered along with Bedaquiline and Delamanid for treating *DR-TB.* Previous reports suggest that impairing the function of mycobacterial isocitrate lyase [ICL gene – Rv0462] improves the efficacy and shortens the duration of first line anti-TB drugs.

One such drug is *Salicylanilide* which is an anti-helminthic drug with the amide of salicylic acid and aniline. It is classified as both a salicylamide and an anilide [US National Library of Medicine]. *In vitro* activity of *Salicylanilide* benzoates and pyrazine-2-carboxylates can act against *M, tuberculosis*. Furthermore, salicylanilide derivatives acts as inhibitors against mycobacterial isocitrate lyase with a MIC as low as 0.5µmol/L.

We systematically evaluated the comparative growth kinetic profile of drug sensitive laboratory H37Rv and 8 different clinical isolates of *M. tuberculosis*. In addition, DST of new anti-TB drugs along with the lead compound *Salicylanilide* for drug sensitive laboratory strain H37Rv and one DR clinical isolate (H-Mono) was performed.

Aim:

(i) To characterize the activity of new anti-TB drugs against MTB clinical isolates having different pattern of drug resistance

Methods:

Mycobacterial cultures: The study was performed on a drug sensitive laboratory strain H37Rv and 8 clinical isolates: R-Mono, H-Mono, MDR (82 & W2), Pre-XDR (81 & W1), XDR (W3 & W4) obtained from NIRT. The cultures were identified as *M. tuberculosis* by

conventional culture and biochemical tests.

Growth kinetics: Mycobacterial growth was monitored by measuring optical density at 600nm for 30 days with a regular intervals of time and each cultures were diluted by PBS (Phosphate Buffer Saline) with dilution of N (Neat), 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 5µl of these diluted cultures were spotted in Middlebrook 7H11 agar (0.5% glycerol, 10% oleic acid, albumin, dextrose and catalase supplement) plates where the colony forming units (CFU) were recorded, incubated at 37^{o}C for 4 weeks.

Resazurin microtitre assay: The resazurin microtiter assay (REMA) was performed in 7H9-S medium which contained Middlebrook broth, 0.1% Casitone, 0.5% glycerol supplemented with 10 % OADC. The drug solutions BDQ (100µg/mL), DEL (80µg/mL), MFX (100µg/mL) and INH (100µg/mL) were thawed and diluted in 7H9-S medium. Growth controls lacked antibiotic and sterility controls without inoculation of culture were also included. A change from blue to pink color indicated growth of the bacteria (reduction of resazurin to resorufin). MIC was defined as the lowest concentration of drug that prevented colour change.

Results: Analysis of the growth kinetics observed between these strains showed that there was a significant difference between the laboratory drug sensitive strain H37Rv and drug resistant clinical isolates with a P value < 0.05 (Figs. 13 & 14). A representative figure has been shown which implies that on 0^{th} day, 8 DR clinical isolates of MTB showed significant difference with the drug sensitive laboratory strain H37Rv ($P<0.05$).

The comparison of mean OD values at 600nm and log CFU values for H37Rv and clinical isolates of MTB which was recorded at regular intervals of 30 days were consolidated (Table 50). The individual MICs of BDQ, DEL, MFX, Clofazimine and *Salicylanilide* were evaluated against laboratory drug sensitive H37Rv and DR clinical isolate (H-Mono) of MTB by REMA assay. The MIC of the above mentioned drugs fell within the previously published range against drug sensitive and DR clinical isolates of MTB. The published MIC of Bedaquiline against drug sensitive H37RV strain was reported as 0.03 to 0.12 µg/ml. The MIC for DEL, MFX and clofazimine against *M. tuberculosis* are 0.006–0.024 mg/L, 0.5 – 2.0 µg/mL and ~0.5µg/ml.

The MIC of the drugs BDQ, DEL, MFX, Clofazimine and *Salicylanilide* were found to be 0.06µg/ml, 0.06µ/ml, 0.125µg/ml, 0.06µg/ml and 30µg/ml respectively against drug sensitive laboratory strain (H37Rv) and the MIC of BDQ, DEL, MOX, Clofazimine and *Salicylanilide* (30µg/ml) were found to be 0.125µg/ml, 0.25µ/ml, 2µg/ml, 0.015µg/ml and 30µg/ml respectively for drug resistant clinical isolate (H Mono) (Fig. 15). Our results provide evidence that the MIC of *Salicylanilide* may decrease when it is administered along with the new anti-TB drugs. The drug interaction profile of *Salicylanilide* with the new anti-TB drugs against drug sensitive laboratory strain H37Rv and DR clinical isolates of MTB will be carried out in future. The study is ongoing.

Fig. 13: Growth kinetic profile of drug resistant clinical isolates of MTB against drug sensitive laboratory strain H37Rv under aerobic condition over time period.

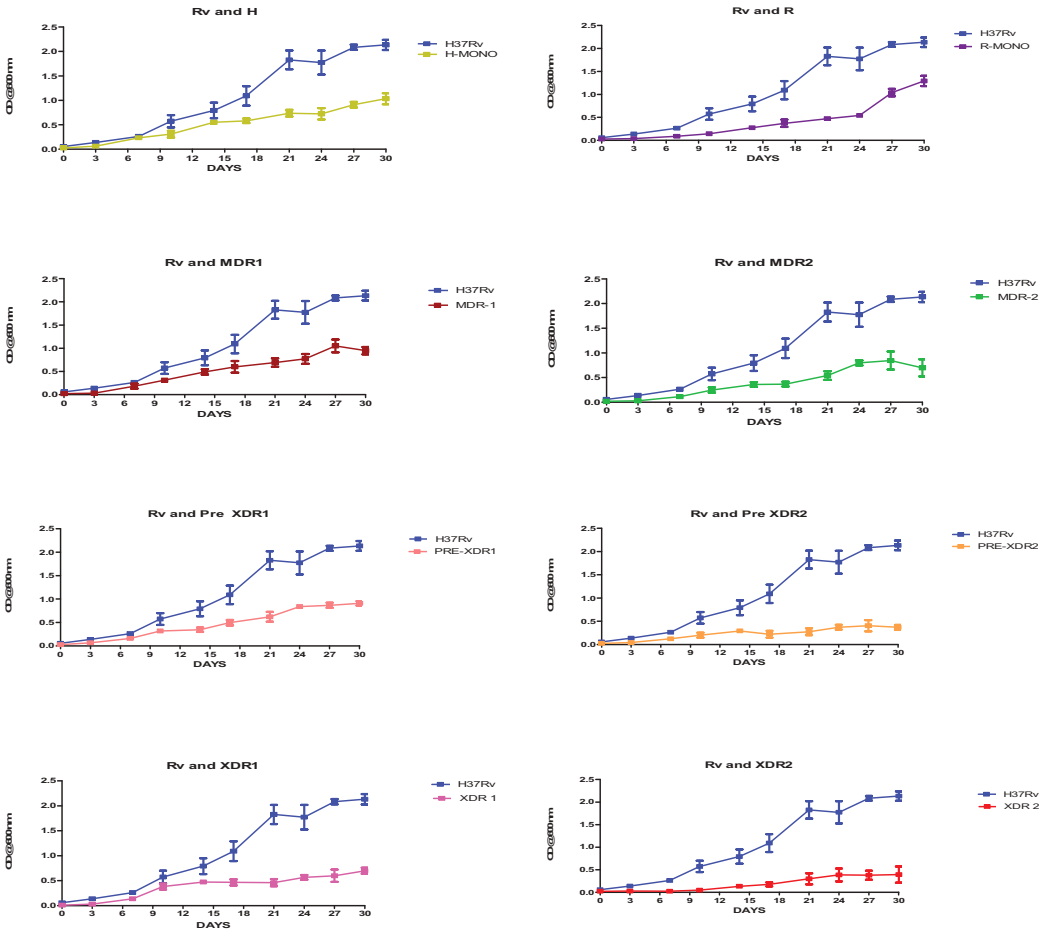


Fig. 14: Comparative growth kinetic analysis by colony forming unit between drug sensitive and drug resistant strains

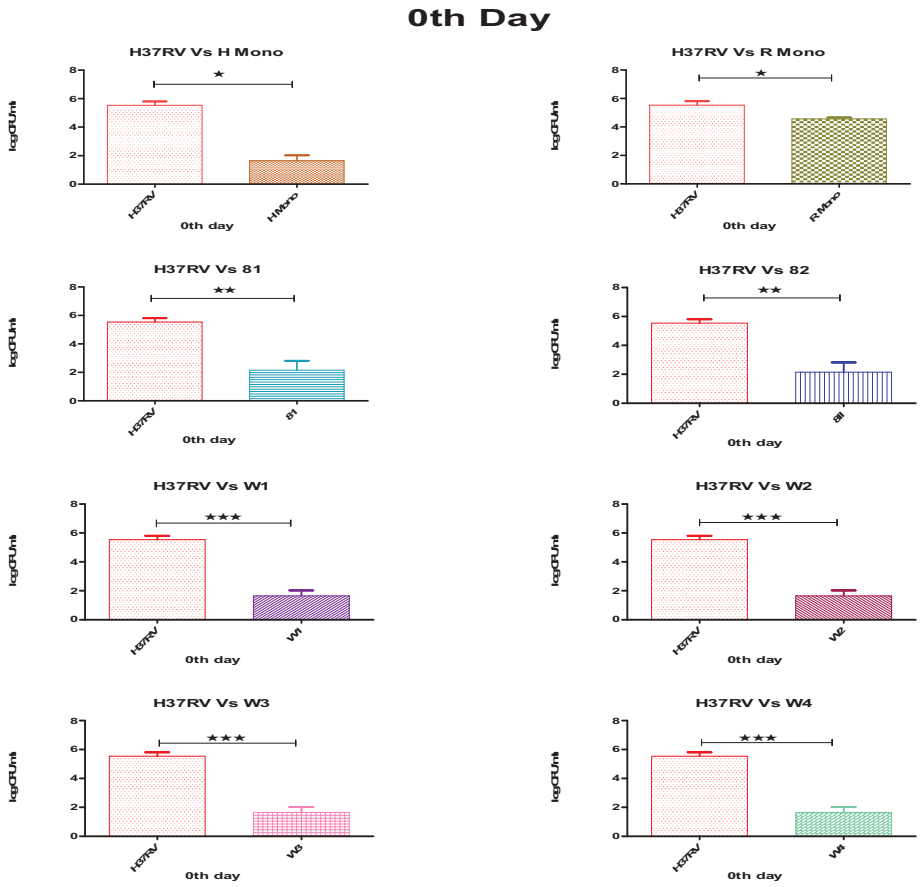
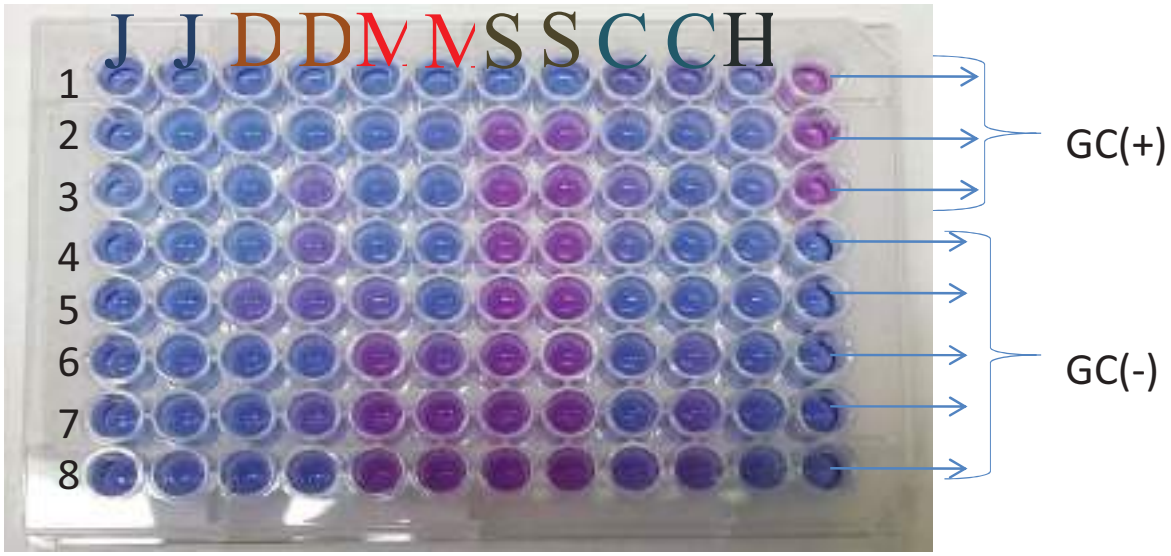


Table 50: Comparison of mean OD (600nm) and mean log CFU/ml -0th Day

Strains	Mean OD (600nm)	Mean log CFU/ml
H37Rv	0.05	5.5×10^{8}
HMono	0.03	4.6×10^{6}
RMono	0.03	4.5×10^{5}
MDR1	0.02	2.1×10^{2}
MDR2	0.02	1.6×10^{2}
Pre-XDR1	0.03	2.1×10^{2}
Pre-XDR2	0.025	1.6×10^{2}
XDR1	0.01	1.6×10^{2}
XDR2	0.02	1.6×10^{2}

Fig. 15: Representative figure for drug sensitive H37Rv strains by REMA assay



J- Bedaquiline, D- Delamanid, M- Moxifloxacin, S- Salicylanilide, C- Clofazimine, H- isoniazid.

I-7: Early bactericidal activity of anti-TB drugs

Principal Investigator	:	Dr. K. R. Uma Devi (umadevi.r@nirt.res.in).
Collaborators	:	Dr. Peter Cegielski; Dr. Sarah Smith Center for Disease control, Atlanta.
Source of funding	:	CRDF (CDC)
Period	:	2017-2019

Background and Rationale: Several MDR-TB treatment patients have DST results showing resistance to one drug within a class of drugs but susceptibility to other drugs in the same class. Examples include (1) resistance to RIF and susceptibility to rifabutin, (2) resistance to kanamycin but susceptibility to amikacin or capreomycin (or vice versa), (3) resistance to OFX but susceptibility to MFX and (4) resistance to standard concentrations of INH but susceptibility to higher concentrations. In the

preserving effective TB treatment study, the following were observed: 32% were susceptible to rifabutin but resistant to RIF, 7% were susceptible to amikacin and 41% were susceptible to capreomycin but resistant to kanamycin, 39% were susceptible to MFX but resistant to OFX and 45% were susceptible to higher INH concentrations (5.0 mcg/ml) but resistant to standard (0.2 mcg/ml) concentrations. Other investigators have reported similar results. Experts agree there are no solid clinical data for evidence-based treatment decisions in these situations.

General approach: We propose a novel approach to developing clinically relevant evidence in a short time period using a variation on the method of EBA studies. In conventional EBA studies, a newly diagnosed, sputum smear positive TB patient is treated with a single drug for the first 3 days to 14 days of treatment. Quantitative cultures are performed before treatment and several times during the period of monotherapy, comparing the change in bacterial burden (quantified as CFU per ml of sputum) with pre-treatment burden and with controls treated with a reference standard. After the period of monotherapy ends, patients receive normal multidrug therapy.

In the novel approach proposed here, smear-positive patients would be screened with Gene Xpert or another rapid, molecular test. In patients with RIF or INH resistance according to the rapid molecular test results, while completing the pre-treatment evaluation, phenotypic DST for the drugs named above will be set up right away using the direct method in the MGIT 960 automated system. Hence results would be available within 1-2 weeks. In case phenotypic DST results demonstrate any of the patterns described above, the patient will be invited to participate in the study. Time is critical at this point in the process because CFU/ml will start decreasing as soon as treatment starts. The phenotypic DST results must be available and the patient enrolled within 1 week by the time the pretreatment evaluation is completed. The study subject would be treated with one drug for 6 days, the drug to which the patient's isolate was susceptible *in vitro*. The drug's effect in vivo will be measured by serial quantitative cultures. After the 6 days are over, the patient would be treated according to national guidelines. DST will be repeated on day 6 and 2 months later to ensure there was no acquired resistance.

Primary objective:

(i) To determine the bactericidal activity of RBT in patients whose baseline DST results demonstrate susceptibility to RBT and resistance to RIF

(ii) To determine the bactericidal activity of high-dose INH in patients whose baseline DST results demonstrate susceptibility to high concentrations of INH and resistance to low concentrations of INH

(iii) To determine the bactericidal activity of MFX in patients whose baseline DST results demonstrate susceptibility to MFX and resistance to OFX

(iv) To determine the bactericidal activity of amikacin and capreomycin in patients whose baseline DST results demonstrate susceptibility to these either of these two drugs and resistance to kanamycin and

(v) To determine the bactericidal activity of RIF when an approved molecular assay demonstrates genetic mutations associated with RIF resistance, but the phenotypic testing demonstrates susceptibility to RIF

Sample size required:

15 - 20 patients for each of the five drug resistance pattern.

Methods: Potential subjects are screened with an approved rapid, molecular test to confirm the species as *M. tuberculosis* and detect mutations associated with RMP resistance and possibly INH resistance.

Among those found to have RMP or INH resistance, DST is carried out in liquid media by the direct method, which yields results in 7-14 days. Drugs to be tested include at least RMP, rifabutin, OFX, MFX, kanamycin, amikacin, capreomycin and 3 concentrations of INH in addition to any other standard anti-TB drugs such as ETH, PZA, ethionamide and PAS. The laboratory technicians are trained by a consulting microbiologist from CDC in standardized methods for counting CFU on Middlebrook agar plates and time-to-detection in MGIT 960. Quantitative bacteriology is carried out on days 0, 2, and 4, using a 16-hour overnight sputum specimen. In addition, lab technicians are also trained in rapid direct method DST using MGIT 960. Standard phenotypic DST using the indirect method for the drug used during the period of monotherapy is repeated on specimens collected in the morning after the last day of monotherapy and again 2 months later for all patients who remain culture positive at those points in time.

Results: The recruitment details of study participants are shown in Table 51.

Update on the participant screening status:

No. of valid Line probe assay positive and smear positive	:	1453
No. of direct DSTs (MGIT 960) performed	:	357
No. of valid direct DST results	:	283
No. patients with any DST pattern of study interest	:	125

Table 51: Status update of the participants recruited in the study

	High Dose INH arm	RBT arm
	MGIT results of INH-R@0.1 mcg & INH-S@0.4 or 2.0 mcg	MGIT results of RIF-R & RBT-S
No. of patients with DST results of interest	125	4
No. of patients who met baseline clinical eligibility	6	1
No. of patient consented to study	6	1
No. of patients started on monotherapy	6	1
No. of patients for whom 5 pooled sputum collections have been completed	6	1
Completed monotherapy	6	1
Completed 1 & 2 month follow-up DST	3	0

So far, seven patients were recruited in the study and 1st and 2nd month follow up completed for 3 patients.

The follow up DST results for these participants are provided in Table 52.

Table 52: DST results

Patient Number	DST for first Month follow up specimen			DST for 3rd Month follow up specimen econd Month		
	INH 0.1	INH 0.4	INH 2.0	INH 0.1	INH 0.4	INH 2.0
1	Resistant	Sensitive	Sensitive	Resistant	Sensitive	Sensitive
2	Resistant	Sensitive	Sensitive	Resistant	Sensitive	Sensitive
3	Resistant	Sensitive	Sensitive	Resistant	Sensitive	Resistant

The study is ongoing.

I-8: Whole genome sequencing and transcriptome analysis of *M. tuberculosis* clinical isolates from bovine and human origin

Mentor	:	Dr.P.Kannan (email: kannanp@nirt.res.in)
Principal Investigator	:	Dr. Ahmed Kabir Refaya (email: refaya@nirt.res.in)
Source of funding	:	Extramural – DST- SERB (N-PDF)
Study Period	:	2017 - 2019

Background: Bovine TB is one of the important areas of concern because of the serious impact it causes on economic losses and public health. Reverse zoonosis due to *M. tuberculosis* in bovine is increasingly being reported around the world. It was earlier believed that *M. tuberculosis* is not capable of establishing active infection in bovine models and there were many reports substantiating the fact that bovine animals are resistant to *M. tuberculosis* infection. But in the recent years, there are various reports of *M. tuberculosis* being isolated in animals

such as cattle, dogs, cats and even in wildlife reservoirs. The TB epidemiological survey of cattle farms in Chennai city identified a significant incidence of *M. tuberculosis* infection in cattle. This situation warrants an urgent need of understanding the reason behind *M. tuberculosis* transmission from human to cattle. The transmission of this pathogen could be possible only by genetic alterations in *M. tuberculosis* which has resulted in establishing active TB infection in cattle and other animals. Now, the situation is alarming because of increasing prevalence of *M. tuberculosis* infection in cattle which opens up a new area for consideration under TB control program. Transmission of these *M. tuberculosis* strains between cattle and livestock workers are considered as epidemic and the whole genome and transcriptome sequencing data of these clinical isolates will provide valuable resources to understand the transmission and virulence mechanism of TB.

Objectives: (i) To identify the drug susceptibility patterns of the cattle and human *M. tuberculosis* clinical isolates

(II) To study the genomic patterns of *M. tuberculosis* clinical isolates from cattle and livestock workers using whole genome sequencing

(iii) To assess the host transcriptome response generated by cattle and human *M. tuberculosis* clinical isolates in peripheral blood mononuclear cells (PBMCs) of cattle and human respectively and

(iv) To validate the top 10 differentially expressed genes using quantitative real-time PCR (qPCR) in PBMCs

Methodology:

- **Drug Susceptibility Testing:** DST was performed for 12 *M. tuberculosis* clinical isolates from bovine and humans with standard concentrations of first line drugs using automated liquid medium-based system Bactec MGIT960 (Becton Dickinson).
- **DNA Preparation and Whole Genome Sequencing:** Six *M. tuberculosis* strains each from bovine and human origin were grown and genomic DNAs was extracted by hexadecyl- trimethiammonium bromide (CTAB) method. Sequencing libraries were prepared with TrueSeq Nano DNA library preparation kit according to the manufacturer's recommendation. Paired end whole genome sequencing was performed in Illumina HiSeq X Ten system. The data generated were mapped with *M. tuberculosis* reference genome H37Rv and bioinformatics analysis was

performed to identify SNPs, InDels and Phylogeny.

Results: A total of 12 samples were collected and analyzed. Six of these isolates were from cattle and 6 isolates from human (Animal Handlers). Majority of the samples were collected from the organized cattle farms around Chennai (Table 53). Initially these samples were subjected to DST with standard concentrations of first line drugs using Bactec MGIT960 system. All the strains were identified to be sensitive for the first line drugs except 143 which was resistant to both INH and RMP and hence classified as MDR strain. Another strain 129 showed resistance to EMB and RMP (Table 53). Both the strains were isolated from cattle.

Whole genome sequences of all the 12 isolates were captured and used for further analysis. Among the 12 isolates, 10 of the samples were identified to be *M. tuberculosis* strains belonging to a single lineage L1 (East African Indian) strain. The remaining 2 isolates (129 & 144) were identified to be *M.orygis* belonging to the MTBC complex and this requires more in-depth and detailed analysis to proceed further (Table 53).

The analysis was carried forward with the 10 sequences identified as *M. tuberculosis*. The sequences were further screened for contamination with Kraken software before mapping it with the *M.tuberculosis* reference genome $H_{37}Rv$ (NC_000962) using BWA and Samtools. The BAM files generated were processed with Genome Analysis Toolkit and QualiMAP to generate a Variant Calling File (VCF). The VCF files were further filtered with a python script based on Qual Scores >=50; Mapping Quality >=30; Read depth >=5; Alternative Base call >=0.75 for 1 read in each strand.

The filtered VCF files were used for identifying the lineage of *M. tuberculosis* strains based on Region of Difference and single nucleotide polymorphisms (SNPs). These SNPS were further matched with existing resistance database and resistance prediction was done for all the samples. The Bedtools were used to mask the PE/PPE genes in the BAM file of each sample and a pseudo -ref sequence was generated for all the 10 sequences by replacing all the SNPs of the reference sequence with the sample sequence. These pseudo- ref sequences was then compared with each other to identify the transmission of infection between cattle - cattle and cattle-human. Transmission is considered possible when the difference in SNPs between the strains <=10. A

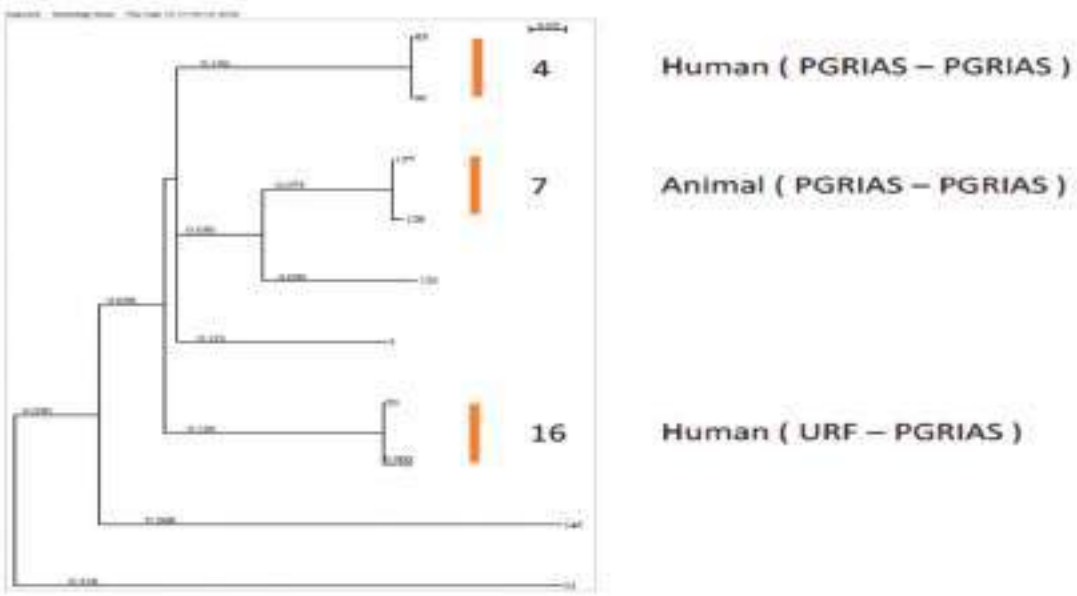
phylogenetic tree was generated based on the SNPs (Fig. 16).

The difference between the *M.tb* strains isolated from bovine and human origin was approximately 150-350 SNPs. Further analysis is currently being carried out to identify the mutations and its relatedness for the adaptability of the strains in selection of the host.

Table 53: Data collected from sample subjected to whole genome sequencing

Strain No.	Farm	Origin	MTBC complex	Lineage	Drug resistance MGIT	Drug resistance WGS
3	Organized Farm 1	Human	*M.tuberculosis*	L1	Sensitive	Sensitive
29	Organized Farm 1	Human	*M.tuberculosis*	L1	Sensitive	Sensitive
69	Organized Farm 2	Human	*M.tuberculosis*	L1	Sensitive	Sensitive
85	Organized Farm 2	Human	*M.tuberculosis*	L1	Sensitive	Sensitive
90	Organized Farm 2	Human	*M.tuberculosis*	L1	Sensitive	Sensitive
91	Organized Farm 2	Human	*M.tuberculosis*	L1	Sensitive	Sensitive
126	Organized Farm 2	Bovine	*M.tuberculosis*	L1	Sensitive	Sensitive
127	Organized Farm 2	Bovine	*M.tuberculosis*	L1	Sensitive	Sensitive
128	Organized Farm 2	Bovine	*M.tuberculosis*	L1	Sensitive	Sensitive
129	Organized Farm 2	Bovine	*M.orygis*	NA	Emb^R, Rif^R	NA
143	Organized Farm 1	Bovine	*M.tuberculosis*	L1	Inh^R, Rif^R (MDR)	Inh^R, Rif^R (MDR)
144	Organized Farm 1	Bovine	*M.orygis*	NA	Sensitive	NA

Fig. 16: Phylogenetic tree based on SNP difference



The study is ongoing.

I-9: Molecular analysis of monocyte subsets from humans infected with *M. tuberculosis*

Principal Investigator	:	Dr. Ramalingam B. (ramalingam.b@nirt.res.in)
Source of Funding	:	DBT
Study Period	:	2015-2020

Background: The mechanisms for mononuclear phagocyte-mediated protective immunity against *M. tuberculosis* infection have not yet been completely deciphered. Multiple changes have been documented in both the innate and adaptive immune responses with mononuclear phagocytes. Monocytes differentiate into macrophages and dendritic cells, with macrophages being a key component of innate immune system, while dendritic cells interface with adaptive immune system and modulate immune responses. Human blood monocytes are heterogeneous and can be conventionally distinguished into two major subsets based on expression of CD16 and CD14. Recently, new official

nomenclature has subdivided human blood monocytes into three subsets based on these markers, namely: (i) Classical (CD14++ CD16-); (ii) Non-classical (CD14+ CD16++) and (iii) Intermediate monocytes (CD14++ CD16+). It will be very useful to generate data about the potential roles of each of the monocyte subsets by studying their phenotypic and functional differences along with their transcriptome analysis during different stages of the disease such as active disease, latent TB infection, resistant TB infection and disease relapse.

Specific Aims:

(i) To phenotypically characterize and analyze the different monocyte subsets from PBMC of TB patients, by immunophenotyping based on cell surface marker expression and comparison to normal healthy subjects

(ii) To study the transcriptome profiles of monocyte subsets within the study subjects and to validate the differentially expressed genes by quantitative real time PCR and

(iii) To identify the most promising candidate biomarker genes and pathway networks by comparing the transcriptomic profile of the monocyte subsets from active TB patients to the healthy subjects

Study subjects:

Healthy subjects: Asymptomatic for TB with normal chest X-ray, and negative for Interferon Gamma Release Assay (IGRA).

Latent TB infection: Asymptomatic, healthy adults with positive IGRA and normal chest X-ray.

Active TB cases: PTB patients with typical clinical and radiological presentation and bacteriologically confirmed by Zielhl-Neelsen staining of sputum smear and culture positivity for *M. tuberculosis*, seronegative for HIV. Heparinized blood is collected from these patients before the start of ATT. MDR-TB patients are identified by drug sensitivity test for INH & RMP.

Experimental approach: As per Aim 1, in order to determine the cell phenotype of human monocyte subsets together with dendritic cells, we have used flow cytometric analysis of peripheral blood from the above mentioned study subjects. Heparinized blood is stained for different panels of monoclonal antibodies tagged with fluorescence for monocytes and dendritic cells. Antibody cocktail is prepared for individual subset and added to 250 µl of human whole blood and incubated for 30 minutes. Following antibody binding, lysis of RBC is performed by adding BD FACS

lysing solution and incubated for 10 minutes, and then washed with 2ml of PBS and the cells were pelleted upon centrifugation. The final pellet is fixed with 2% paraformaldehyde solution and stored at 4^{o}C till acquisition. The fixed samples are acquired in FACS Canto II (BD, USA) instrument using FACS DIVA software (version 6.0) and the data are analyzed by Flowjo software.

For Aim 2, peripheral blood mononuclear cells are isolated from the whole blood of study subjects by using Ficoll density centrifugation, using sepmate tubes. Monocytes are sorted by magnetic beads and stored together with RLT buffer at -80 degree for RNA Seq studies.

Results:

Table 54: Demographic data for the recruited study subjects

Study Demographics	HC	LTB	PTB	DR-TB
No of subjects recruited	35	21	50	11
Gender (Male/Female)	17/18	9/12	35/15	8/3
Age (years) (Median Range)	40 (20-57)	33 (21-55)	44 (21-65)	41 (18-59)

Overall, target sample size for the study is 50 in each group. With reference to Aim 1, we have recruited around 117 samples so far in the study (Table 54), out of which 35 are healthy controls (HC), 21 are LTB infected individuals and 50 are PTB diseased individuals and 11 individuals are resistant to first line drugs (MDR-TB). Increased monocyte numbers and monocyte to lymphocyte ratio could be observed among patients with active disease when compared to healthy contacts.

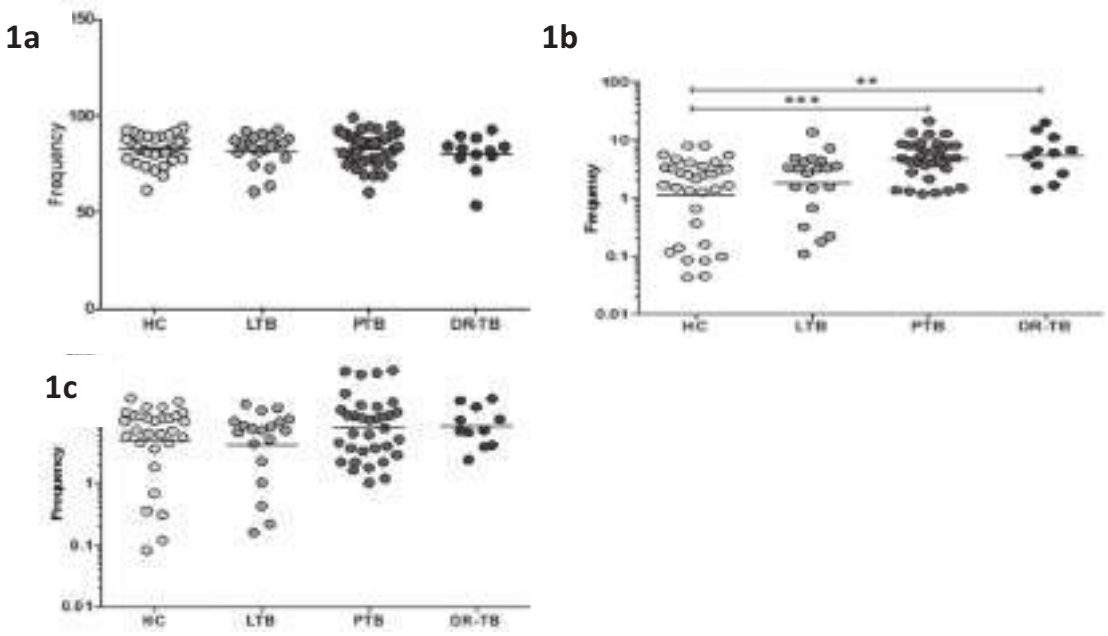
When subsets of monocytes were analyzed by immunophenotyping studies, significant difference could be observed within the intermediate subsets between HC vs PTB and HC vs MDR-TB. No difference was observed with classical and non-classical monocyte subsets among four groups (Table 55 and Fig. 17).

Further studies will focus towards carrying out RNA sequencing, network and pathway analysis for the sorted monocytes from four groups. The study is ongoing.

Table 55: Mean frequencies of monocyte subsets (Mean ± SD)

Monocytes	HC	LTB	PTB	DR-TB
Classical Monocytes	82.54 ± 0.21	82.5 ± 0.40	78.11 ± 0.39	82.92 ± 0.54
Intermediate Monocytes	2.3 ± 0.06	3.12± 0.15	4.12 ± 0.09	5.58 ± 0.41
Non-classical Monocytes	7.5 ± 0.18	6.69 ± 0.27	10.18 ± 0.34	8.41 ± 0.58

Fig. 17: Frequency distribution of monocyte subsets



I-10: Vitamin D binding protein gene polymorphisms in PTB

Principal Investigators	:	Dr. M.Harishankar; Dr. Ramalingam B. (email:ramalingam.b@nirt.res.In; harishankarm@nirt.res.in)
Source of Funding	:	Intramural, NIRT/ DBT.
Study Period	:	2018-2019

Background: The bioavailabity of 25(OH)D_3 is determined by vitamin D binding protein (VDBP) levels, which binds to vitamin D and its plasma metabolites and transports them to target tissues. Polymorphisms in VDBP are shown to alter its binding affinity for vitamin D and susceptibility to TB.

Aim:

(i) To find out whether allele and genotype frequencies of exon 11 rs7041(T/G) and rs4588 (C/A) of VDBP) gene variants are associated with susceptibility or resistance to PTB in healthy controls and PTB patients

Methodology: Isolated Genomic DNA by simple salting out procedure is used for genotyping. Both the VDBP polymorphisms will be genotyped by polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP) method.

Study subjects:

The study population consists of:

Healthy controls – 100

PTB patients – 100

Genomic DNA extraction: The human genomic DNA is isolated from the granulocytes (white blood cells) stored with 0.5 M EDTA in RPMI-1640 tissue culture medium at -80°C. The extraction is done by a simple salting out procedure. Finally, the DNA is resuspended in Tris-EDTA (TE) buffer (pH 8.0) and stored at -80 ºC until use. Stored genomic DNA is used for VDBP genotyping by PCR-RFLP method.

rs7041 genotyping: The PCR product size of 483bp is digested with the restriction enzyme *Hae*III at 37ºC for 16hrs. The homozygous frequent genotype "GG" containing the restriction site yields 2 bands of 297 bp and 186 bp sizes. The homozygous infrequent genotype "TT" which lacks the restriction site yields a single band of 483 bp size. The heterozygous genotype "GA" yields 3 bands of 483 bp, 297 bp and 186 bp sizes.

rs4588 genotyping: The 483bp PCR product is digested with the restriction enzyme *Sty*I at 37ºC for 16hrs. The homozygous frequent genotype "CC" which lacks the restriction site yields a

single band of 483 bp size. The homozygous infrequent genotype "AA" containing the restriction site yields 2 bands of 305 bp and 178 bp sizes. The heterozygous genotype "CA" yields 3 bands of 483 bp, 305 bp and 178 bp sizes.

Analysis: The allele, genotype and haplotype frequencies will be estimated using SNP stats software. Pearson $\chi 2$ test will be used to find out whether the genotype frequencies are in Hardy–Weinberg equilibrium. The 2X2 table, p-value with Yates correction and odds ratio (OR) with 95% confidence intervals will be analyzed using epi info version 6.04. For genotypic associations, p-values with OR adjusted for gender and age will be calculated by logistic regression under co-dominant, dominant, recessive and over-dominant models using the online SNP stats program. The best fitting model of association will be determined using the Akaike information criterion and Bayesian information criterion provided by the software. The model with lowest AIC and BIC values will be considered as the best fitting model. A p value ≤0.05 will be considered statistically significant.

The study is in progress.

I-11: A novel molecular approach for diagnosis of common non- tuberculous mycobacteria

Principal Investigator	:	Dr. K.R. Uma Devi (email: umadevi.r@nirt.res.in)
Source of funding	:	ICMR Intramural
Study period	:	2017-2018

Background: Non-tuberculous Mycobacteria (NTM) which contains more than 150 species, belongs to the genus *Mycobacterium*. Among those 150 species, more than 45 species are of clinical importance. Previous studies reported that incidence of disease due to NTM has increased worldwide including India. In India, it has already been shown that NTM prevalence rate is ranging from 0.5 % to 8.5%. It also shows that prevalence of NTM is high in south India, where species such as *M. kansasii, M. chelonae, M. fortuitum, M.*

avium, M. intracellulare and *M. abscessus* are reported as clinically relevant.

The clinical presentation of pulmonary disease due to NTM may or may not resemble PTB and extrapulmonary TB. Since many of the NTM are not amenable to routine anti-TB therapy, it is important to correctly diagnose the causative agents.

Currently, the diagnosis of NTM infection relies on conventional phenotyping, biochemical, HPLC, Polymerase chain reaction and Mass spectrometry methods. Previous reports have stated that, these methods show either poor accuracy and / or long period of laboratory testing. The above described methods used for identification of NTM species has got its own disadvantages. In this context, it is mandatory to find alternatives to achieve an accurate identification affordable for majority of laboratories. Previous studies have also shown that to accomplish an accurate identification of NTM at the species level, more than one gene can be used as a genetic marker.

Objectives:

(i) To identify species-specific genome sequence among different species of NTM strain using Dot-plot matrix analysis and

(ii) To develop and evaluate an in-house PCR based method for the identification of NTM species

Methodology:

Dot plot analysis using Microbial Genome Comparative Tool (MGCT): It is a graphical method which allows the comparison of two whole genomes and identify the region of similar and unique genome sequence. This in house comparative tool was developed by AU-KBC bioinformatics centre and it will be outsourced to them. The FASTA sequence for all *M.* species will be retrieved from NCBI database and these sequences will be used for constructing a BLAST protein database for each species. After construction of the database, NCBI stand alone blast will be run against H37Rv. The output file will be generated for each species and will be stored as BLAST output. A list of unique gene sequences against H37Rv is obtained. In order to reconfirm, secondary confirmation will be done by PROTEIN BLAST. The sequences given by MGCT will be manually withdrawn from NCBI for different NTM strains and PROTEIN-BLAST will be carried out. Based on the results given by PROTEIN BLAST, an algorithm will be created to identify the unique genes.

Preparation of Reference NTM strains: All isolates are sub-cultured on Lowenstein–Jensen agar (LJ medium) and the organisms are incubated at 37°C, with the exception of *M. ulcerans* and *M. marinum*, which are incubated at 30°C, and *M. xenopi* are incubated at 42°C for 4 weeks.

Isolation of DNA from cultures by modified heat killed method: One loop full of culture is taken and dissolved in 500µl of TE buffer and kept at 80ºC for heat killing of bacteria. To this, 500µl of TE and 300µl of Triton X 100 mix is added and mixed slowly to avoid any froth formations. This is incubated at 80ºC for 35 min and centrifuged at 7,000 rpm for 15 min. The supernatant is transferred into a fresh vial and this mixture is used for PCR amplification.

Polymerase chain reaction: Chromosomal DNA of 15 NTM strains are extracted using modified boiling method. PCR of the species-specific genome sequence as identified by MGCT is amplified. The PCR products are detected by 2% agarose gel electrophoresis.

Results:

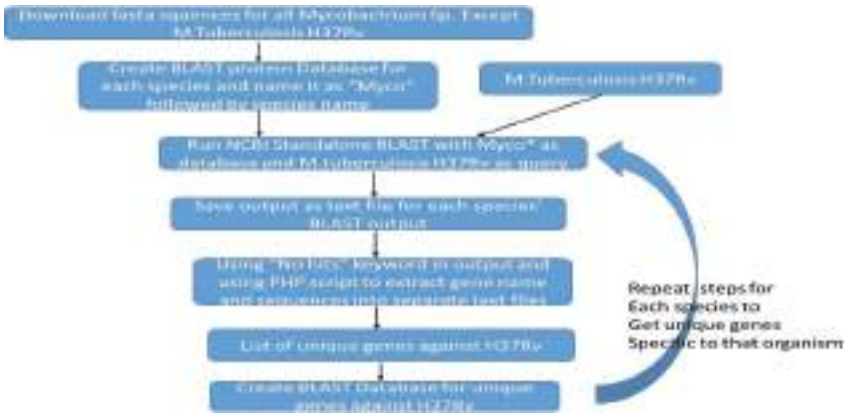
Genome Difference analysis by MGCT software:

The complete genome sequences of 15 reference NTM strains were retrieved from NCBI databases in FASTA format and were compared using the MGCT software to find out species specific genes as shown in Fig. 18 Table 56 shows a representative list of genome sequence id identified by MGCT software. We identified a total of 19 genome sequences from *M. avium*, 10 genome sequences from M. *africanum*, 1 from *M. abscessus*, 15 from *M. intracellulare*, 8 from *M. fortuitum*, 12 from *M. kansasii*, 29 from *M. chelonae*, 5 from *M. gordonae*, 7 from *M. simiae*, 8 from *M. bovis*, 9 from *M. smegmatis*, 6 from *M. xenopi*, 7 from *M. ulcerans*, 5 from *M. marinum* and 15 from *M. chitae*. Among the genome seqences identified, were hypothetical proteins, membrane proteins, putative proteins, PE-PGRS family proteins, cyclase, cytochrome, glycine rich proteins, acyl transferase, DNA replication/ repair proteins recF, phosphogluconate dehydrogenase, pyruvate dehydro-genase subunit B, CoA transferase, alanine racemase, DnaJ protein, recA protein, heat shock protein, elongation factor Tu partial, PE family proteins, haloacid dehalogenase, fattyacid CoA ligase, ABC transporter, ribosome biogenesis, mammalian cell entry protein, peptidase, diacylglycerol, O acyltransferase etc.

Table 56: List of unique gene Id obtained by MGCT software

M.abscessus	*M.africanum*	*M.chelonae*	*M.fortuitum*	*M.intracellulare*	*M.marinum*	*M,ulcerans*	*M.avium*
YP_001700938.1	CCC25682.1	WP_046252071.1	WP_054600674.1	YP_005335969.1	YP_001848863.1	YP_904815.1	ABK67736.1
	CCC25452.1	WP_046252133.1	WP_054600697.1	YP_005338140.1	YP_001849143.1	YP_905253.1	ABK69039.1
	CCC25579.1	WP_030093447.1	WP_054600650.1	YP_005335727.1	YP_001850256.1	YP_905380.1	ABK68304.1
	CCC26111.1	WP_030093448.1	WP_054600651.1	YP_005335802.1	YP_001851092.1	YP_905999.1	ABK69505.1
	YP_004723775.1	WP_030093459.1	WP_054600654.1	YP_005335807.1	YP_001852139.1	YP_906690.1	ABK67535.1
	YP_004723776.1	WP_030093470.1	WP_054600660.1	YP_005335897.1		YP_907376.1	ABK67986.1
	YP_004724668.1	WP_046251970.1	WP_054600661.1	YP_005336104.1		YP_907996.1	ABK69264.1
	YP_004725286.1	WP_046251990.1	WP_054600681.1	YP_005337861.1			ABK68638.1
	YP_004725287.1	WP_046252005.1		YP_005338139.1			ABK69185.1
	YP_004725288.1	WP_046252006.1		YP_005338373.1			K66296.1
		WP_046252008.1		YP_005338668.1			ABK66170.1
		WP_046252009.1		YP_005339027.1			ABK66894.1
		WP_046252010.1		YP_005339304.1			ABK67183.1
		WP_046251985.1		YP_005339458.1			ABK65600.1
		WP_046252016.1		YP_005339473.1			ABK66617.1
		WP_046252030.1					ABK66899.1
		WP_046252034.1					ABK68803.1
		WP_046252037.1					ABK68324.1
		WP_046252038.1					ABK66418.1
		WP_046252046.1					
		WP_046252050.1					
		WP_046252051.1					
		WP_046252053.1					
		WP_046252057.1					
		WP_046252066.1					
		WP_046252075.1					
		WP_046252078.1					
		WP_046252079.1					
		WP_046252081.1					

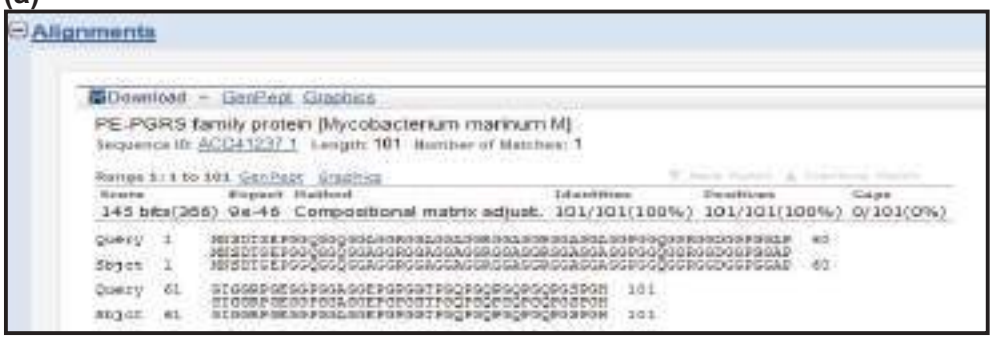
Fig. 18: Schematic representation of gene identification using MGCT software



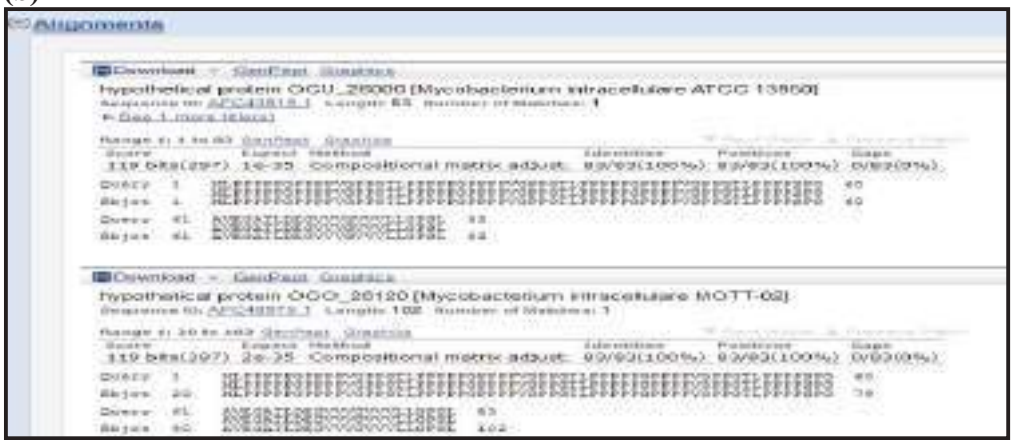
Identification of Unique genes using BLASTp- A secondary confirmation: The unique genome sequences as identified by MGCT software were subjected to manual confirmation using BLASTp tool. PSI-Blast search was performed for each unique genome sequence against 15 most prevalent NTM species. Figure 19 shows that the top BLAST hit represents a sequence similarity less than 35%; query cover 100% between query and the subject sequence was considered positive for unique gene. Upon confirmation, a BLAST algorithm (Fig. 20) was created for each species.

Fig. 19: BLAST alignment results (a) *M. marinum*, (b) *M. intracellulare*, (c) *M. ulcerans*, (d) *M. abscessus*, (e) *M. xenopi*, (f) *M. avium*, (g) *M. fortuitum* and (h) *M. chelonae*.

(a)



(b)



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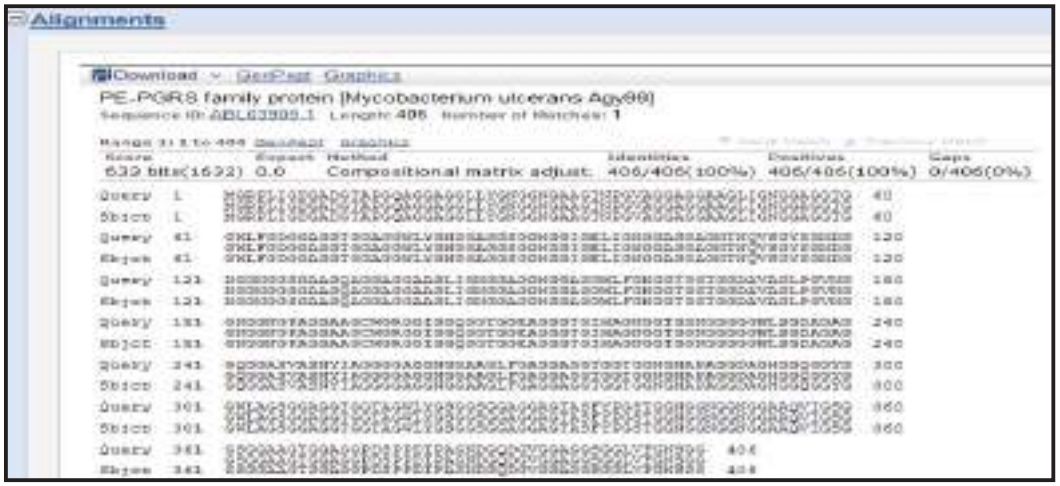
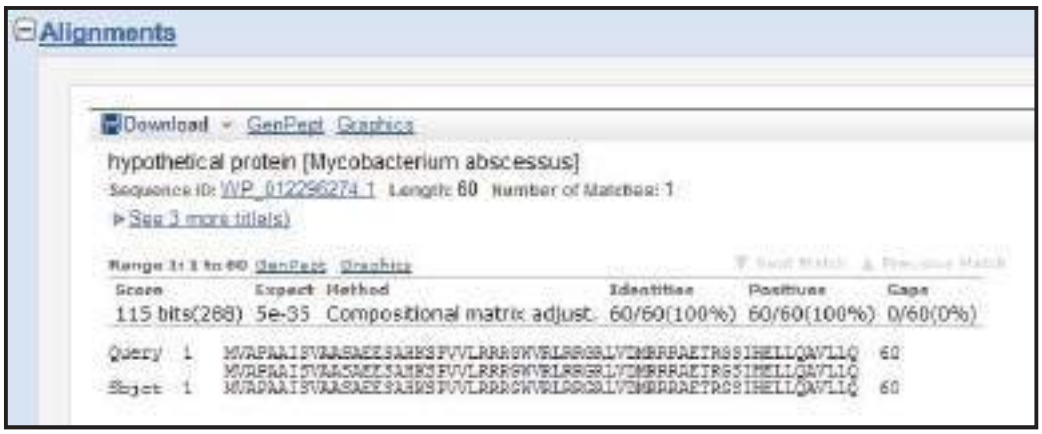
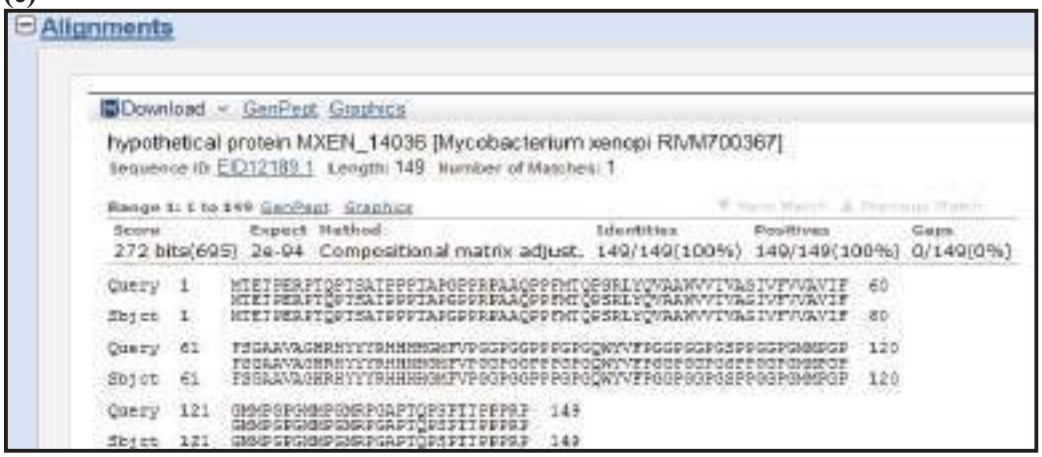

Alignments
Download GenPept Graphics
PE_PGRS family protein [Mycobacterium ulcerans Agy99]
Sequence ID: ABL03989.1 Length: 406 Number of Matches: 1
Score Expect Method Identities Positives Gaps
639 bits(1693) 0.0 Compositional matrix adjust. 406/406(100%) 406/406(100%) 0/406(0%)

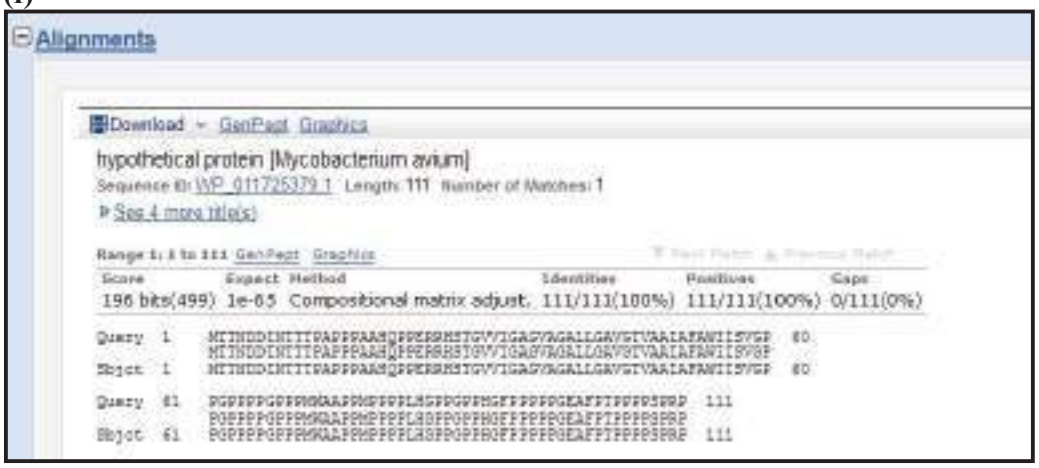
(d)


Alignments
Download GenPept Graphics
hypothetical protein [Mycobacterium abscessus]
Sequence ID: WP_012296274.1 Length: 60 Number of Matches: 1
See 3 more title(s)
Score Expect Method Identities Positives Gaps
115 bits(288) 5e-35 Compositional matrix adjust. 60/60(100%) 60/60(100%) 0/60(0%)
Query 1 MVRPAAISVAASAEESAAWSFVVLRRRGWVRLRRGRLVIMRRRAETRGSIHELLQAVLLQ 60
Sbjct 1 MVRPAAISVAASAEESAAWSFVVLRRRGWVRLRRGRLVIMRRRAETRGSIHELLQAVLLQ 60

(e)


Alignments
Download GenPept Graphics
hypothetical protein MXEN_14036 [Mycobacterium xenopi RIVM700367]
Sequence ID: EID12189.1 Length: 149 Number of Matches: 1
Score Expect Method Identities Positives Gaps
272 bits(695) 2e-94 Compositional matrix adjust. 140/140(100%) 140/140(100%) 0/140(0%)

(f)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hypothetical protein [Mycobacterium avium]
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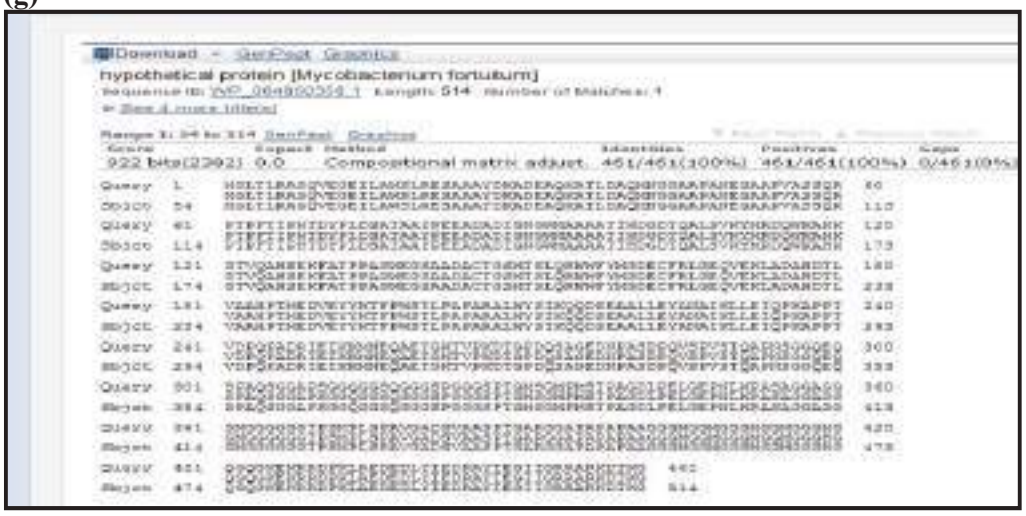
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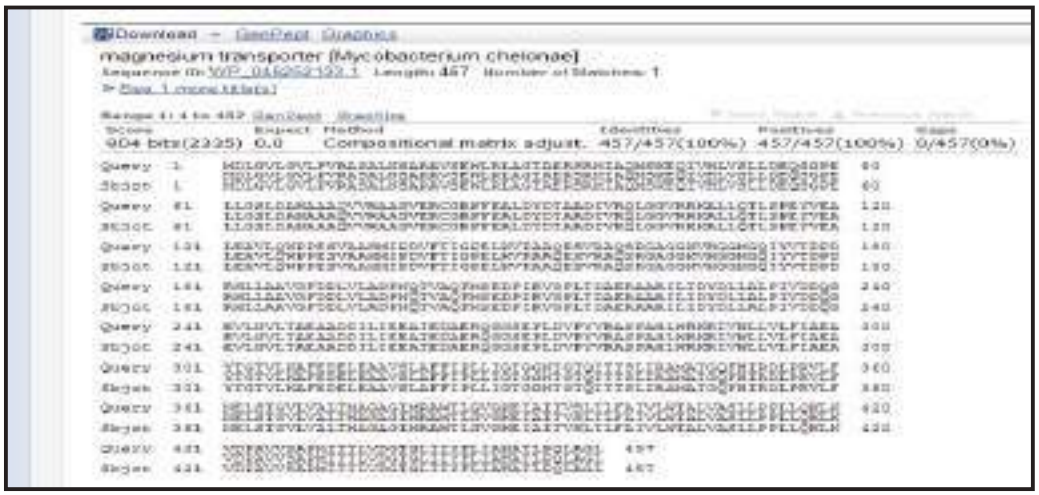
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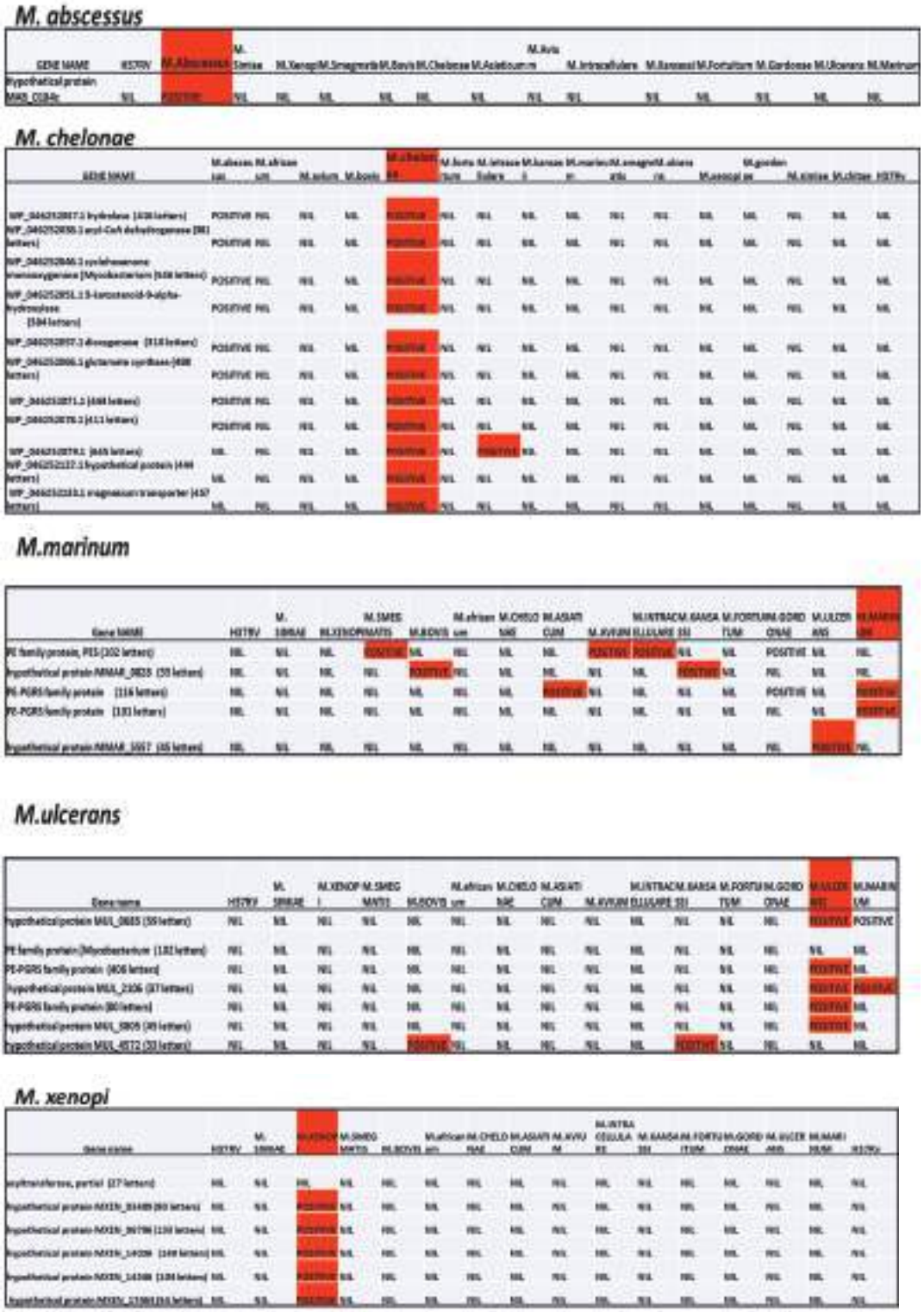
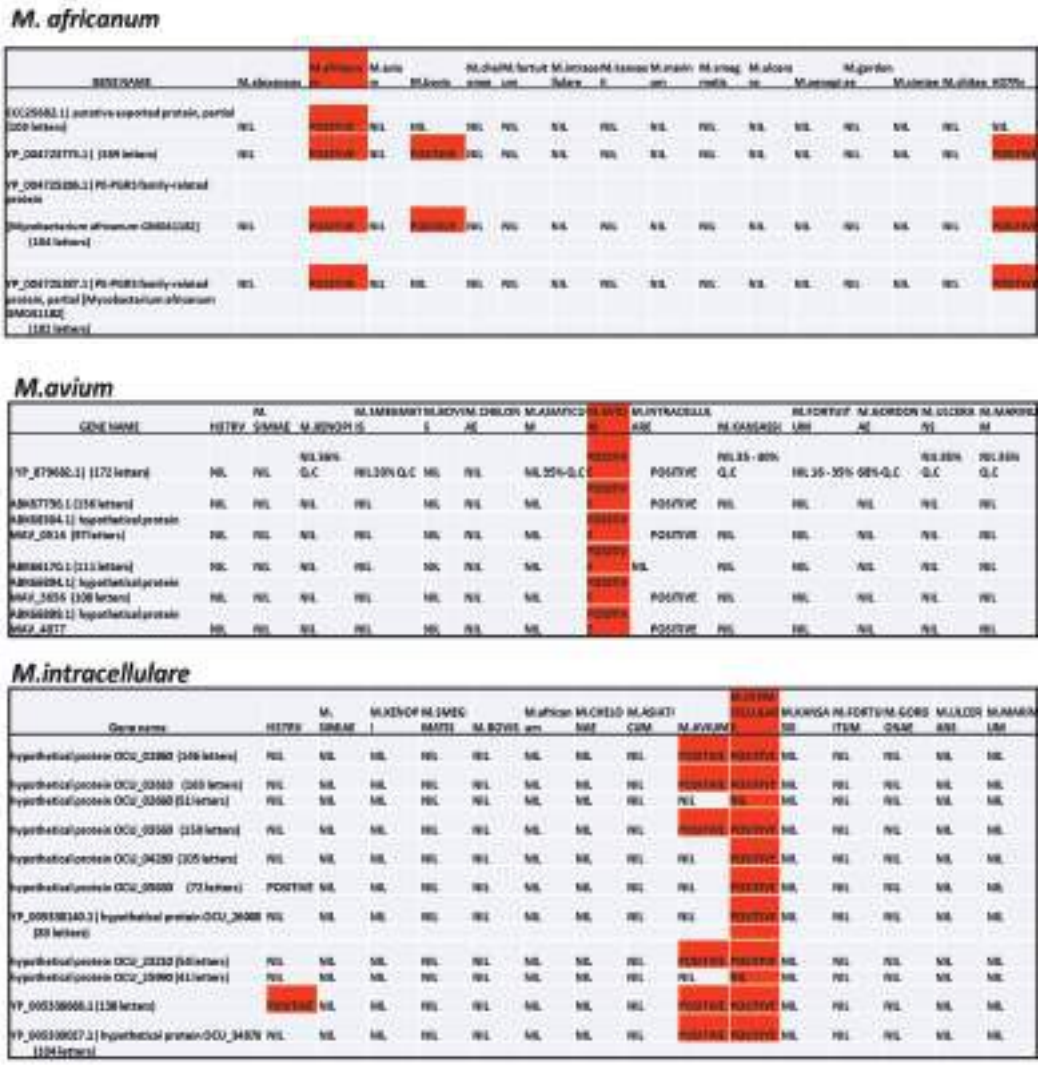
Fig. 20: (Title ????)





Identification of reference NTM strains using species specific primers: After screening the potential unique genes, primers were carefully designed in such a way that each primer gives product of different size to avoid ambiguous results. Genomic DNA from reference strains were used as template for PCR-based detection. Amplification of the genomic DNA using primers specific for *M. abscessus* under conditions shown in Table 57 produced a product of length 182bp (Fig. 21). It also shows that the primer did not amplify sequences of other species such as *M. avium, M. intracellulare and M.fortuitum*. Similarly, amplification of the genomic DNA using primers specific for *M. marinum* under conditions shown in Table 56 produced a product of length 341bp (Fig. 22). The figure also shows that the primer did not amplify sequences of other species.

Table 57: PCR conditions for amplification using species specific primer

Contents	Concentration
PCR Mastermix	10µl
Forward primer	1µl
Reverse primer	1µl
Template DNA	3µl
Molecular grade water	10µl

PCR conditions- 35 cycles	Temperature	Duration
Initial denaturation	94^0C	10 min
Denaturation	94^0C	30 s
Annealing	56^0C	30s
Extension	72^0C	1 min
Final Extension	72^0C	10 min

Fig. 21: Identification of reference strain *M. abscessus* using unique primers

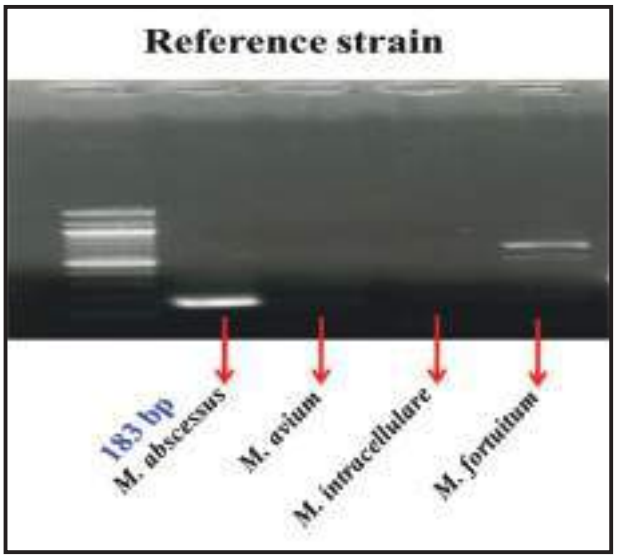
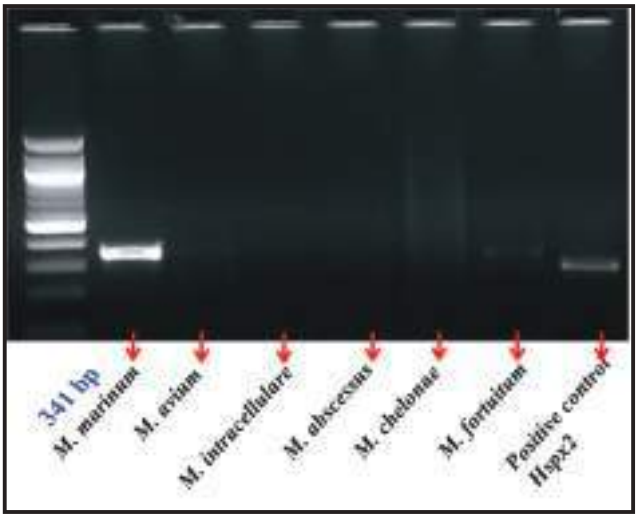


Fig. 22: Identification of reference strain *M. marinum* using unique primers



The study is ongoing.

I-12: Protecting and improving public health globally: Building laboratory, surveillance and workforce capacity to detect, respond to and prevent DR-TB in India

Principal Investigator	:	Dr K R Uma Devi (email: umadevi.r@nirt.res.in)
Collaborators	:	Central TB Division, NITRD and NIRT
Source of funding	:	CDC India
Study period	:	2015-2020

Background: The proposed activities in this project will build capacity to prevent, detect, respond to, and control the growing problem of DR-TB in India and prevent antimicrobial resistance, and strengthen surveillance systems, national laboratory systems, and workforce development.

Developing a facility capable of Next Generation Sequencing (NGS) will substantially strengthen overall laboratory capacity in India and this will contribute to prevention and control of DR-TB. Current methods for

detection of TB drug resistance are limited either by a long delay between sample submission to result (typically 2-6 weeks for culture based methods) or by number of drugs that can be assessed (1 for GeneXpert to 2 for Hain Line Probe Assay). Early detection of the specific pattern of drug resistance is critical to ensure that patients are appropriately treated, and that they do not develop additional acquired resistance and also do not transmit DR-TB to others. NGS capacity is key:

(1) To understand the molecular epidemiology of DR TB in India – thus far the database in India is very limited;

2) To assess the existing methods for molecular detection of TB and drug resistance;

3) To develop new molecular methods for detection of TB and drug resistance;

4) To contribute to the global database of drug resistance mutations by contributing matched molecular, clinical, and phenotypic drug susceptibility testing data and

5) To assist in outbreak investigations by supporting molecular epidemiology studies.

Aims and objectives:

(i) To develop laboratory capacity to conduct NGS and perform NGS for the nationally representative DR-TB samples

(ii) To develop a real-time laboratory-based surveillance for drug-resistant TB

(iii) To develop the capacity to conduct TB outbreak investigations and

(iv) To conduct training on MDR-TB management for clinical providers

Progress of activities performed at NIRT:

<u>Activity 1:</u> Develop laboratory capacity for next generation sequencing (NGS):

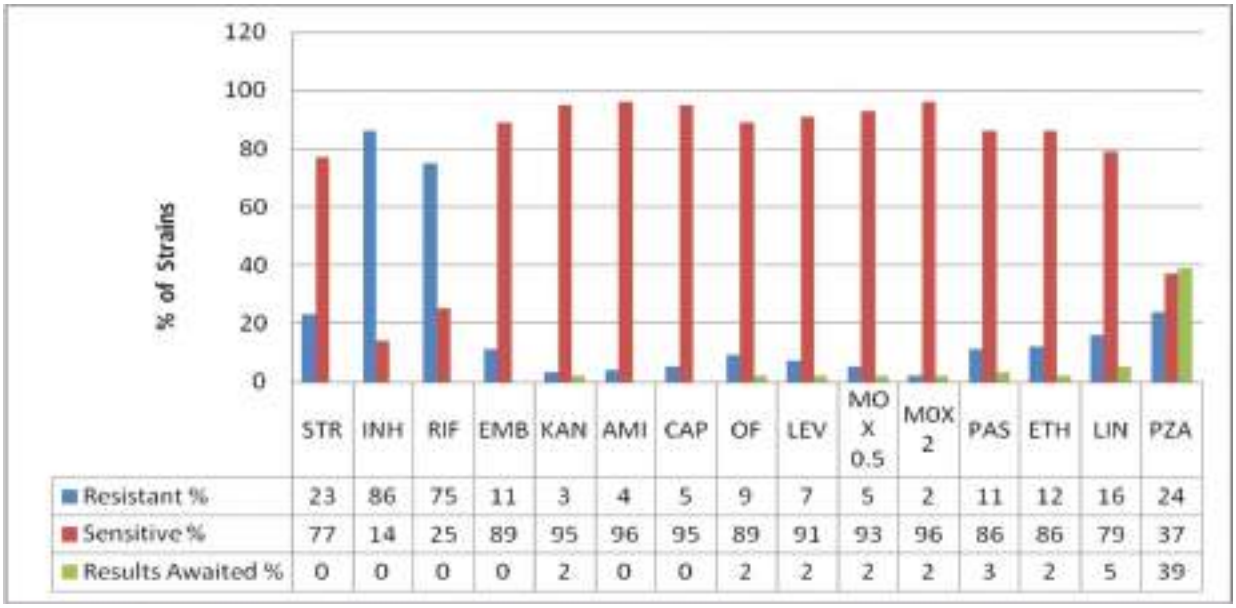
- Established the whole genome facility and analysis capabilities for *M. tuberculosis* at NIRT.
- Conducted an International symposium on "Application of AMD in Public Health Partners in India".
- The IQA and EQA validation plans for NGS sequencing and MGIT DS has been developed and approved by the CDC technical officers.
- For the prospective drug resistant strain collection for a national representative of DR-TB strains, a list

of IRLs has been identified by Central TB division.

DST pattern for MDR-TB culture isolates from Chennai collected for outbreak investigations:

As a part of outbreak investigations and to validate NGS using the DST by Bactec MGIT 960, 158 MDR-TB isolates that were collected in Chennai during the years 2013 and 2014 were received. However, out of these 158 stored isolates, 74 isolates have been retrieved so far. These isolates were then subjected to phenotypic DST for first line and second line drugs at specified concentrations. Phenotypic DST has been completed for 57 isolates. Of the 57 isolates, 86 % and 75% of the isolates were resistant to INH and RMP respectively (Fig. 23). In total, 72% of the isolates were resistant to both INH and RIF. Another 23% of the isolates were resistant to Streptomycin. Two of the isolates were resistant to all three injectable aminoglycosides Kana-mycin, Amikacin, Capreomycin. Four of the isolates were resistant to both Ofloxacin and Levofloxacin. Two of the isolates were resistant to MFX at lower concentration (0.5µg/ml) but was sensitive to the higher concentration of the drug (2µg/ml).

<u>Fig. 23:</u> Drug susceptibility pattern for MDR-TB isolates from Chennai for the 78 retrieved strains



	STR	INH	RIF	EMB	KAN	AMI	CAP	OF	LEV	MOX 0.5	MOX 2	PAS	ETH	LIN	PZA
Resistant %	23	86	75	11	3	4	5	9	7	5	2	11	12	16	24
Sensitive %	77	14	25	89	95	96	95	89	91	93	96	86	86	79	37
Results Awaited %	0	0	0	0	2	0	0	2	2	2	2	3	2	5	39

<u>Activity 2:</u> Establish capacity for DR-TB outbreak investigations to determine burden of transmitted DR-TB and assess risk factors

The objectives of the study are:

(i) To use genotype results from a cohort of DR-TB isolates stored at NIRT from 2013–2016 to determine the proportion of isolates with the same (i.e. clustered) TB genotype, suggesting recent transmission among the cases

(ii) To screen all household contacts from this cohort of DR-TB cases to estimate the proportion of household transmission

(iii) To identify risk factors for transmission, including spatial relatedness between the homes of clustered DR-TB cases versus DR-TB cases with unique genotypes

(iv) To conduct investigations of cases with clustered genotypes to assess transmission links among them and determine possible transmission locations and social networks outside the household where additional persons might be at risk for acquiring DR-TB

For the DR-TB molecular epidemiology and outbreak investigation objective of the study, data available from 2013 to 2016 has been abstracted from LPA register in NIRT and DRTB patient treatment cards in Chennai.

▲The data has also been re-checked with respective DTC in Chennai and final list of 557 patients has been developed.

▲A preliminary Georeferenced map has been created based on the line list and grouped based on the respective TU.

▲Epi info forms have been created for entry of the data abstracted from PMDT card and LPA register.

▲Abstracted data from PMDT card and LPA register has been entered into EpiInfo forms and a database has been created. Training session for NIRT field staff regarding filling the questionnaires and consenting process has been done.

▲The CDC and NIRT teams met with the DTC for preliminary visit to TU to get oriented towards the workflow of TU.

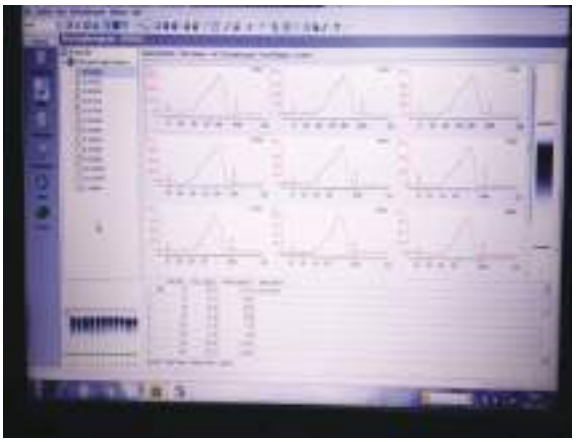
▲Standard operating procedures for data collection and management has been developed.

▲Pilot study for the household contact investigation of the DR-TB patients has been completed and the main study will be initiated.

▲Based on the TB genotyping study, cluster investigation and the risk factors for transmission of the TB

cases will be done in the next few months. The study is ongoing.

Pictures of NGS facility at NIRT (Activity1):



I -13: Cambridge-Chennai Centre Partnership on antimicrobial resistance in TB: Focus on novel diagnostics and therapeutics

Activity 1: Bacterial genomics as a diagnostic tool in DR-TB

Principal Investigator	:	Dr Mohan Natrajan (email: mohan.n@@nirt.res.in)
Collaborator	:	Dr. Sharon Peacock, University of Cambridge
Source of funding	:	MRC (UK) & DBT (India)
Study period	:	2016-2019

Background: Whole genome sequencing (WGS) of *M. tuberculosis* can be used for phylogenetic analysis (e.g. to investigate transmission, mixed or re-infection), and to predict drug resistance. Rapid genetic DST would bring greatest benefit to patients with MDR and XDR-TB, since conventional DST of second line drugs is lengthy (weeks/months). Rapid tests are increasingly used in India to provide a predictor of MDR based on rifampicin resistance (e.g. Xpert MTB/RIF). These are of limited scope in terms of the number of tests performed in a single assay, but ease of use and increasing availability means that its use as a screening tool for resistance can identify an appropriate subset for more extensive testing using WGS. This cost-effective, targeted approach to implementation of WGS will capture patients with DR-TB who most require rapid and accurate antimicrobial therapy. This would be predicted to lead to more rapid conversion to TB smear-negativity and resolution of disease, and reduced risk of onward transmission.

Planned activities:

▲Create a database of *M. tuberculosis* genomes for isolates from India (n=700);

▲Define the accuracy and clinical applicability of genetic DST;

▲Define the frequency of mixed infection in patients with DR-TB in India

▲Establish sustainable knowledge transfer by establishing sequencing as a research tool in Chennai

This study will involve sequencing of 50 drug sensitive TB isolates and 100 drug resistant isolates that are prospectively collected along with 500 isolates from the bacterial repository, in order to capture the full range of resistance patterns, geographical case distribution, and genetic diversity based on existing genotyping data.

Method: WGS are performed by Miseq Illumina sequencer after DNA extraction and library preparation and analyses of data performed by mapping genomes to a reference (*M. tuberculosis H37Rv),* followed by detection of variants (SNPs), insertions, deletions).

DST for 13 drug panel has been done on MGIT 960 and the phenotypic data has been provided to the clinician for diagnosis; genotypic data will only be used for creating the database.

Results:

From the prospective strain collection, phenotypic DST has been completed for 47 samples and WGS has been completed for 89 strains. Among the 500 retrospective strains identified for the study, culture retrieval and subculture has been done for 250 strains. Among them, phenotypic DST has been completed for 191 strains. DNA extractions from these strains are in progress.

The drug resistance prediction pipeline is being developed for predicting drug resistance from the sequenced data that will be obtained from this study.

The study is ongoing.

<u>Activity 3</u>: Population based study of gene repertoire associated with drug tolerance and their *in vivo* expression

Principal Investigator	:	Dr Mohan Natrajan (email: mohan.n@@nirt.res.in)
Collaborator	:	Dr. Lalitha Ramakrishnan, University of Cambridge
Source of funding	:	MRC (UK) & DBT (India)
Study period	:	2016-2019

Background: In India, an estimated 2.2% of new cases and 15% of previously treated cases have multi-drug resistant-TB (MDR-TB). Compared to infection with drug-sensitive TB where ~90% of individuals have successful TB treatment, patients with MDR- TB have only a 46% chance of treatment success (WHO Global TB report 2015). Previous findings from our collaborators have shown that *M. tuberculosis* becomes tolerant to anti-tubercular drugs upon entering host macrophages. This macrophage-induced tolerance results from bacterial efflux pumps that are induced inside host macrophages to counter macrophage defences, thereby permitting intracellular bacterial growth. Thus, the pumps primarily serve as classical virulence factors. However, they also cause the efflux of anti-tubercular drugs tested to date, including first-, second- and third-line agents. Importantly, we have shown that tolerance to INH, RMP, MOX and bedaquiline are inhibited by verapamil, a calcium channel blocker with a more than 40-year history of safe clinical use for wide-ranging problems. Verapamil reduces both bacterial growth and tolerance, consistent with the dual function of the pumps, a characteristic that we expect will allow verapamil to synergize with standard antitubercular chemotherapy. The verapamil-sensitive efflux pumps also mediate intracellular bacterial growth. Indeed, the dual role of efflux in promoting intracellular *M. tuberculosis* growth in the absence of antibiotics and antibiotic tolerance provides the best explanation for longstanding clinical data linking increased *M. tuberculosis* burden to the need for longer curative treatment. Thus, verapamil should have dual effects *in vivo*: potentiating antibiotic killing, and killing intracellular bacteria independently of antibiotics. Supporting our findings and our model, verapamil has since been observed to improve bacterial killing in mouse models of TB and to shorten the

duration of treatment. Based on this fact, the following objective was studied.

Objective:

(i) To gain a fundamental understanding of the mechanism of macrophage induced efflux pump-mediated drug tolerance by conducting laboratory-based studies

Planned activities:

1. To understand macrophage induced RIF and INH tolerance of different M. tb lineages and the effect of Verapamil on tolerance;
2. To understand macrophage induced MOX tolerance of Pre-XDR and MDR strains and the effect of Verapamil in tolerance reduction;
3. To estimate the drug tolerance exhibited by mycobacteria directly from the patient's sputum and
4. To perform transcriptome analysis in mycobacteria directly isolated from patient's sputum.

1. To understand macrophage induced RIF and INH tolerance of different M. tb lineages and the effect of Verapamil on the mycobacterial strains

Methods: Macrophage tolerance assay was performed for lineages 1, 2 and 3 (M1338, NIRT203 Beijing and CC0111) with H37Rv as control strain. Lineage strains were grown in 7H9 medium until log phase and checked for sterility. THP1 cells were plated and infected with different lineage strains. *M. tuberculosis* grown inside the macrophages were treated with antibiotics (RMP and INH) for 2 and 96 hours. For 96 hours samples, antibiotic effect was studied in the presence or absence of Verapamil. Tolerance was calculated from CFU counts and any difference due to addition of verapamil was noted. Experiments will be repeated to get independent results and the same will be consolidated.

Results: Macrophage tolerance assay was performed for lineage 1 (Fig.24a.) and 2 (Fig.24b.) (M1338 and NIRT203 Beijing) with H37Rv as control strain. It was observed that RIF tolerance was observed in M1338 and as expected no tolerance was observed in NIRT203 Beijing. Upon addition of verapamil, reduced RIF tolerance was observed with L1 but not L3. However, no significant effect was observed with INH tolerance in both L1 and L3.

Figs. 24a & b: **Macrophage induced drug tolerance**

Fig 1a: Lineage 1

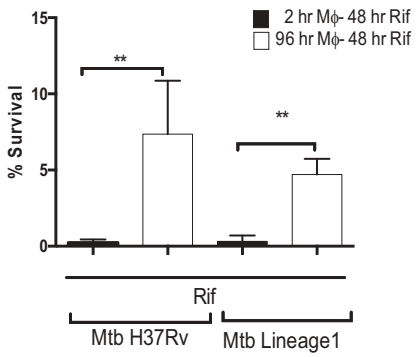
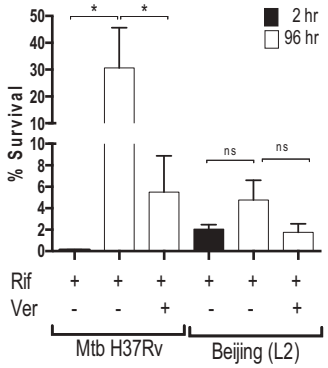


Fig 1b: Lineage 2



2. To understand macrophage induced Moxifloxacin tolerance of Pre-XDR and MDR strains and the effect of Verapamil in tolerance reduction

Methods: Protocol followed here will remain the same as stated above for lineage strains. However, *M. tuberculosis* taken from macrophages will be treated with MOX instead of RMP or INH for the same period of time.

Pre-XDR strains were collected, sub-cultured and stocked. MIC was estimated using resazurin assay for those strains and tabulated (Table 58). For estimation of MIC, various *M. tuberculosis* isolates were grown in 7H9 medium with drugs to be tested at different concentrations. The concentrations were chosen higher and lower than MIC concentration reported for the standard laboratory strain H37Rv. After growth in 96 well plate with drugs for 7 days, resazurin was added to the cells. Resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) is a blue dye which is irreversibly reduced to the pink colored and highly red fluorescent resorufin. It is used as an oxidation-reduction indicator in cell viability assays. Visual quantitation of viability can be observed and photographed. The lowest concentration where no growth is observed in presence of drug is considered the MIC for that particular drug for the strain.

Experiments are in progress.

Table 58: Pre-XDR strains and their MIC

	MIC (ug/ml)	
Strains	Mox	Verapamil
NIRT1622	**<0.125**	200
NIRT1638	2	200
NIRT1748	2	200
NIRT1765	2	200
NIRT2053A	ND	ND
H37Rv	0.25	**200**

MDR strains were collected, sub-cultured and stocked

3. To estimate the drug tolerance exhibited by mycobacteria directly from the sputum of TB patients

Method: Fresh sputa were collected from four patients and bacilli were isolated. *M. tuberculosis* isolated from sputa were treated with rifampicin and isoniazid up to 48 hours and plated. CFUs were counted after growth on plates and tolerance was estimated. The 4 clinical samples were grown in 7H9 medium and stocked for further use. The 4 clinical samples were tested for their broth tolerance and macrophage tolerance with the same two antibiotics. For broth tolerance, log phase cultures in 7H9 were treated with RIF and INH up to 48 hours and plated. CFUs estimated after growth on plates and tolerance was estimated. THP1 cells were plated and infected with 4 clinical samples. *M. tuberculosis* was grown inside the macrophages and treated with antibiotics for 2 and 96 hours. Tolerance was calculated from CFU counts. Experiments are repeated to get independent results and the same will be consolidated.

In all the sputum samples collected, no decrease in percent survival was observed with addition of verapamil in presence of RIF. However, with 2 samples (32S and 33B), decrease in percent survival with addition of verapamil in presence of INH was observed. All the clinical isolates were grown in culture and stocked. MIC was estimated to be 0.2 ug/ml for RIF.

The stdy is ongoing.

4. To perform transcriptome analysis in mycobacteria directly isolated from sputum of TB patients

RNA samples were isolated from fresh sputa and extraction procedures are being standardized for the same.	The study is ongoing

<u>Activity 5:</u> Manipulating T-cell exhaustion: new therapies to improve outcomes in resistant TB

Principal Investigator	:	Dr Mohan Natrajan (email: mohan.n@@nirt.res.in)
Co-Principal Investigators	:	Dr. Alena Srinivasan; Dr.C. Padmapriyadarsini; Dr. Annapurna V.; Dr.K.R. Uma Devi
Collaborators	:	Dr. Eoin McKinney; Dr. Ken Smith University of Cambridge
Source of funding	:	MRC (UK) & DBT (India)
Study period	:	2016-2019

Background: The capacity of the immune response to control infection becomes of paramount importance in patients with drug-resistant TB. This capacity is in large part controlled by the phenomenon of T-cell exhaustion, a process resulting in reduced immune capacity and driven by persistent antigen exposure in the face of inadequate T-cell co-stimulation. As treatment failure due to MDR- and XDR-TB, allows infection to persist, host defences may be further eroded through exhaustion, compounding treatment resistance and making recovery even less likely. We have recently found that T-cell exhaustion is a critical factor in controlling autoimmune disease, and a biomarker measuring it can predict long-term

patient outcome and is entering clinical trials in Crohn's disease. Preliminary data suggests that exhaustion is also important in responses to TB. Therefore, the main objectives of the study are listed below:

Key Objectives:

1. Investigate the association between exhaustion and treatment outcome in 50 MDR-TB and 100 drug susceptible TB patients;
2. Identify the T-cell co-stimulatory pathways responsible for controlling exhaustion in the context of TB;
3. *In silico* screen for existing pharmaceuticals that limit or reverse exhaustion at a transcriptional level

Methodology:

1. Whole blood intracellular cytokine staining (ICS) assay and multiparameter flow cytometry

Whole blood culture was performed as described in the previous report. On the day of staining, cryopreserved whole-blood samples were thawed in a water bath at 37°C and cells were initially stained with antibody cocktail containing cell surface antibodies, followed by staining with antibody cocktail containing intra-cellular markers. Finally, cells were washed and resuspended in 100µl 1% paraformaldehyde.

2. Flow cytometry analysis

Samples were acquired on BD FACSAria™ Fusion flow cytometer and BD FACSDiva™ version 8.0.1 software (BD Biosciences). Cytometer Setting and Tracking (CST) beads (BD Biosciences) were acquired before each experiment to ensure that cytometer parameters remained consistent across all experiments. Data was analysed using FlowJo version 9.9.4 software (Treestar, Ashland, OR). Background subtractions were performed in Pestle version 1.8.

3. Thawing of PBMC and CD4+, CD8+ T-cell and CD14+ sorting:

Vials of frozen PBMC were taken out from liquid nitrogen and thawed in a 37°C water bath and washed in pre-warmed complete media and total contents were transferred to 50 ml tube. Cells were pelleted down and washed twice with complete media and re-suspended in complete media and rested in 6 well plates. Live/Dead discriminating dye Avid was added to the cells and washed with 1 ml of PBS (2% FBS). Staining cocktail comprising the following was added to the cells: FcR Block and Anti- CD3 FITC, Anti-CD4 APC, Anti- CD8 PE and Anti-CD14 PE-Cy7; incubated with staining cocktail for 20 min at room

temperature. Finally, cells were washed in PBS (2% FBS), resuspended in the same buffer (total volume – 200 µl) and transported on ice for sorting.

Sorting was carried out using a BD FACS Aria Fusion with a 100 µm nozzle, 10 – 20 psi, 100 rpm agitation flow rate of 3000 – 6000 events/sec. Sorted CD4+ T-cells, CD8+ T-cells and CD14+ monocytes were collected in 1 ml complete RPMI media. Collection was done in polystyrene FACS tubes. Appropriate single colour stained and unstained samples were used as compensation controls. CD3+CD4+ cells were sorted as CD4+ T-cells, CD3+CD8+ were sorted as CD8+ T-cells and CD3-CD14+ cells were sorted as monocytes. Purity of sorted cells was gauged by performing a post-sort analysis.

Work Progress:

1. In this phase we have mainly achieved the PBMC, plasma and PAX-Gene tubes from 125 [57 Drug Resistant (DR) and 68 Drug Sensitive (DS)] patients which will be used for other experiments to fulfill our goal. We have also archived the plates from 7day whole blood culture which we will analyze for IFN-γ secretion. Intra cellular staining of whole blood stimulation (20-22 hr culture) samples (Baseline and follow up) will be performed to find peptide specific immunophenotyping (Table 59).

Table 59: Number of samples archived at various time points

Months	DR-BL	DR-2M	DR-6M	DS-BL	DS-2M	DS-6M	Total
PBMC archived	21	19	17	17	30	21	**125**
Whole Blood Stimulation (Intra cellular staining)	10	0	15	13	0	12	**50**
Whole Blood 7 day culture (IFN-γ release assay)	10	0	15	5	0	11	**41**

2. **P2 (Mutant peptide pool) is augmenting superior immune response in CD4+ and CD8+ T-cells than P1 (parent peptide pool) in Drug Sensitive patient group:** We have used 16 color flow cytometer to check the activation status of CD4 and CD8 cells of 23 drug sensitive (DS) TB patients. PPD was used as positive control for all experiments. Flow cytometry result clearly shows that CD4 cells in drug sensitive patients are secreting more IFN-γ and IL-17 after stimulation,, and response is expressively better in mutant peptide pool (Figs. 25 and 26). Supporting this result, IL-2 secretion is also enhanced after stimulation and significantly superior result is observed for mutant peptide pool. TNFα and MIP1-β levels were found to be increased after stimulation but no significant difference was noted between parent and mutant peptide pool. This result suggests that peptide pools are enhancing both Th1 and Th17 response resulting in better treatment outcome. Status of IL-4 remained unaltered even after stimulation.

Superior immune response in CD4+ T-cells on stimulation with P2 (Mutant peptide pool) than P1 (parent peptide pool) in drug sensitive patient group

Fig.25:

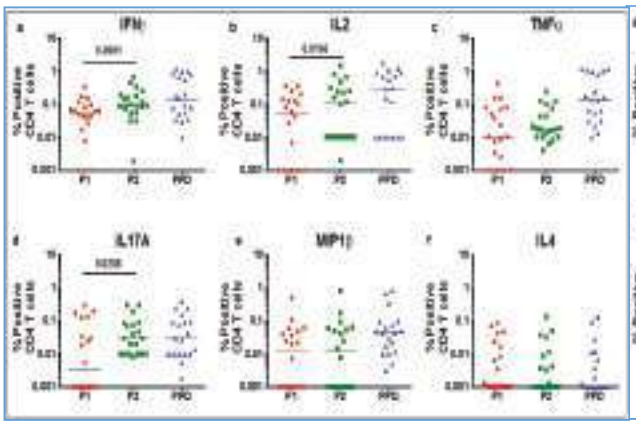
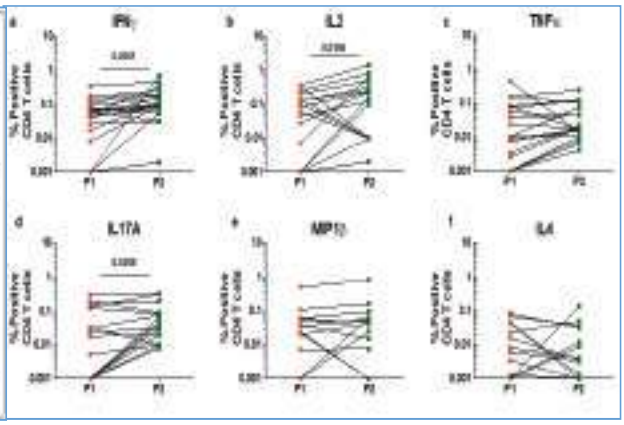


Fig.26



Flow cytometry result also revealed that CD8 cells in drug sensitive patients are also secreting more IFN-γ and IL-2 after stimulation and response is log fold higher in mutant peptide pool (Figs. 27 & 28). In contrary to CD4 cells, IL-17 and TNFα level was found to be unaltered after stimulation whereas no significant difference was noted in IL-4 status between parent and mutant peptide pool. Enhanced IFN- γ secretion by

CD8 T cells reflecting superior effector response by mutant (P2) peptide pools than parent (P1) peptide pool.

Superior immune response in CD8+ T-cells on stimulation with P2 (Mutant peptide pool) than P1 (parent peptide pool) in drug sensitive patient group

Fig 27:

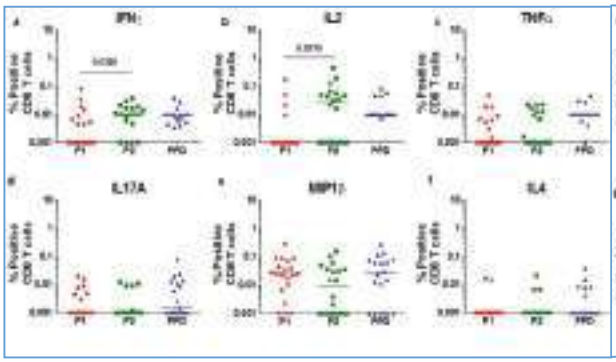
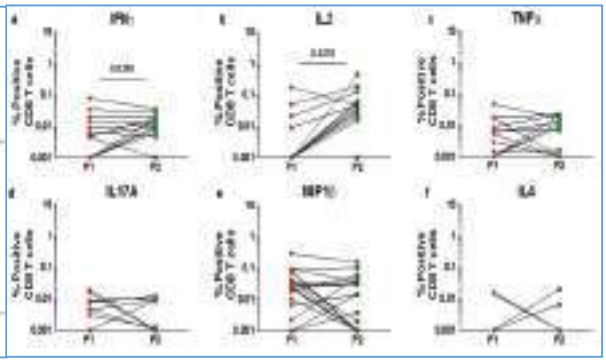


Fig. 28:



P2 (Mutant peptide pool) is augmenting superior immune response in CD4+ and CD8+ T-cells than P1 (parent peptide pool) in DR patient group: We have used 16 color flow cytometer to check the activation status of CD4 and CD8 cells of 22 drug resistant (MDR-TB) patients. PPD was used as positive control for all experiments. Flow cytometry results clearly show that CD4 cells in DR patients are secreting very high levels of IFN-γ, IL-17 after stimulation and response is radically higher in mutant peptide pool (Figs. 29 & 30). Supporting this result, IL-2, TNFα and MIP1-β secretion are also enhanced after stimulation and significantly better results were demonstrated by mutant peptide pool quite consistently. However, IL-4 level was found to be elevated after stimulation but no significant difference was noted between parent and mutant peptide pools. This result suggests that peptide pools are enhancing both Th1 and Th17 response in drug resistant patient pool making P2 peptide pool a good candidate for novel TB vaccine.

Superior immune response in CD4+ T-cells on stimulation with P2 (Mutant peptide pool) than P1 (parent peptide pool) in drug resistant patient group

Fig.29:

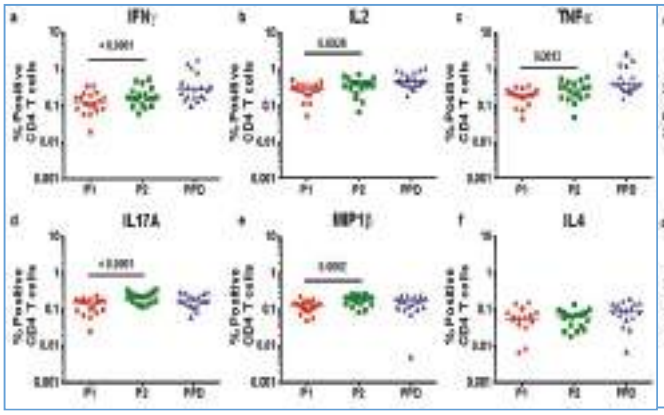
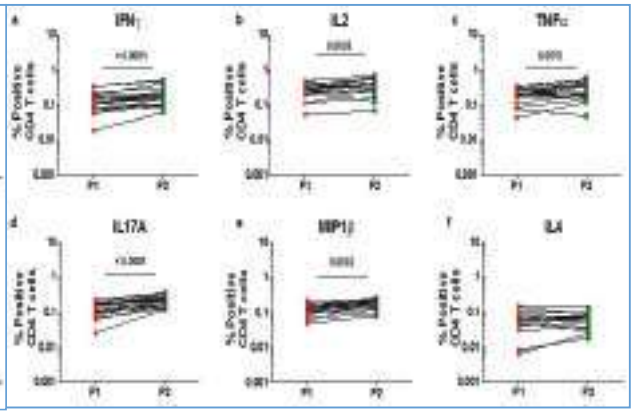


Fig.30:



Flow cytometry results also revealed that CD8 cells in DR patients are also consistently secreting high levels of all effector cytokines i.e. IFN-γ, IL-2, TNFα, IL-17 and MIP1-β after stimulation and response is significantly higher in mutant peptide pool (Figs. 31 & 32). However, no significant difference was noted in IL-4 status between parent and mutant peptide pools. Enriched IFN- γ and IL-17A secretion by CD8 T cells revealed superior effector response by mutant (P2) peptide pools than parent (P1) peptide pool.

Superior immune response in CD8+ T-cells on stimulation with P2 (Mutant peptide pool) than P1 (parent peptide pool) in drug resistant patient group

Fig. 31:

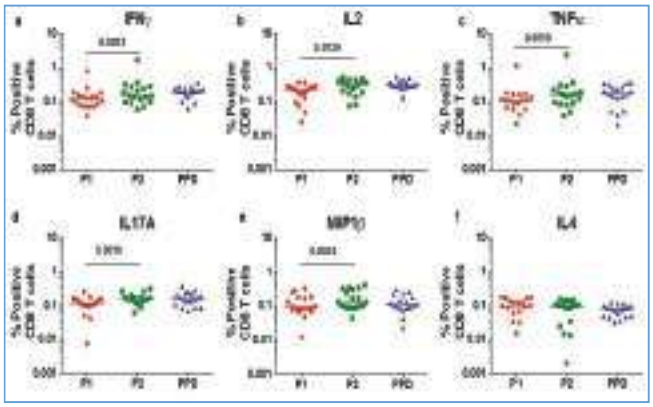
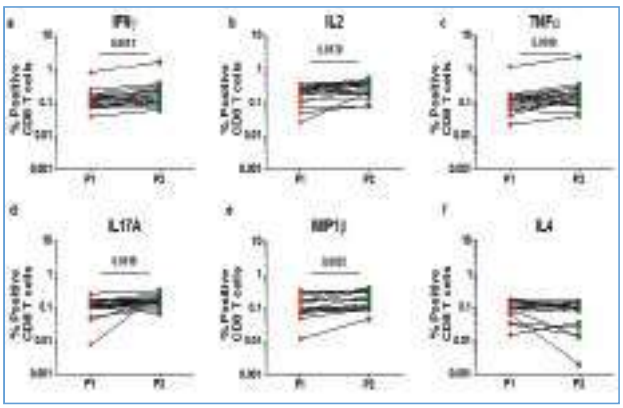


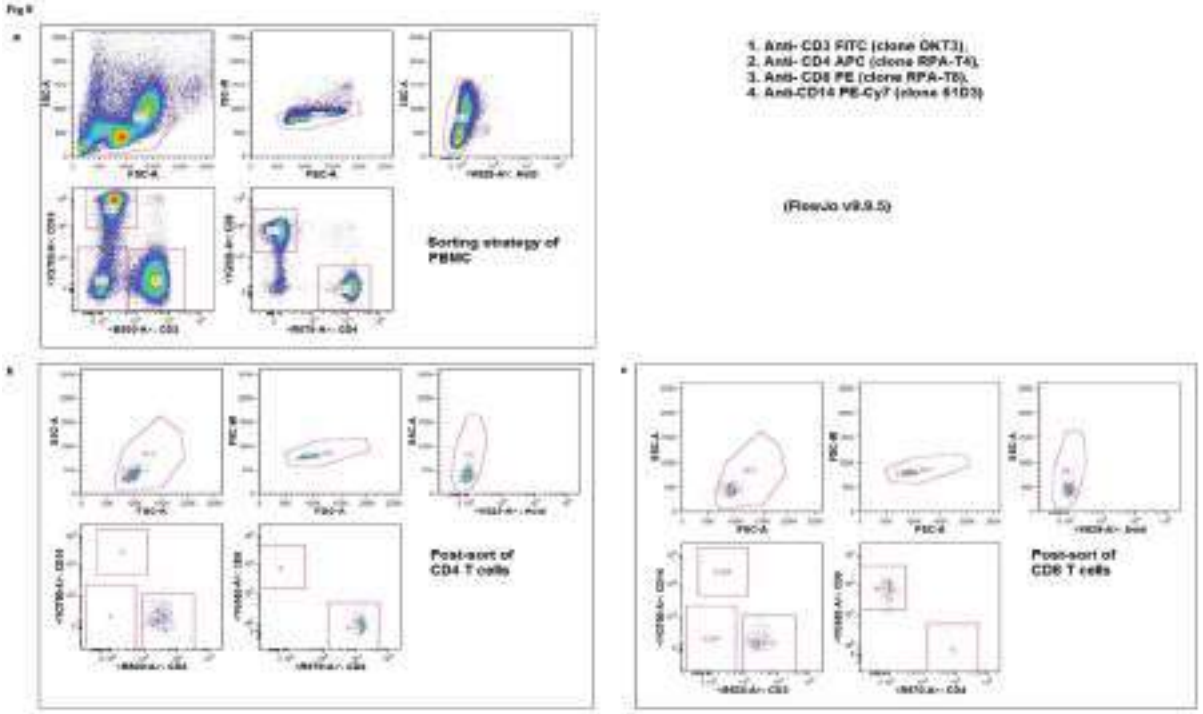
Fig. 32:



3. Sorting of different immune cell population following RNA isolation and library preparation for NGS:

We have performed sorting of CD4+, CD8+ and CD14+ cells from 67 blood samples (i.e. 28 DR patient and 39 DS patient) and RNA was isolated and stored in -80^{0}C for NGS analysis following library preparation and quality control. The purity of sorted population was found to be perfect for library preparation (Fig. 33).

Fig. 33:



The study is in progress

DEPARTMENT OF STATISTICS

COMPLETED STUDIES:

S1: Decision tree classification and model evaluation for breast cancer survivability: A data mining approach

Principal Investigator : Dr.C. Ponnuraja
(email: cponnuraja@gmail.com)
Source of funding : Intramural
Study Period : 2017-2018

Background: In survival analysis, there are many clinical examples where a patient may experience a variety of intermediate events. Competing risks models analyze the time until a first event and the event type that occurs at that time. Competing risks also model the endpoint type but it does not model subsequent events such as multiple events. To perform this, more complex multistate models are needed which also investigate covariate effects for each specific transition between states

The data provided by Three intermediate events are included in the model: Recovery (Rec), an Adverse Event (AE) and a combination of the two (AE and Rec). It is to be expected that recovery improves the prognosis and an adverse event deteriorates it. The model is suitable to show the size of these effects, and to capture the influence of their timing and of the covariates on the prognosis. It shows what happens when both the positive and negative event take place, as compared to one or none of them.

Aim:

(i) To explain use and interpretation of both non proportional hazard Cox (non PH) and proportional Cox (PH)-type multistate models for the suitability of assessing different covariate effects in situations of multiple endpoints and also different baseline hazard assumptions in a comparison manner for the European Group for Blood and Marrow Transplantation competing risks data

Methodology: We have considered 2279 patients who were treated between 1985 and 1998. Four prognostic factors were known at baseline for all patients. They were: donor-recipient match (where gender

mismatch is defined as female donor, male recipient), prophylaxis (Treatment), year of transplant and age at transplant in years. All these covariates were treated as time-fixed categorical covariates. The distribution of the values of the covariates over the patients in the data set is shown in Table 60.

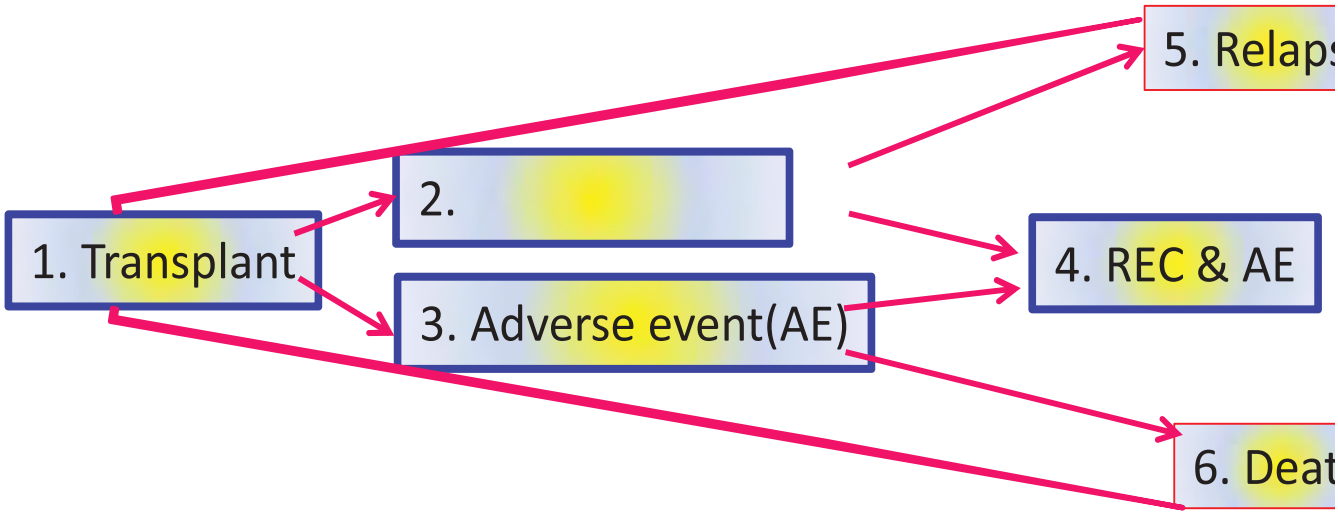
Table 60: Prognostic factors for all patients

	N	%
Year of transplant		
1985-1989	634	27.8
1990-1994	896	39.3
1995-1998	749	32.9
Age at transplant (years)		
<=20	551	24.2
20-40	1213	53.2
>40	515	22.6
Prophylaxis		
no	1730	75.9
yes	549	24.1
Donor recipient		
gender mismatch	545	23.9
no gender mismatch	1734	76.1

A multi-state approach is particularly appropriate for these data, since it can help to model both the disease-related and the treatment-related morbidity and mortality. These were modeled here by including the intermediate events, recovery and adverse event. Information about the occurrence of these events was used to update the prognosis of the patients. Total cases recovered were 1218 (53.4%), with adverse event 1134 (49.8%) and both recovered and with adverse event in 660 (29%), relapse in 370 (16.2%) and death in 838 (36.8%). We considered the following six-state model for leukemia patients after bone marrow transplantation (Fig. 34). The six-state model has the following factors with six-states:

1. Transplantation
2. Recovery
3. Adverse event
4. Recovery and Adverse event
5. Relapse
6. Death

Fig. 34: Diagram of Six state model



The detailed states have been illustrated as in Figure 34 as follows:

State1. Alive and in remission, no recovery or adverse event;
State2. Alive in remission, recovered from the treatment;
State3. Alive in remission, occurrence of the adverse event;
State4. Alive, both recovered and adverse event occurred;
State5. Alive, in relapse (treatment failure);
State6. Dead (treatment failure).

Table 61: Data structure and frequencies

id	from	to	trans	Tstart	Tstop	time	status	proph	year	agecl
1 1	1	2	1	0	22	22	1	no	1995-1998	20-40
2 1	1	3	2	0	22	22	0	no	1995-1998	20-40
3 1	1	5	3	0	22	22	0	no	1995-1998	20-40
4 1	1	6	4	0	22	22	0	no	1995-1998	20-40
5 1	2	4	5	22	995	973	0	no	1995-1998	20-40
6 1	2	5	6	22	995	973	0	no	1995-1998	20-40
7 1	2	6	7	22	995	973	0	no	1995-1998	20-40

Frequencies

	to							
from	Tx	Rec	AE	Rec+AE	Rel	Death	no event	total entering
Tx	0	785	907	0	95	160	332	2279
Rec	0	0	0	227	112	39	407	785
AE	0	0	0	433	56	197	221	907
Rec+AE	0	0	0	0	107	137	416	660
Rel	0	0	0	0	0	0	370	370
Death	0	0	0	0	0	0	533	533

According to the Table 61, Starting from state 1, the patient is at risk for transitions 1,..., 4. This means that the patient can move to states 2, 3, 5 and 6. At time 22, the patient moves to state 2 (Recovery), from where the patient is at risk for a further transition to state 4, 5 and 6 (i.e., transitions 5, 6 and 7). None of these occur and the patient is censored at time 995. The patient has no rows for transitions 8-12 because the patient has never been at risk for these. The value of time is equal to “Tstop” ”Tstart”; it is of use in 'clock reset'-models, where the time t refers to the time spent in the current state. The numbers of transitions, both in terms of frequencies and percentages, are given subsequently. This process was shown by a selection of the long format data for the first patient, leaving out the values at baseline and the transition-specific counterparts of “match”, “proph” and “agecl”. Zero having factors like year1.1 to year1.12 were omitted.

The original covariates were retained to consider models with any mixture of basic and transition-specific covariates. It was considered for both non-parametric and semi-parametric models. For the estimation of the cumulative hazard, it is assumed to have data with independent censoring. This Cox model has separate baseline hazards for each of the transitions and no covariates. In principle, the transition intensities could also be estimated separately, but the combined use of long format data and a single stratified “*coxph”* object makes further calculations easier.

Co-variances of the estimated cumulative hazards may be computed in two different ways: by means of the Aalen estimator or by means of the

Greenwood estimator. An advantage of the Greenwood estimator is the fact that it yields exact multinomial standard errors for the transition probabilities when there is no censoring. The head and tail of the cumulative hazards of all transitions along with time (time in years) are presented in Table 62A.

Table 62A: Greenwood method of stimation

Hazards				Variances of hazards			
time	Haz	trans		time	varHaz	trans1	trans2
1 0.002737851	0.000000000	1		1 0.002737851	0.000000e+00	1	1
2 0.008213552	0.000000000	1		2 0.008213552	0.000000e+00	1	1
3 0.010951403	0.000000000	1		3 0.010951403	0.000000e+00	1	1
4 0.013689254	0.000000000	1		4 0.013689254	0.000000e+00	1	1
5 0.016427105	0.000443066	1		5 0.016427105	1.962205e-07	1	1
6 0.019164956	0.001333142	1		6 0.019164956	5.919853e-07	1	1
...				...			
6199 12.48460	0.3800455	12		40321 12.48460	0.002461034	12	12
6200 12.61602	0.3800455	12		40322 12.61602	0.002461034	12	12
6201 13.02396	0.3800455	12		40323 13.02396	0.002461034	12	12
6202 13.10609	0.3800455	12		40324 13.10609	0.002461034	12	12
6203 13.12799	0.4255001	12		40325 13.12799	0.004433236	12	12
6204 17.24572	0.4255001	12		40326 17.24572	0.004433236	12	12

The last time point in the list indicates the last time point in the data, either of an event or of a censoring. The graph (Fig. 35A) using Greenwood method illustrates all estimated cumulative hazards in different colours.

Fig. 35A: Greenwood method ilustraton: estimated cumulative hazards

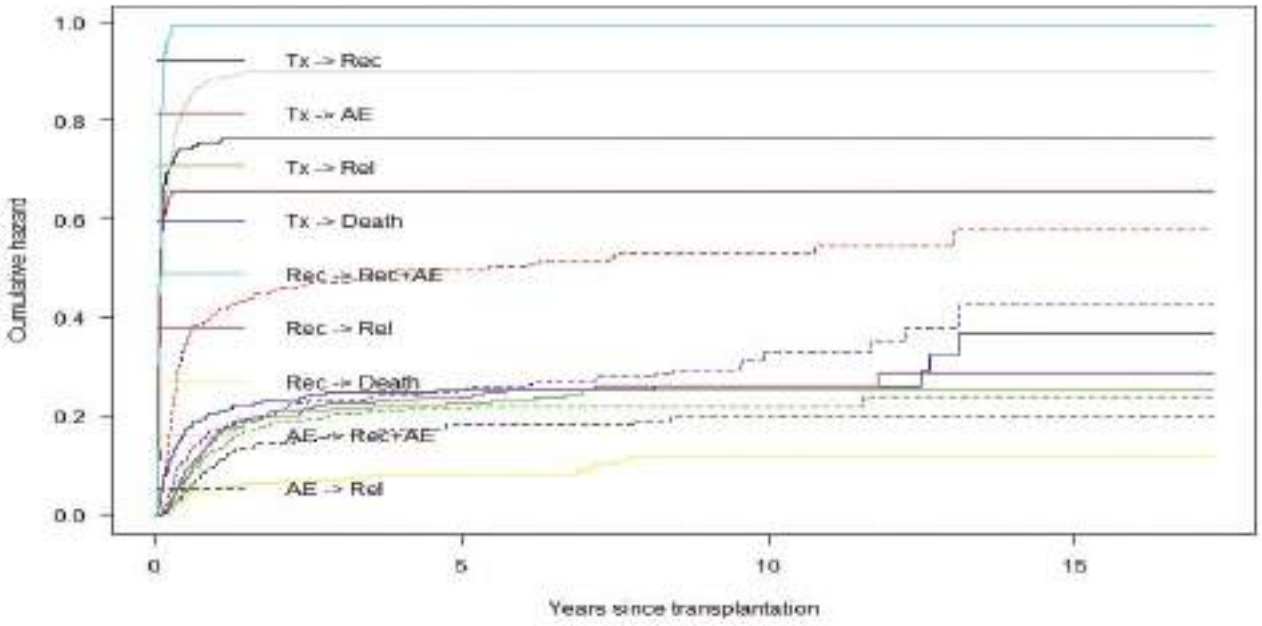
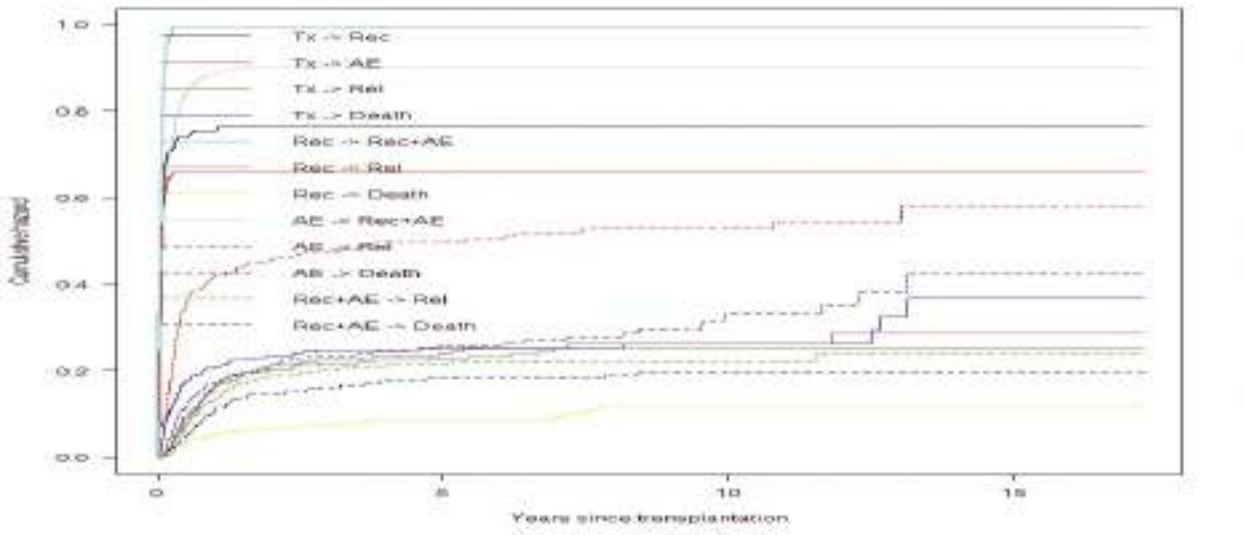


Table 62B: Aalen method of estimation

Hazards				Variances of hazards			
time	Haz	trans		time	varHaz	trans1	trans2
1 0.002737851	0.000000000	1		1 0.002737851	0.000000e+00	1	1
2 0.008213552	0.000000000	1		2 0.008213552	0.000000e+00	1	1
3 0.010951403	0.000000000	1		3 0.010951403	0.000000e+00	1	1
4 0.013689254	0.000000000	1		4 0.013689254	0.000000e+00	1	1
5 0.016427105	0.000443066	1		5 0.016427105	1.963075e-07	1	1
6 0.019164956	0.001333142	1		6 0.019164956	5.924248e-07	1	1
…				…			
6199 12.48460	0.3800455	12		40321 12.48460	0.002502928	12	12
6200 12.61602	0.3800455	12		40322 12.61602	0.002502928	12	12
6201 13.02396	0.3800455	12		40323 13.02396	0.002502928	12	12
6202 13.10609	0.3800455	12		40324 13.10609	0.002502928	12	12
6203 13.12799	0.4255001	12		40325 13.12799	0.004569044	12	12
6204 17.24572	0.4255001	12		40326 17.24572	0.004569044	12	12

The last time point in the list indicates the last time point in the data, either of an event or of a censoring. Both these methods mimic each other but there is difference in their estimation. Fig. 33B using Aalen method illustrates all estimated cumulative hazards in different colours (Table 62B).

Fig. 35B: Aalen method illustration: Estimated cumulative hazards



There is a need to calculate the estimated transition probabilities, and optionally the standard errors and/or the covariances of the transition probabilities. The Stacked transition probabilities (an informative ordering of the transition probabilities) are shown in Figs. 35A & B with plots using Greenwood and Aalen estimators respectively. These figures show the distance between two adjacent curves representing the probability of being in the corresponding state. The particular order chosen makes it possible to combine the probabilities of recovery and recovery + AE, and of AE and recovery + AE.

Semi-parametric models (A model with transition-specific covariates) showed the prediction of the transition probabilities, which can be improved by taking covariates at baseline into account. Events were of the same `type' or `stratum' if they shared a baseline hazard. We considered a model in which `type' was equivalent to transition: each transition had its own baseline hazard. In the second session of part, we considered a so-called `proportional baseline hazards model'. In both models, covariates can have the same effect for all transitions or different effects for different transitions; in the latter case, transition-specific covariates are needed. We refer to this model as the full model.

Table 63: Regression co-efficients (and standard errors) for the full model; covariate effects significant at 0.05 and 0.01 levels are indicated with(*).

	Coef	exp(coef)	se(coef)	z	p
match.1*	-0.16740	0.84586	0.08530	-1.96	0.04971
match.2	-0.11056	0.89533	0.07879	-1.40	0.16054
match.3	0.19559	1.21602	0.22378	0.87	0.38212
match.4	-0.00346	0.99654	0.18131	-0.02	0.98476
match.5	0.19044	1.20979	0.15293	1.25	0.21303
match.6*	0.42575	1.53074	0.21397	1.99	0.04661
match.7	0.24448	1.27696	0.40481	0.60	0.54588
match.8	0.12589	1.13416	0.11294	1.11	0.26499
match.9	-0.41437	0.66075	0.35218	-1.18	0.23936
match.10	0.00820	1.00823	0.16750	0.05	0.96095
match.11	-0.30128	0.73987	0.24825	-1.21	0.22490
match.12*	0.57151	1.77093	0.17942	3.19	0.00145
*proph.1**	*-0.36579*	*0.69365*	*0.09288*	*-3.94*	*8.2e-05*
*proph.2**	*-0.27760*	*0.75760*	*0.08319*	*-3.34*	*0.00085*
proph.3	*0.38495*	*1.46955*	*0.22714*	*1.69*	*0.09012*
proph.4	*-0.05639*	*0.94517*	*0.17875*	*-0.32*	*0.75239*
proph.5	*-0.28184*	*0.75439*	*0.19626*	*-1.44*	*0.15098*
proph.6	*0.26760*	*1.30682*	*0.22087*	*1.21*	*0.22568*
proph.7	*-0.00757*	*0.99245*	*0.37778*	*-0.02*	*0.98400*
proph.8	*0.12495*	*1.13309*	*0.12488*	*1.00*	*0.31706*
proph.9	*0.15889*	*1.17221*	*0.32079*	*0.50*	*0.62037*
proph.10	*0.32360*	*1.38209*	*0.16638*	*1.94*	*0.05178*
proph.11	*0.01226*	*1.01234*	*0.24709*	*0.05*	*0.96042*
proph.12	*-0.11176*	*0.89426*	*0.21739*	*-0.51*	*0.60718*
year1.1*	0.40111	1.49348	0.10015	4.01	6.2e-05
year1.2	0.02298	1.02324	0.08388	0.27	0.78414
year1.3	0.44194	1.55572	0.24466	1.81	0.07086
year1.4	-0.35866	0.69861	0.19303	-1.86	0.06316
year1.5	-0.09469	0.90966	0.19133	-0.49	0.62067
year1.6	-0.21006	0.81054	0.26343	-0.80	0.42522
year1.7*	-0.83628	0.43332	0.39828	-2.10	0.03575
year1.8*	0.52824	1.69594	0.13534	3.90	9.5e-05
year1.9	-0.31090	0.73279	0.29970	-1.04	0.29956
year1.10*	-0.64392	0.52523	0.17330	-3.72	0.00020
year1.11	-0.02429	0.97601	0.25262	-0.10	0.92341
year1.12	-0.36239	0.69601	0.22839	-1.59	0.11257

year2.1*	0.52122	1.68409	0.10300	5.06	4.2e-07
year2.2	-0.11391	0.89234	0.09119	-1.25	0.21162
year2.3	0.22095	1.24726	0.30204	0.73	0.46445
year2.4*	-0.47558	0.62152	0.21760	-2.19	0.02884
year2.5	-0.15092	0.85991	0.18997	-0.79	0.42693
year2.6	0.05517	1.05672	0.25848	0.21	0.83099
year2.7*	-0.97962	0.37545	0.44233	-2.21	0.02678
year2.8*	0.93043	2.53559	0.14057	6.62	3.6e-11
year2.9	-0.58037	0.55969	0.43328	-1.34	0.18042
year2.10	-0.21282	0.80830	0.19459	-1.09	0.27409
year2.11	-0.38956	0.67735	0.27724	-1.41	0.15998
year2.12	-0.35202	0.70327	0.23844	-1.48	0.13985
agecl1.1	0.04911	1.05034	0.08888	0.55	0.58054
agecl1.2	0.12334	1.13127	0.08283	1.49	0.13648
agecl1.3	-0.09361	0.91064	0.23218	-0.40	0.68682
agecl1.4*	0.76603	2.15121	0.22856	3.35	0.00080
agecl1.5	0.29238	1.33961	0.18750	1.56	0.11892
agecl1.6	-0.25542	0.77459	0.22306	-1.15	0.25218
agecl1.7	0.15025	1.16213	0.49093	0.31	0.75956
agecl1.8*	-0.39316	0.67492	0.11630	-3.38	0.00072
agecl1.9	0.17250	1.18827	0.36679	0.47	0.63814
agecl1.10	0.23758	1.26818	0.20526	1.16	0.24709
agecl1.11	0.41404	1.51292	0.25018	1.65	0.09793
agecl1.12*	0.75952	2.13726	0.27229	2.79	0.00528
agecl2.1	0.19944	1.22072	0.10244	1.95	0.05155
agecl2.2	0.06731	1.06963	0.10094	0.67	0.50488
agecl2.3	-0.23217	0.79281	0.32229	-0.72	0.47128
agecl2.4*	0.93422	2.54524	0.26441	3.53	0.00041
agecl2.5	0.47007	1.60010	0.20528	2.29	0.02203
agecl2.6	-0.10072	0.90419	0.26413	-0.38	0.70296
agecl2.7*	1.46451	4.32544	0.48111	3.04	0.00233
agecl2.8*	-0.32763	0.72063	0.14244	-2.30	0.02144
agecl2.9	0.42286	1.52631	0.43253	0.98	0.32825
agecl2.10*	0.49466	1.63993	0.23683	2.09	0.03674
agecl2.11	0.25618	1.29199	0.30415	0.84	0.39963
agecl2.12*	1.33674	3.80663	0.28701	4.66	3.2e-06
Likelihood ratio test=288 on 72 df, p=0					
n= 15512, number of events= 3255					

The estimated regression coefficients of the covariates and their standard errors for each of the transitions are shown in Table 63. For each covariate, the estimated effects were positive for some transition hazards and negative for others. The use of transition-specific covariates is very convenient to observe such effects. It also enables to make different kind of predictions for individual patients and in dynamic prediction of 10-year relapse-free survival (RFS) probabilities (Table 64). It can be obtained that these prediction probabilities change as more information about intermediate events become known in the course of time. We wanted to study 10-year RFS probabilities changing when the patient experiences an adverse event 60 days (0.164 years) post-transplant and is recovered from the treatment 80 days (0.219 years) post-transplant.

Table 64: Dynamic prediction of 10-year relapse-free survival (RFS) probabilities for specific patients

	time	pstate1	pstate2	pstate3	pstate4	pstate5	pstate6
1	0.000000000	0.2027821	0.2011028	0.03643172	0.2243403	0.2202891	0.1150540
2	0.002737851	0.2028442	0.2011644	0.03642153	0.2242138	0.2203215	0.1150345
3	0.008213552	0.2030930	0.2014111	0.03638068	0.2237070	0.2204516	0.1149566
4	0.010951403	0.2033788	0.2016946	0.03634618	0.2232383	0.2206213	0.1147208
5	0.013689254	0.2040161	0.2023266	0.03626654	0.2221685	0.2209954	0.1142269
6	0.016427105	0.2046623	0.2028231	0.03618690	0.2209705	0.2212890	0.1140682
	time	**se1**	**se2**	**se3**	**se4**	**se5**	**se6**
1	0.000000000	0.03251177	0.03348491	0.01310601	0.03201052	0.03621101	0.01778362
2	0.002737851	0.03252166	0.03349510	0.01310245	0.03200975	0.03621862	0.01778126
3	0.008213552	0.03256129	0.03353594	0.01308821	0.03200663	0.03624914	0.01777181
4	0.010951403	0.03260681	0.03358284	0.01307626	0.03200914	0.03628623	0.01776477
5	0.013689254	0.03270819	0.03368732	0.01304868	0.03201329	0.03636863	0.01774802
6	0.016427105	0.03281083	0.03377366	0.01302117	0.03200179	0.03644010	0.01772962

<u>Fig. 36:</u> Standard errors for the transition probabilities AE to Relapse and AE to Death (starting time: 100 days), both for the full model and the proportional baseline hazards model

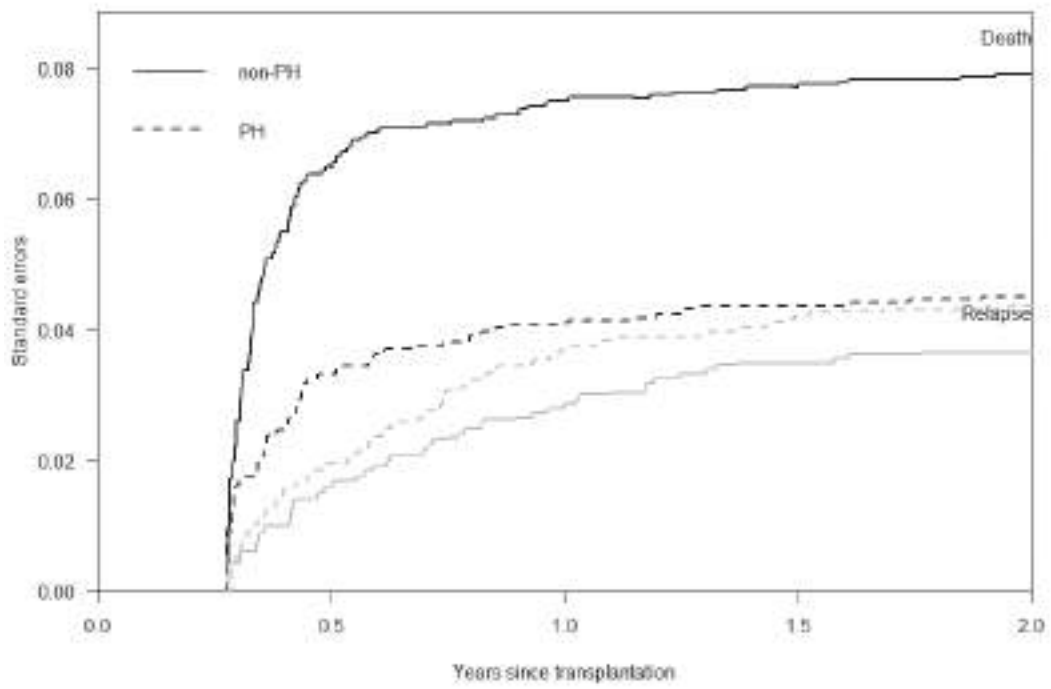


Figure 36 shows the standard errors of the transition probabilities from state 3 (AE) at time 100 days to states 5 (Relapse) and 6 (Death), for the full Cox model (non-PH) and for the proportional baseline hazards model (PH) and also shows that the proportional hazards assumption does not always decrease the standard errors of the predictions in either of categories. The main advantage of proportional baseline hazards models is the accuracy with which we obtain such a measure for the impact of intermediate events on the final outcome.

Conclusions: Multi-state models are a very useful tool to answer a wide range of questions in survival analysis, especially in the competing risks aspects. We have explored the different phases of a multi-state analysis: model building, data preparation, exploration of different covariate effects and baseline assumptions, estimation of hazards, transition probabilities and associated standard errors using *ebmt* data. In particular, it has been explained how

predictions can be rationalized with dynamic prediction if supplementary information is considered. This possibility is an important added feature of multi-state models compared to classical survival models.

STUDIES IN PROGRESS:

S2: Modelling spatial disease pattern and disease clustering of TB in Chennai

Principal Investigator	:	Dr. R.Srinivasan (email: srinivast_r@nirt.res.in)
Source of funding	:	Intramural
Study Period	:	2017-2019

Background: Studying spatial pattern of TB is very important for identifying the distribution of disease and also to identify high risk areas in Chennai. Despite RNTCP programme being successfully implemented to control TB cases in India from 1992, in Tamil Nadu, around 42,000 new cases were registered under RNTCP and every year 6000-7000 newly diagnosed cases are being registered in Chennai Corporation. Prevention and control of this disease would be assisted by spatial or spatio-temporal methods system using Geographical Information System (GIS). GIS is useful to identify location of the disease with characteristics of the location to examine reasons for spread of the disease in that particular location.

Objectives:

(i) To identify areas of high prevalence (hot spot) in Chennai, to test their statistical significance, and reasons behind the high prevalence of the disease

(ii) To detect spatial clusters and to test whether disease clustering is statistically significant and

(iii) To understand the underlying factors contributing to the spatial variation of disease in Chennai

Methodology: Chennai Corporation has been implementing the RNTCP and DOTS through a network of clinics and hospitals in 15 zones consisting of 200 wards. Patients aged > 15 years registered during 2014 to 2016 under the RNTCP programme in Chennai Corporation were considered for this study. The patient street address was geocoded by GIS. The following details were taken from the RNTCP register; age, sex, category of treatment, type of TB, address, locality of the house, etc with other environmental risk factors of air pollution. ArcGIS software was used for disease mapping in Chennai ward and SaTScan was used for cluster

detection of disease. R-Software and WinBUGS software were used for disease mapping, disease clustering and for ecological modeling. The spatial scan statistics method was used to find the disease clustering (hot spot) in Chennai. Multi-level was used to find the spatial variation of disease in Chennai.

Results: Table 65 shows the 15 zones of Chennai with number of wards in each zone, estimated population and TB cases taken for the period 2014 to 2016. The Crude Incidence rate per 1000 population and standardised morbidity ratio were calculated for all zones. The results of the CIR and SMR revealed that zone 4, 5, 6, 8 & 9 are having higher risk area compared to other zones. The highest values of CIR & SMR were found in zone 5 and lowest in zone 15.

Table 65: No of wards in each zone, estimated poplation and TB cases for the peiod 2014-2016

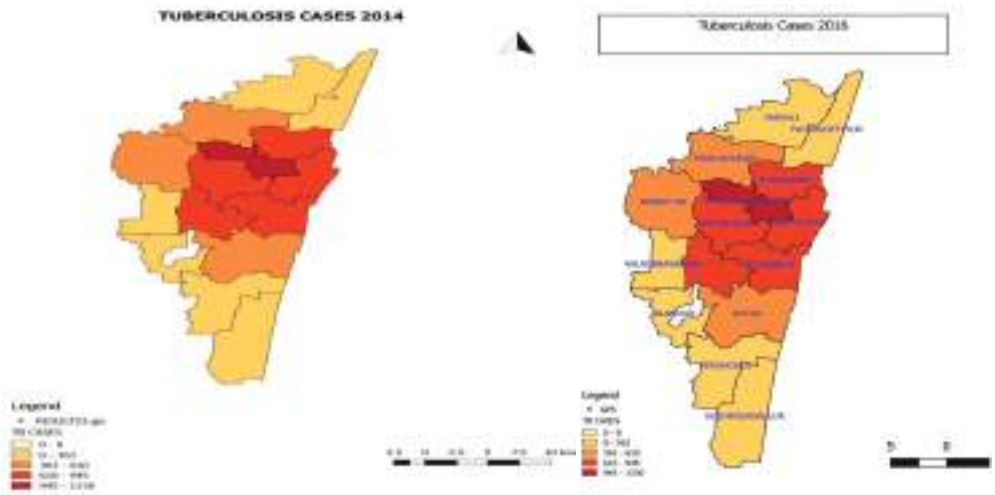
Zone	No.of Wards	No. of UPHC	Estd Popn.2015	TB CASES 2014	TB CASES 2015	TB CASES 2016	CIR (2014-16)/1000	SMR
1	14	6	45714	375	329	326	23.76	0.9
2	7	2	18286	469	328	330	11.32	0.43
3	12	4	27429	454	433	420	12.03	0.45
4	15	15	100571	1330	869	840	36.29	1.37
5	15	13	73143	0	665	765	43.86	1.66
6	15	14	91429	1067	1019	1005	36.73	1.39
7	15	9	73143	0	413	424	13.04	0.49
8	15	15	91429	1198	670	648	28.92	1.09
9	18	16	100571	914	919	919	29.46	1.11
10	16	15	100571	878	754	726	25.07	0.95
11	13	5	54857	821	256	283	12.94	0.49
12	12	5	36571	346	204	175	16.08	0.61
13	13	11	82286	0	612	569	26.55	1
14	11	5	36571	0	174	234	20.67	0.78
15	9	5	27429	0	333	288	8.35	0.32
Total	200	140	960000	7852	7978	7952		

Spatial clustering was tested using spatial scan software. Those areas having higher risk zones in Chennai were confirmed (Table 66).

Table 66: Spatial clustering tested using spatial scan software and confirmed areas having higher risk zones in Chennai

Risks areas 4,5,6,8 & 9	
Coordinates / radius.....	: (13.089700 N, 80.278900 W) / 7.46 km
Number of cases.....	: 15823
Expected cases.....	: 12090.00
Annual cases / 100000.....	: 1153.5
Observed / expected.....	: 1.31
Relative risk.....	: 1.82
Log likelihood ratio.....	: 1284.927103
P-value.....	: < 0.00000000000000001
Isotonic step-wise information:	
Radius for each step.....	: 0 km, 4.80 km, 5.68 km, 7.46 km
Number of cases by step.....	: 3208, 7008, 2963, 2644
Expected cases by step.....	: 1934.40, 5077.80, 2659.80, 2418.00
Observed/expected by step...	: 1.66, 1.38, 1.11, 1.09
Relative risk by step.....	: 2.31, 1.92, 1.55, 1.52

Fig. 37: Identifying higher risk clutering areas



Analysis reveals that the zones 4,5,6, 8 & 9 had higher risk for disease clustering areas in Chennai and spatial temporal changes between 2014 and 2016 does not exist (Fig. 37).

Conclusions: Identifying higher risk clustering areas are useful for early detection of TB diseases and screening the population will improve the control of TB, finding the factors behind the spread of the disease, and making suitable policies to control these factors. The study is ongoing and possible factors for higher risk in these areas are being studied.

S-3: Identifying TB cluster and the etiology behind it in Tamil Nadu using model based approach and GIS

Principal Investigator	:	Dr. K.Chandrasekaran (email: chandrasekarank@nirt.res.in)
Co-Principal Investigators	:	Dr. Srikanth Prasad Tripathy, Dr.G.Arivarignan (Prof (Retd), Dept. of Applied Mathematics and Statistics, Madurai Kamaraj University, Madurai.)
Source of funding	:	Intramural
Study period	:	2017-2019

Background: The RNTCP Annual Status Report, 2016 and WHO Global TB Report, 2015 emphasize the importance of more initiatives in India to bring TB under control. In this direction, identification of TB cluster in Indian regions with model based approach, clear understanding on the etiology behind the high TB risk in the cluster and taking effective measure to control the disease in the identified area are needed.

Study objectives:

(i) To identify TB clusters in Tamil Nadu

(ii) To estimate TB risk in each of the districts of Tamilnadu and then to

(iii) To identify the etiology behind the high risk and possible reasons for low TB risk in the identified districts

Methods: We have identified the spatial TB clusters using discrete Poisson

distribution and tested its significance using Log Likelihood Ratio. We have considered the population percentage of cluster size as fifteen and cluster with relative risk more than 1.2 as high risk. We have used SatScan software, version 9.4.4 for this purpose.

Results: We have considered RNTCP data for all the Districts in Tamilnadu for the year 2011 and 2016 to identify clusters for various forms of TB such as new sputum positive (NSP), new smear negative (NSN), previously treated sputum positive (Re-Rx) and extra PTB patients (EPT).

The identified most likely and secondary clusters for the year 2011 and 2016 with corresponding log likelihood ratio and p-value are shown in tables 67 & 68. In each TB category, the most likely clusters (having highest LLR) are denoted in bold letters.

Table 67: TB clusters in Tamilnadu for the year 2011

Sl_no	TB category	Cluster Districts	Log likelihood ratio (LLR)	Relative risk	p-value
1.	NSP	Thiruvannamalai, Vellore, Viluppuram	83.45	1.22	<0.01
2.	NSN	Viluppuram, Cuddalore	154.94	1.49	<0.01
3.		Theni, Virudhunagar	153.10	1.68	<0.01
4.		Dindigul	46.66	1.43	<0.01
5.		Thiruvarur	36.95	1.49	<0.01
6.		Karur, Tiruchirapalli	33.18	1.27	<0.01
7.	Re-Rx	Chennai	74.12	1.56	<0.01
8.		Viluppuram, Cuddalore, Tiruvannamalai	35.46	1.4	<0.01
9.		Madurai	23.46	1.37	<0.01
10.		Erode	6.11	1.21	0.03
11.	EPT	Chennai	217.76	1.79	<0.01
12.		Vellore	178.09	1.77	<0.01
13.		Tiruchirapalli	55.92	1.49	<0.01
14.		Kancheepuram	54.90	1.40	<0.01
15.		Cuddalore	43.86	1.44	<0.01
16.		Dindigul	36.87	1.43	<0.01
17.		Thiruvallur	19.88	1.24	<0.01
18.		Viluppuram	18.74	1.24	<0.01

Table 68: TB clusters in Tamilnadu for the year 2016

Sl_no	TB category	Cluster Districts	Log likelihood ratio	Relative risk	p-value
1.	NSP	Theni, Virudhunagar, Madurai and Dindigul.	229.08	1.4	<0.01
2.		Thiruvarur	89.05	1.61	<0.01
3.	NSN	Vellore	207.44	1.54	<0.01
4.		Viluppuram	197.05	1.56	<0.01
5.		Chennai _North	171.92	1.76	<0.01
6.		Chennai_South and Chennai_Central	74.07	1.31	<0.01
7.	Re-Rx -	Chennai_North, Chennai_Central and Chennai_South.	81.95	1.43	<0.01
8.		Madurai	46.63	1.43	<0.01
9.		Thiruvarur	27.53	1.51	<0.01
10.		Thanjavur	14.81	1.27	<0.01
11.	EPT	Vellore	243.91	1.94	<0.01
12.		Chennai_North	131.63	2.05	<0.01
13.		Chennai_South and Chennai_Central	86.46	1.52	<0.01
14.		Viluppuram	45.3	1.4	<0.01
15.		Perambalur	19.61	1.41	<0.01

With regard to NSP, TB clusters for the year 2011 were Thiruvannamalai, Vellore and Viluppuram with RR 1.22 and the cluster for the year 2016 were Theni, Virudhunagar, Madurai and Dindigul with RR 1.40. The most likely cluster for NSP for the year 2016 was a secondary cluster in the year 2011 with RR 1.16 and it was not included in the Table since the RR was less than 1.2.

The NSN clusters for the year were Viluppuram and Cuddalore with RR 1.49 and for the year 2016 was Vellore with RR 1.54. One of the most likely clusters District Viluppuram for NSN with RR 1.49 for the year 2011 was a secondary cluster with RR with RR 1.54 in 2016.

As far as Re-Rx clusters were concerned, the cluster for the year 2011 was Chennai (three Districts combined) with RR 1.56 and it was Chennai North, Chennai Central and Chennai South with reduced RR 1.43 in 2016.

For EPTB, the primary and secondary clusters for the year 2011 were Chennai (three Districts combined) and Vellore with RR 1.79 and 1.77. In the year 2016, the most likely and secondary clusters have interchanged with RR 1.94 and RR 2.05 (Chennai North with increased risk).

A survey will be conducted in the identified TB cluster Districts and the Districts having low risk for the year 2016, to understand the etiology behind the high TB risk.

The study is ongoing.

DEPARTMENT OF EPIDEMIOLOGY

STUDIES IN PROGRESS:

E-1: The prevalence survey of PTB in Tiruvallur district

Principal Investigator	:	Dr.C.K. Dolla (email: chandrakumar.d@nirt.res.in)
Source of fundnig	:	WHO-MDP
Study Period	:	2015-2018

Background: TB prevalence surveys, if periodically done, are useful to estimate the trend in TB prevalence in the community and the impact of control strategies, un-diagnosed cases, and the extent to which people with TB are being treated by health-care providers that are not linked to the NTP. This information can be used to identify strategies for active case finding and to improve the proportion of TB cases being captured by routine surveillance data. Other strategies that may be identified as relevant based on the results of a prevalence survey include expansion of active case-finding and contact tracing to specific high-risk groups, and all those with signs and symptoms suggestive of TB.

The study of prevalence of PTB by disease surveys started from the year 1999 in Tiruvallur district among adults aged >= 15 years. Later TB prevalence surveys were conducted as repeat surveys during the years 2003, 2006 and 2009 at an interval of two and half years. From 1999-2006, the prevalence of culture-positive TB decreased from 607 to 307 per 1, 00,000 population with decline of 12% per annum after implementation of DOTS strategy in 1999. However, there was an increase in the prevalence of culture positive TB from 307 to 388 per 1, 00,000 in the survey conducted during 2006-2008 in the same area. In order to determine whether the increased prevalence has remained same or changed after interventions, one or more prevalence surveys are proposed to be conducted after a gap of 7 years in the same area. Also, it is necessary to conduct surveys to see the trend of disease in the community, to formulate future control policies and to assess the impact of ongoing control programs.

Primary:

(i) To estimate the prevalence of PTB among adults in Tiruvallur district in 2015 and

(ii) To determine the prevalence of TB among contacts less than 10 years in TB affected households

Secondary:

(i) To determine the association between PTB and tobacco smoking, alcohol consumption, biomass fuel usage and BMI and

(ii) To know the geographical distribution of tuberculosis cases in Tiruvallur district.

Methodology:

Study design: Community based cross-sectional study in the villages previously surveyed during 2006-2008.

Study setting: Five blocks of Tiruvallur district, in which the impact of short course chemotherapy and DOTS is being monitored over a period of 15 years under Model DOTS project was studied.

Study population: Adults aged 15 >= years and above and contacts of TB patients less than 10 years of age formed the study population**.**

Data collection: All persons from each household were included in the study from the selected villages registered by door to door census. After obtaining informed consent from those aged 15 years and above, information about chest symptoms were collected using a questionnaire. Height in centimeters, weight in kilograms, blood pressure using digital sphygmomano-meter were recoded for all participants > 30 years age. Data on biomass fuel usage was collected from the head of the family in each household. Details regarding tobacco smoking and alcohol use were collected from all male participants.

Chest radiograph (digital X-ray) was taken for all participants in the field through Mobile X-ray Unit. The radiographs were read independently by two readers. In case of disagreement between these two readers, it was read by a third reader. For those with abnormal radiographs and symptoms, attempts were made to collect two sputum samples. Sputum samples were examined by fluorescence microscopy and cultured on Lowenstein-Jensen medium. Sputum samples were also tested with Gene-X-pert. Those yielding growth on culture were subjected to identification tests for *M. tuberculosis* and drug susceptibility tests. Further,

spoligo-typing of the identified species was done.

If the sputum sample was positive for TB based on smear and/or culture, the respective person was informed about his disease condition and referred to the nearest RNTCP center for further management.

If a participant was diagnosed of TB, we screened the household contacts who were less than 10 years by symptom screening, Mantoux test, smear and radiological examination for diagnosis of TB. If there were children less than 5 years of old living with sputum and or culture positive cases diagnosed from our survey, we refered them to RNTCP for Isoniazid prophylaxis. The GPS coordinates for the surveyed houses was collected for finding the geographical distribution of the TB cases.

Sample size: The sampling design which was used in the baseline survey (1999) and the subsequent repeat surveys was adopted for this survey. The required sample size was estimated to be 82000 adults aged 15 years for an annual incidence of culture-positive TB of 260 per 100000 population, a precision of 20% at 95% confidence level, a proportion examined (coverage) of 90% and a design effect of 2. The baseline survey was undertaken in a random sample of 50 of 208 villages and three of 10 urban units separately in the five blocks in 1999–2001. Based on the growth rate in the survey area, it has been estimated that an adult population of 1, 00,867 (year 2014) will be covered for this current survey.

Current status of survey is reflected in tables 69 & 70.

Main findings:

Table 69: Information on the current status of subject registration and x-ray activity

	Team I	Team II	Total
No of Working Days	528	528	528
No. Census	44064	44016	88080
No. Ineligible (0 - 14 years)	9317	9422	18739
No. Eligible for Xray	34697	34667	69364
No. Xrayed	29754 (85.75%)	30755 (88.72%)	60509 (87.23%)
Average X-ray per working day	56	58	115
No of BP readings	30272	30730	61002
No of Random Symptoms	3041	3113	6154

Table 70: Information on the current status of subject registration and x-ray activity

	Regular	Additional Collection	Total
No. eligible for sputum collection	6121	11	6132
By symptom	5089	0	5089
By Xray	1032	0	1032
No. of individuals from whom sputum collected	5706	11	5717
No. of specimens collected	11331	0	11331
Spot Specimens	5706	0	5706
Overnight Specimens	5625	0	5625
No. of working days	654	0	654
Average collections per working day	17	0	17

Regular sputum collection has been completed in 28 villages. Out of the 11331 specimens, 146 were smear positive and 255 were culture positive.

Paediatric contacts of treatment referrals: Children among the household contacts of a bacteriologically TB positive case detected in this survey (Children aged <10 yrs) were examined for TB symptoms along with Mantoux

and x-ray examination. If any of these were abnormal, sputum examination is done. The details of the contacts identified and action taken are given in Table 71.

Table 71: Contact details

Contacts identified	80
Contacted	77
Symptoms present	1
Mx done	60
Mx reading done	60
X-ray done	61
Sputum	1
GL/LN	0
Refused	10

No. of patients referred for treatment : 184 (2.65 per 1000's) among X-ray eligible persons

Recruitment of participants to the survey has been completed. Data analysis is in progress.

E-2: Retrospective observational time series study for fifteen years TB relational impact with climatic factors: Multi-centric study

Principal investigator : K Rajendran (email: rajendran.k@nirt.res.in)
Source of Funding : Intermural
Study period : 2015-2018

Objective: The objectives of the study are to determine the relation between TB cases and climatic factors such as temperature, relative humidity, wind direction, dew points, rainfall and air pollution in Chennai and Madurai from 2000 to 2015. From the comparative statistical analyses, the characteristics of the climatic factors will be elucidated for the more effective prediction and disease management as given below:

(1) Application of time series models to find out the TB disease pattern and long term variation

(2) Analytical strategies of seasonal composition factors for Time series models and

(3) Relate the influence of climatic factors on TB infection -Application of General Linear model

Study progress: Fifteen years of (2001-2015) climate data for factors like temperature, relative humidity. sunshine duration, wind direction, dew points and rain fall have been procured from Department of Meteorology for Chennai and Madurai. The individual TB *cases* data are being collected from NIRT and RNTCP centres at Chennai. Entry of about 7054 cases has been completed for the years 2008, 2009, 2010, 2012, 2014 and 2015. Climate data has been cleaned and is made ready for analysis after checking the nature of variables. TB patient's data collection is ongoing in the RNTCP centre at Chennai. Preliminary analysis has been initiated to build a time series model. Figs. 38 & 38A shows seasonal influence of TB patients with climate factors.

The study is ongoing.

<u>Fig. 38:</u> Seasonality influence TB with climate factors

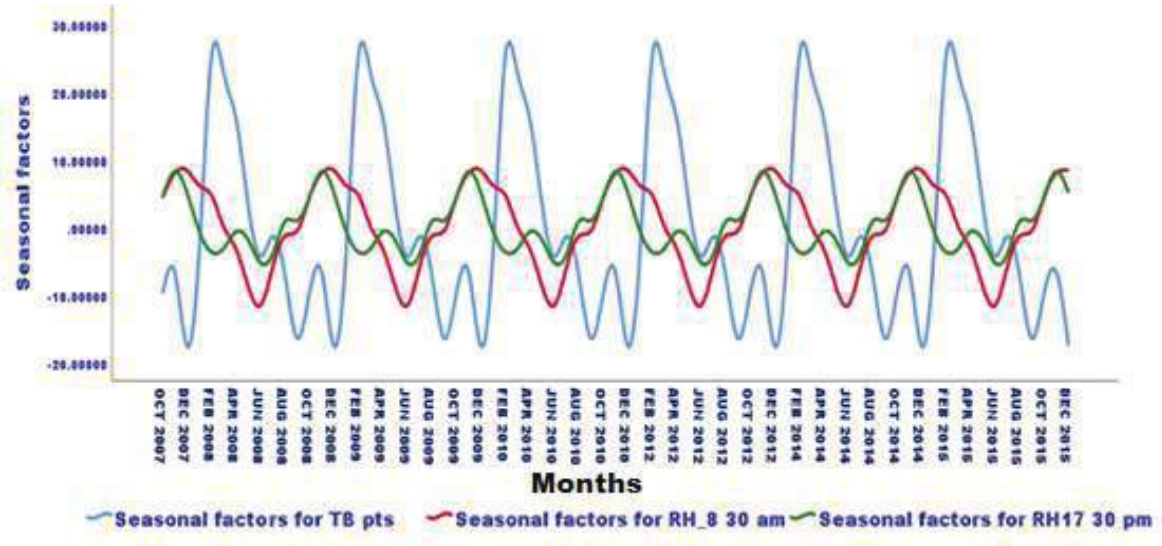
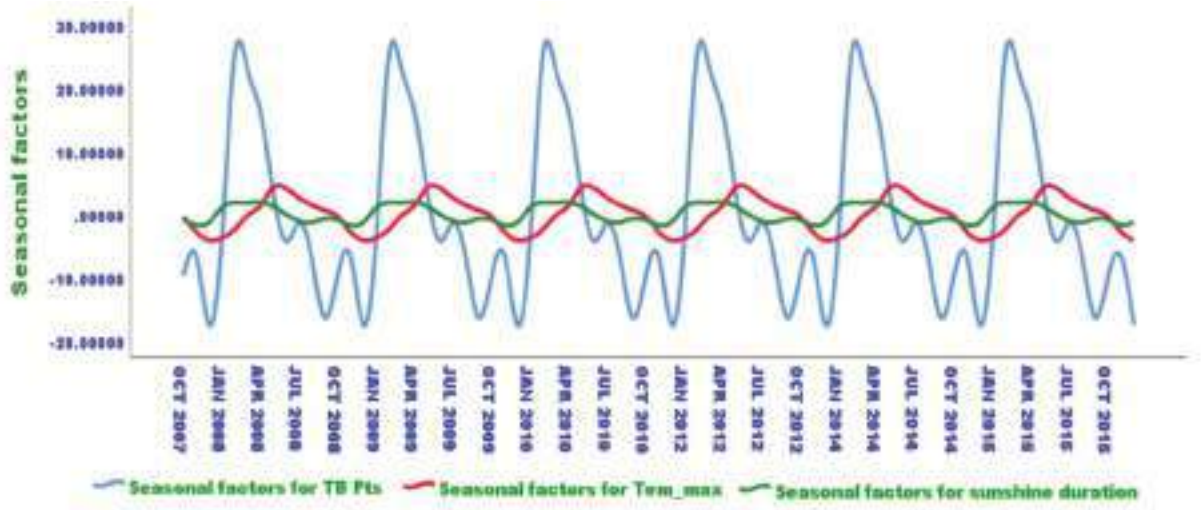


Fig. 38A: Seasonality influence TB with climate factors?



E-3: Tuberculosis among homeless persons, Chennai city

Principal Investigator	:	Dr C K Dolla (email: chandrakumar.d@nirt.res.in)
Source of Funding	:	Extramural ICMR
Status of Study	:	Ongoing
Study duration	:	2017-2020

Background: TB disease/infection in homeless persons is significant because of TB outbreaks and emergence of DR-TB among this group. TB outbreaks occur due to the risk factors they are exposed to, such as, overcrowding in shelters, poor ventilation, HIV infection, alcohol, drug use and under- nutrition. Further, homeless persons are more likely to be diagnosed late. Emergence of DR-TB in homeless people is likely due to difficulty in taking medications on a regular basis. TB infection rates among the homeless persons are 20 times more than in the general population. High prevalence of inactive TB (LTBI) is important, as the risk of reactivation is about 90% due to the impoverished living conditions and poor nutritional status among the homeless.

Objectives:

(i) To estimate the prevalence of TB (active disease and latent infection) among homeless people of different zones in Chennai city and

(ii) To identify the distribution of risk factors associated with TB among homeless people

Sample size: 4936 homeless persons

Study design: Cross-sectional community prevalence survey.

Methods: All the homeless persons were screened for signs & symptoms of PTB and X-ray chest PA view was taken in eligible individuals i.e. ≥ 15 years age group. Those who refused to participate in study and with morbid conditions were not included. Two spot sputum samples were collected from symptomatics and with abnormal X-ray. The sputum samples were subjected to smear and culture examinations. The smear positive samples were further tested for RMP resistance in gene X-pert machine.

Findings: Three groups were Identified: Gr.1.Sheltered homeless, Gr. 2.Foot path dwellers and pavement dwellers, Gr.3. Homeless persons sleeping in the night under bridges, flyovers etc.

The recruitment details and summary findings are given in Table 72.

Table 72: Recruitment details

Groups identified	No. Screened	X-ray +ve	*Sputum +ve	*Culture +ve	Gene X-pert
Shelter	1076	55	1	1	Sensitive
#Pavement/Road side dwellers	318	8	2	2	Sensitive

*same individuals. # screening underway

Conclusion: There was low rate of active TB in shelter homeless group (one smear positive in 1076 persons), compared with other groups (2 smear positive among 318 pavement dwellers). The study is in progress.

BIOMEDICAL INFORMATICS

COMPLETED STUDIES:

B-1: Database for drug resistant TB (DDRTB)

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Source of funding	:	ICMR Biomedical Informatics Project
Study period	:	2014-2018

Background: Drug resistant TB has become a global threat and public health priority. On the one hand, a large amount of valuable clinical, biomedical and molecular data is being generated by medical professionals employed in the management of DR-TB as well as researchers working in this field. If this data is systematically captured in an electronic format and made available in the form of an integrated database it could prove highly useful for understanding various aspects of the pathogenesis and profile of the disease and help in devising strategies for the control and spread of the disease.

Aim:

(i) To develop an integrated database containing systematically captured clinical, socio-demographic, microbio-logical, and molecular data on DR-TB cases.

Method and outcome: The above mentioned data were captured for over 200 variables categorized as demographic data, molecular data, laboratory investigations, drug resistance pattern, X-ray reports and pharmacokinetic data. The database currently contains data on 37 XDR and 74 MDR-TB patients captured longitudinally at every single time point of follow-up during the entire course of treatment. All the data currently present in the database are from participants enrolled at NIRT only. However, the scope of the database is not restricted to viewing available data alone, but for use as an online portal to deposit data on DR-TB cases from across the country. Users will also be able to perform comprehensive analysis of drug resistance related data using tools and links provided in the database. The database will be made available online shortly.

B-2: *In silico* analysis of the variable loops of HIV gp120 protein to aid in the differentiation of R5, X4 and R5X4 HIV viruses

Principal Investigator	:	Dr. Luke Elizabeth Hanna (email: hanna@nirt.res.in)
Source of funding	:	ICMR Biomedical Informatics Project
Study period	:	2014-2017

Backround: Co-receptor usage and tropism of HIV isolates is of clinical significance because of its relevance in HIV disease progression and its potential as an important drug target. This has led to the development of several *in silico* tools for predicting tropism based on V3 loop sequences. However, it has become evident that although the existing methods can distinguish between the R5 tropic viruses that use CCR5 as their co-receptor, and X4 tropic viruses that use CXCR4 as the co-receptor, with a high degree of sensitivity, the accuracy of prediction of dual tropic viruses that are capable of using more than one co-receptor is very poor. This led us to undertake a detailed investigation of all the five 5 variable regions (V1-V5) of the HIV-1 envelope in order to identify molecular determinants for expanded co-receptor usage that can be incorporated in to the existing tools to improve the sensitivity of prediction and enable accurate prediction of dual tropic viruses.

Methodology: For this purpose, full length gp120 sequences of the 3 groups of viruses, R5-tropic (n=1260), X4-tropic viruses (n=650) and R5X4-tropic viruses (n=493), were obtained from published literature and databases. Hidden Markov Model based alignment was performed with the V1-V5 variable loop region sequences. Sequences were then coded for features such as hyrophobicity, bulkiness and polarity for discriminant analysis. One-way ANOVA was performed using the total charge of the protein, length and glycosylation sites.

Results: Discriminant statistical analysis of the V1-V5 regions of the HIV-1 gp120 revealed a few significant differences between the 3 tropic groups (R5, X4, R5X4). While the V4 and V5 loop sequences did not contribute significantly to the differences seen between the 3 viral tropic groups, we

identified unique characteristics in the V1 and V2 loops that were different in the 3 tropic groups, suggesting the importance of the V1/V2 loops in contributing to expanded co-receptor usage in dual tropic virus strains.

B-3: Database of drugs for TB (TB-DRUGS, Version 2.0)

Principal Investigator	:	Dr. Jagdish Chandrabose (email: sjcbose@gmail.com)
Source of funding	:	ICMR Biomedical Informatics Project
Study period	:	2013-2018

Background: Significant success has been achieved in drug repurposing against various clinical conditions. These encouraged researchers to evaluate several known drugs for anti-TB activity, and a number of drugs have been identified to have a potential for repurposing for TB treatment. We collected this information and developed it in the form of a database called 'TB DRUGS'. Version 1.0 of the database was launched online in 2016 (accessible at http://bmi.icmr.org.in/tbdrugs/ and http:// bic.icmr. org.in/tbdrugs/).

Aim:

(i) To expand the existing database by incorporating more number of drugs which are being explored for repositioning/repurposing for TB treatment

Methodology and Outcome: The second version of the database TB DRUGS contains information on 33 additional drugs reported to have potential for repurposing for TB. The mechanisms of action of these drugs include inhibition of efflux pumps of *M. tuberculosis*, stimulating autophagy and acceleration of bacterial clearance, thus enhancing the activity of anti-TB drugs. Along with many of the variables used in the first version of the TB drugs, 'relevance to TB' (a brief description about how these drugs are useful for TB) and 'primary use' are provided for the drugs included under the 'drug repurposing' tab.

Significance: The TB DRUGS database (version 2.0) will facilitate clinical researchers in selection of drugs for further evaluation towards repurposing for TB tratment.

B-4: Database of genome-wide variations in *M. tuberculosis* from India

Principal Investigator	:	Dr. Jagdish Chandrabose (email: sjcbose@gmail.com)
Source of funding	:	ICMR Biomedical Informatics Project
Study period	:	2013-2018

Background: Emergence of DR-TB worldwide has caused major concern for the control of TB. Delay in the diagnosis of the drug resistance leads to increase in the burden of DR-TB. Though, newer molecular methods including GeneXpert and Line Probe Assay enable rapid diagnosis of DR-TB, they are limited to few drugs and their accuracy is yet to be matched precisely to the phenotypic determinants of drug resistance in *M. tuberculosis*. To improve the power of rapid diagnosis of DR-TB, we need to have a complete catalogue of mutations that can accurately explain/define phenotypic drug resistance. Globally, efforts to sequence the whole genome of *M. tuberculosis* isolates have grown. Some of the mutations associated with drug resistance are known to be specific to different regions and countries and also to different lineages of *M. tuberculosis*. In this context, we recently identified some novel mutations potentially associated with drug resistance in one of our studies based on WGS of 223 *M. tuberculosis* strains from south India. These evidences support the need for country-specific diagnostic methods for accurate and rapid detection of DR-TB.

We initiated an effort to develop a comprehensive database of genome-wide mutations from *M. tuberculosis* using whole genome sequence data generated from Indian studies, with the

hope that this could serve as a useful resource for TB researchers involved in discovery of novel drugs and diagnostics. However, the study could not be completed because the project term ended before the completion of the study.

B-5: Sequence and structural analysis of IDH1 and IDH2 proteins of glioma patients

Principal Investigator	:	Dr. Sameer Hassan (email: sameerhassan1@gmail.com)
Collaborators	:	Sher-i-Kashmir Institute of Medical Sciences)
Source of funding	:	ICMR Biomedical Informatics Project
Study period	:	2013-2018

Background: Mutations in the Isocitrate Dehydrogenase isoforms 1 and 2 (IDH1 and IDH2) have received considerable attention since the discovery of their relation with human gliomas. Mutations in IDH 1 and 2 been reported in a large number of low grade gliomas and secondary high grade gliomas. These mutations that occur early in gliomagenesis, change the function of the enzymes, causing them to produce 2-hydroxyglutarate, a possible oncometabolite, instead of NADPH.

Aim:

(i) To identify mutations in the IDH1 and 2 genes of glioma patients and investigate the impact of the mutations on protein structure and function

Methodology: Collection of blood samples from human glioma patients and sequencing of IDH1 and IDH2 genes were carried out at the collaborating institute. Analysis of sequence data was undertaken at NIRT.

Findings: In addition to the hot spot mutations already reported, two novel mutations were identified in IDH1.

B-6: Synthesis and evaluation of novel NO donors for treatment of type II diabetes and inhibition of aldose reductase activity

Principal Investigator	:	Dr. Sameer Hassan (email: sameerhassan1@gmail.com)
Collaborators	:	Prof.P.K. Zubaidha, Head, School of Chemical Sciences, Swami Ramanand Teerth Marathwada University, Nanded, Maha

Source of funding	:	ICMR Biomedical Informatics Project
Study period	:	2013-2018

Background: Type 2 diabetes, one of the major life-threatening diseases worldwide, continues to progress at an incremental rate every year and most of the research work to control the disease target either enzymes or proteins. Aldose reductase the key enzyme in the polyol pathway gets activated under hyperglycemic conditions and reduces glucose to sorbitol. Aldose reductase is an important drug target and their inhibitors are promising therapeutic agents to combat the development of late diabetic complications such as neuropathy, nephropathy, retinopathy and cataract.

Methodology: Inhibitory effect of NO donors on aldose reductase purified from bovine lens was investigated. The experimental study revealed that 20 of the synthetic molecules tested had good inhibitory activity. *In silico* studies including molecular docking were performed for the 20 molecules to investigate the binding conformation within the active site of the enzyme. Molecules with the highest docking scores were tested *in vitro* for biological activity in terms of aldose reductase inhibition.

Results and Conclusion: Molecular docking studies performed to understand the plausible binding modes of the synthesized K and S schema compounds within the active site of the aldose reductase protein, identified K2 and K9 as well as S5 and S6 molecules with highest docking score. These molecules were also observed to have the highest biological activity in *in vitro* studies with the aldose reductase obtained from bovine lens, suggesting that these molecules could be potential leads for treating diabetic complications.

B-7: Exploring the drug resistance mechanisms in HIV-1 protease

Principal Investigator	:	Dr. Sameer Hassan (email: sameerhassan1@gmail.com)
Source of funding	:	ICMR Biomedical Informatics Project
Study period	:	2013-2018

Background: The Human immunodeficiency virus encodes an aspartic protease (PR) that cleaves viral polyproteins into mature proteins, leading to the formation of infectious particles. Protease inhibitors are successful virostatics; however their efficiency is compromised by antiviral resistance. The structural conformation of the enzyme is a key determinant of both biological function as well as binding to protease inhibitor molecules.

Methodology: In the present study we analyzed 471 crystal structures of HIV-1 protease to understand the conformational changes induced by mutations or binding to various ligands and substrates. We performed principal component analysis on the ensembles of the HIV-1 protease structures to explore the conformational landscapes.

Results and conclusion: The study identified structural differences between drug resistant and drug sensitive protease structures. We found that though mutations induced conformational changes in the active site of the protease structures, the protease inhibitors currently in use do not undergo conformational changes to enable binding to the protease enzyme. Designing new inhibitors with greater flexibility that can adapt themselves within the active site of protease structure which may be altered by mutations will be highly beneficial. This strategy can lead to the design of a new generation of drug molecules that will be largely resistant to resistance mutations.

B-8: Genome-scale analysis of proteins of pathogens and their ligands enables prioritization of drugs for repositioning

Principal Investigator	:	Dr. Jagdish Chandrabose (email: sjcbose@gmail.com)
Source of funding	:	ICMR Biomedical Informatics Project
Study period	:	2013-2018

Background: Identifying new clinical indications for existing (FDA approved) drugs other than the intended primary use is defined as repositioning or repurposing. Repositioning is a successful process and become popular in drug discovery industry and academia since it reduces the significant amount of time and money spent in clinical trial phase I as the safety related issues in human for the known drugs are already established. The advantages of repositioning have resulted in increased efforts globally to explore the repurposing of drugs for various diseases including cancer, neurodegenerative diseases including Alzheimer's and Parkinson's disease, orphan diseases and rare and neglected diseases. Among the drugs used currently for TB, fluoroquinolones (levofloxacin, MOX, OFX, gatifloxacin), clofazimine, linezolid, meropenem-clavulanate and imipenem-cilastatin were introduced for TB therapy through repositioning. Fluoroquinolones are used against MDR-TB while clofazimine, linezolid, meropenem-clavulanate, imipenem-cilastatin are listed as group V drugs and used against XDR-TB (WHO 2014).

Aim:

(i) To prioritize known drugs with a potential for repositioning for TB treatment

Method: To achieve this aim, we developed a protocol that is summarized as following: the structural genomics of *M. tuberculosis* was defined using three-dimensional structure available for unique proteins, and it was chosen as the defined data set of initial drug targets for *M. tuberculosis*. Sequences of these proteins are compared with 4252 known drug targets (of known drugs) to prioritize drug targets from *M. tuberculosis*. All of the ligands known to be crystalized with initial drug targets were compared with known 1877 drugs.

Findings: The study identified a set of drugs with significant levels of sequence and structural similarity with the natural

ligands. Subsequently, molecular docking identified the five top scoring drug that could be potential candidates and may be explored for anti-TB activity in a subsequent study.

ELECTRONIC DATA PROCESSING DIVISION

Electronic Data Processing

The Electronic Data Processing division plays two major roles within the organization:

(i) It maintains IT equipments and network services and also provides troubleshooting services whenever required;

(ii) It provides data entry/verification support for the research undertaken in the epidemiological unit, clinical division, laboratory and other operational research studies. It also plays a key role in the conduct of Epidemiological surveys.

The Electronic Data Processing division plays a key role in the conduct of Epidemiological surveys. The data collected during the epidemiological survey forms the basis of subsequent data analysis which in turn will help in writing reports for publications. In addition, the division provides data entry/verification support for several studies conducted at the Institute.

The division delivers a supportive role to the Administration in its endeavours to move towards electronic based administrative services like eProcurement and eMarketing, which are internet based avenues provided by the Central Government.

Data entry/verification work:

EpiData Entry is used for simple or programmed data entry and data documentation. Entry handles simple forms or related systems Optimised documentation and error detection features.

The quantum of data records of epidemiological, clinical, laboratory and program based studies entered and verified from April, 2017 to March, 2018 is shown in Table 73.

Table 73: Details of data records

	No. of records entered	No. of records verified
Clinical studies	8024	7823
Thiruvallur TB Prevalence Survey	32582	32582
An experimental study on the effectiveness of a School Health Intervention Programme for Tuberculosis case finding through corporation schools students in Chennai	463	463
Total	41069	40868

REDCap: REDCap is a secure web application for building and managing online surveys and databases. While REDCap can be used to collect virtually any type of data, it is specifically geared to support online or offline data capture for research studies and operations. NIRT has obtained license under the REDCap consortium and is in the process of starting data capture for a study using this web application.

Maintenance of IT equipments: The EDP division maintains an internal network of over 100 desktop workstations, network based equipment, operating systems and servers. All break-down calls of computers and its peripherals are dealt under comprehensive annual maintenance contract. This includes managing installations and ensuring that the computers are maintained and kept up to-date.

The division helps in providing audio-visual system for presentation of research materials during conferences, meetings and training programmes held at the centre.

Management of LAN: The management of the LAN facility is carried out with the support given by NIH-ICER project.

Highlights:

1. User Account with email access – 300 users
2. Radio Frequency Facility to enable Internet Connection through National Knowledge Network Project to Tiruvallur & Madurai.

3. First ICMR Institute to Enable Eduroam (The Educational & Research global free Wi-Fi roaming service) in the Campus
4. First ICMR Institute to Enable Shibboleth IDP which is the world's most widely deployed federated identity solutions, connecting users to applications both within and between organizations in the Campus.
5. Bio-thermic Phase I completed in IAVI Facility, which enables live monitoring of Freezers, Incubators, etc. through the RePort Study Project.
6. Bio-thermic Phase II started in the Main Lab Building, ICER, and Clinic Facility to monitor Freezers, Incubators, humidity, nitrogen Gas.
7. New Access Points for the NIRT Campus project has been started to have a Wi-Fi enabled campus.
8. New Access Layer Managed switch has been installed in most of the locations, replaced few unmanaged switch to Managed switches. New Fibre Uplink provided between ICER Facility to IAVI & NIRT Administrations.
9. New Netapp 21TB Storage installed in the Data Centre for the additional requirement of the Research Faculty.
10. Managed Power Distribution Unit installed in the Data Centre and UPS Power distributed to the Data Centre with proper power input & output coding.
11. Video Conference Facility has been built in Conference room, Main Lab Building with the help of ECHO India, Delhi.
12. We have migrated the following servers into NIRT Domain from old domain.
    - ✓ File Server
    - ✓ SMS Server
    - ✓ Monitoring Tool Server for Labs
    - ✓ Storage Server
13. Based on the requirement of Institute we have created new servers for Research studies and administration which are all in the production.
    - ✓ STHIRA Application for NIRT Administration & Finance
    - ✓ Database Server for STHIRA Application

- ✓ Calibration server for monitoring the Freezers
- ✓ Radius Server for Eduroam
- ✓ New Backup Server
- ✓ Redcap Server
- ✓ Federation Identity Server

14. Project planning for upgrading the Data Centre with additional PAC unit and network cabling. Adding more power requirement for the Data Center from 63AMPS to 250AMPS. Adding more server racks to Data Centre to equip more servers and Storage

15. Project planning for converting one of the conference rooms to Tele-Learning room for online training and conference.

Data conversion: The Electronic Data Processing division has archived datasets of major research projects undertaken by the Epidemiology Unit since 1968. Most of them are data captured using the IBM 80-column punched card. An exhaustive list of all the studies undertaken by the Epidemiology Unit of NIRT since 1968 has been prepared. The Division is now in the process of converting all these available old databases to the latest database formats.

Research publication on the BCG study: This was a 15 year large scale community based randomized controlled clinical trial carried out in Chengelpet district of south India to evaluate the protective effect of BCG against bacillary forms of pulmonary tuberculosis. This was undertaken between 1968 and 1987. Follow-up data of 281159 individuals are available over the 15 year period.

Leprosy Prevention Trial: Leprosy component was added to the then ongoing BCG trial in 1973, five years after the initiation of the trial. All the available population in the BCG trial area was covered for leprosy surveys. In addition to the baseline survey, a total of five surveys at intervals of 2.5 years were undertaken. Data conversion of Leprosy Prevention Trial from the 80 column format to Microsoft Access format is in progress.

INTERNATIONAL CENTRE FOR EXCELLENCE IN RESEARCH

COMPLETED STUDIES:

(I) Immunology of parasitic infections: A Elevated systemic and parasite – antigen stimulated levels of Type III interferons in a chronic helminth infection and reversal following antihelminthic treatment

Principal Investigators	:	Dr. Subash Babu; Dr. P. Paul Kumaran (email:sbabu@nirt.res.in; ppkumaran@ nirt.res.in)
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH)
Study Period	:	2016-2017

Background: Type III interferons (IFNs) are important players in immunity to viral and bacterial infections. However, their association with helminth infections has not been examined.

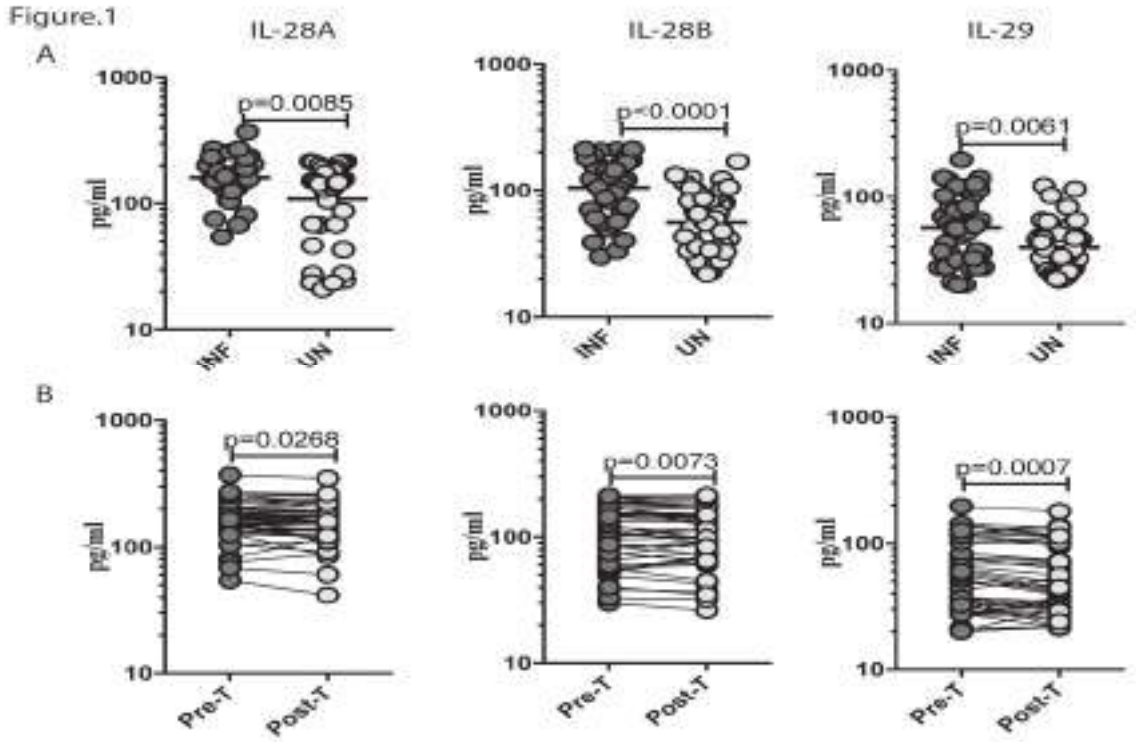
Aim/ Methodology:

(i) To explore the association of Type III IFNs with *Strongyloides stercoralis* (*Ss*) infection, we examined the systemic levels of IL-28A, IL-28B, IL-29 in *Ss* infected (INF, n=44), helminth – uninfected (UN, n=44) and in post treatment INF individuals. We also examined the levels of IL-28A, IL-28B and IL-29 in whole blood culture supernatants stimulated with *Ss* somatic antigens or LPS. Finally, we performed correlations of systemic Type III IFN levels with absolute numbers of dendritic cells subsets

Results: *Ss* infection was characterized by elevated systemic levels of IL-28A, IL-28B and IL-29 (Fig. 39) in comparison to UN individuals with a significant reduction following anthelmintic treatment. *Ss* infection was also characterized by elevated levels of unstimulated or *Ss* antigen stimulated levels of IL-28A, IL-28B and IL-29 and a significant reduction following treatment. In addition, *Ss* infection was characterized by increased numbers of plasmacytoid and myeloid dendritic cells in comparison to UN individuals, with a significant reduction following anti-helminthic treatment of INF individuals. Finally, *Ss* infection exhibited a significant positive correlation between the systemic levels of IL-28A and IL-28B and the numbers of plasmacytoid dendritic cells.

Conclusion: Ss infection is characterized by elevations in systemic and angigen-induced levels of Type III IFNs, which is positively associated with the numbers of plasmacytoid dendritic cells and reversed upon anti-helminthic treatment.

<u>Fig.39:</u> Ss infection is associated with elevated plasma levels of Type III IFNs and reversal following treatment



(A) The plasma levels of Type III IFNs, IL-28A, IL-28B and IL-29 were measured in *Ss*-infected [INF] (n = 44) or un-infected [UN] (n = 44) individuals. The data are represented as scatter plots with each circle representing a single individual. P values were calculated using the Mann– Whitney U-test with Holms correction for multiple comparisons. The horizontal line represents the geometric mean values. (B) The plasma levels of Type III IFNs, IL-28A, IL-28B and IL-29 were measured in *Ss*-infected INF individuals at pre-treatment [pre-T] (n = 44) and 6 months following treatment [post-T] time points. The data are represented as line graphs with each line representing a single individual. P values were calculated using the Wilcoxon signed rank test.

I. Immunology of parasitic infections: B. Elevated systemic levels of Eosinophil, Neutrophil and mast cell granular proteins In *Strongyloides Stercoralis* infection that diminish following treatment

Principal Investigators	:	Dr. Subash Babu; Dr. P. Paul Kumaran (email:sbabu@nirt.res.in; ppkumaran@ nirt.res.in)
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH)
Study Period	:	2015-2017

Background: Infection with the helminth parasite *Strongyloides stercoralis* (*Ss*) is commonly clinically asymptomatic that is often accompanied by peripheral eosinophilia. Granulocytes are activated during helminth infection and can act as immune effector cells. Plasma levels of eosinophil and neutrophil granular proteins provide an indirect measure of granulocyte degranulation and are markedly increased in many helminth-infected patients.

Aims/ Methodology:

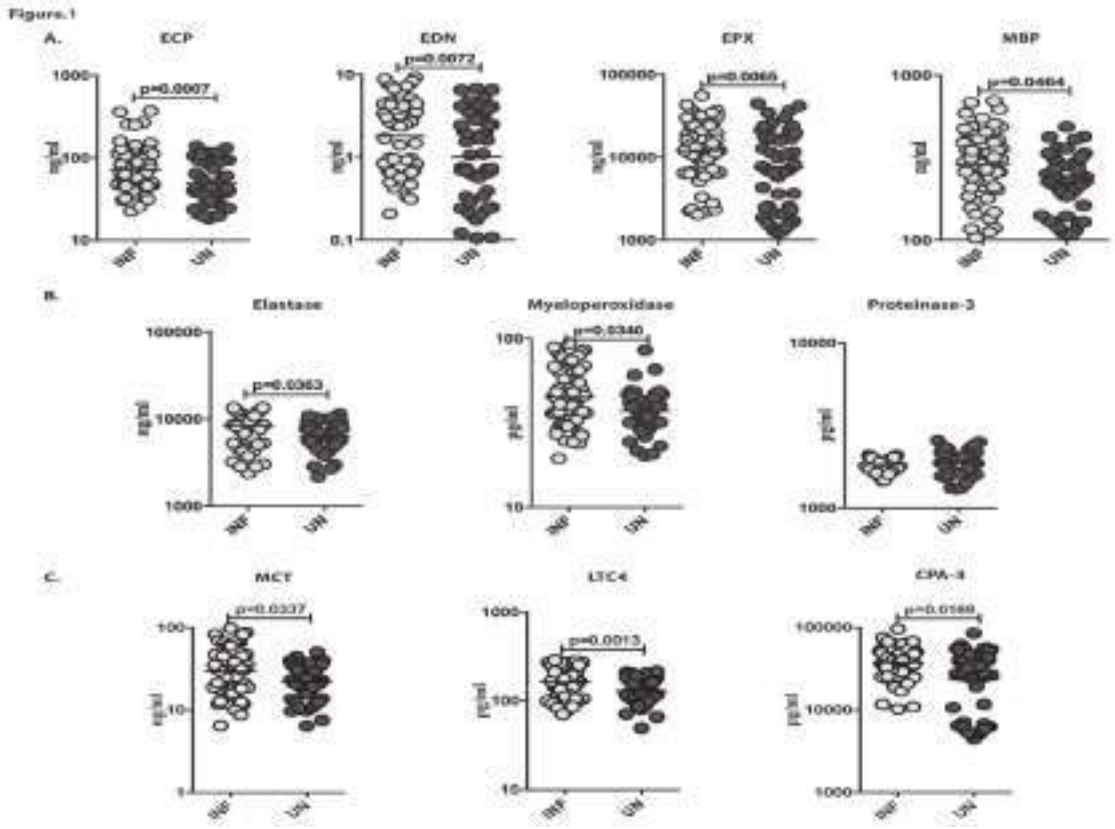
(i) To examine the levels of eosinophil, neutrophil and mast cell activation-associated granule proteins in asymptomatic *Ss* infection and to understand their kinetics following antihelminthic therapy. To this end, we measured the plasma levels of eosinophil cationic protein, eosinophil derived neurotoxin, eosinophil peroxidase, eosinophil major basic protein, neutrophil elastase, myeloperoxidase, neutrophil proteinase-3, mast cell tryptase, leukotriene C4 and mast cell carboxypeptidase-A3 in individuals with asymptomatic *Ss* infection (INF) or without *Ss* infection (UN). We also measured the levels of all of these analytes in infected individuals following definitive treatment of *Ss* infection

Results: INF individuals had significantly higher plasma levels of eosinophil cationic protein, eosinophil derived neurotoxin, eosinophil peroxidase, eosinophil major basic protein, elastase, myeloperoxidase, mast cell tryptase, leukotriene C4 and carboxypeptidase-A3 compared to UN individuals (Fig. 40). Following treatment of *Ss* infection, each of these

granulocyte-associated proteins decreased significantly.

Conclusions: Our data suggest that eosinophil, neutrophil and mast cell activation may play a role in the response to *Ss* infection.

Fig. 40: *Ss* infection is associated with elevated levels of eosinophil, neutrophil and mast cell granular proteins



(A) Plasma levels of Eosinophil cationic protein (ECP), Eosinophil derived neurotoxin (EDN), Eosinophil peroxidase (EPX) and Major basic protein (MBP), from *Ss* infected [INF] (n = 60) or uninfected [UN] (n = 58) individuals were measured by ELISA. Data are shown as scatter plots with the bar representing the geometric mean. P values were calculated using the Mann-Whitney test. (B) Plasma levels of plasma levels of neutrophil elastase (NE), myeloperoxidase (MPO) and proteinase-3 (PTN-3) from *Ss* infected [INF] (n = 60) or uninfected [UN] (n = 58) individuals were measured by ELISA. Data are shown as scatter plots with the bar representing the geometric mean. P values were calculated using the Mann-Whitney test. (C) Plasma levels of plasma levels of mast cell tryptase (MCT), leukotriene C4 (LTC4) and carboxypeptidase A-3 (CPA-3) from *Ss* infected [INF] (n = 60) or uninfected [UN] (n = 58) individuals were measured by ELISA. Data are shown as scatter plots with the bar representing the geometric mean. P values were calculated using the Mann-Whitney test with Holm's correction for multiple comparisons.

I. Immunology of parasitic infections: C. Modulation of $CD4^+$ and $CD8^+$ T-cell function and cytokine responses in *Strongyloides stercoralis* infection by IL-27 and IL-37

Principal Investigators	:	Dr. Subash Babu; Dr. P. Paul Kumaran (email:sbabu@nirt.res.in; ppkumaran@ nirt.res.in)
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH)
Study Period	:	2015-2017

Background: *Strongyloides stercoralis* (Ss) infection is associated with diminished antigen – specific Th1- and Th17-associated responses and enhanced Th2-associated responses. IL-27 and IL-37 are 2 known anti-inflammatory cytokines that are highly expressed in Ss infection.

Aims/ Methodology: We examined the role of IL-27 and IL-37 in regulating $CD4^+$ and $CD8^+$ T cell responses in Ss infection. To this end, we examined the frequency of Th1/Tc1, Th2/Tc2, Th9/Tc9, Th17/Tc17, Th22/Tc22 and Tr1 cells in 15 Ss- infected individuals and 10 uninfected individuals, stimulated with parasite antigen following IL-27 or IL-37 neutralization. We also examined the production of prototypical Type 1, Type 2, Type 9, Type 17 and Type 22 cytokines in the whole blood supernatants.

Principal Findings: Our data revealed that IL-27 or IL-37 neutralization resulted in significantly enhanced frequencies of Th1/Tc1, Th2/Tc2, Th17/Tc17, Th9 and Th22 cells with parasite antigen stimulation. There was no induction of any T-cell response in uninfected individuals following parasite antigen stimulation and IL-27 or IL-37 neutralization. Moreover, we also observed increased production of IFNγ, IL-5, IL-9, IL-17 and IL-22 and decreased production of IL-10 following IL-27 and IL-37 neutralization and parasite antigen stimulation in whole blood cultures.

Conclusions: We demonstrate that IL-27 and IL-37 limit the induction of particular T-cell subsets along with cytokine responses in Ss infections and suggests the importance of IL-27 and IL-37 in immune modulation in a chronic helminth infection.

II. Immunology of TB and its co-morbidities: A. Malnutrition is associated with diminished baseline and mycobacterial antigen – stimulated chemokine responses in LTB infection

Principal Investigators:		Dr. Subash Babu; Dr. Pavan Kumar; Dr. V.V. Banurekha; Dr. Dina Nair (email:sbabu@nirt.res.in; pavankumarn@nirt.res.in; banurekha@nirt.res.in; dinanair@nirt.res.in)
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH)
Study Period	:	2015-2017

Background: Previous studies have demonstrated a diminution in the baseline and mycobacterial antigen – specific cytokines in low body mass index (LBMI) individuals with latent tuberculosis infection (LTBI). We hypothesized that LBMI might be also associated with alteration in the baseline and antigen – stimulated levels of chemokines in LTBI.

Aims/ Methodology: To test this hypothesis, we examined baseline, TB-antigen and mitogen stimulated levels of chemokines in these individuals and compared them with those with LTBI and normal BMI (NBMI).

Results: LBMI with LTBI is characterized by diminished baseline levels of CCL1, CCL4, CCL11, CXCL1, CXCL9, CXCL10 and CXCL11 in comparison to NBMI with LTBI. Similarly, LTBI with LBMI is also characterized by diminished TB-antigen stimulated levels of CCL1, CCL2, CCL3, CCL4, CCL11, CXCL1, CXCL2, CXCL9, CXCL10 and CXCL11. In contrast, there were no significant differences in the mitogen – stimulated chemokine levels between the groups. Finally, there was a significant positive correlation between BMI and CCL1, CCL4, CCL11, CXCL11, CXCL2, CXCL9 and CXCL11 levels in LTBI individuals.

Conclusions: Our data reveal that LBMI are characterized by diminished levels of a variety of important chemokines, providing a novel biological mechanism for the increased risk of developing active TB.

II. Immunology of TB and its co-morbidities: B. Modulation of Th1/Tc1 and Th17/Tc17 responses In PTB by IL-20 subfamily of cytokines

Principal Investigators	:	Dr. Subash Babu; Dr. Pavan Kumar; Dr. V.V. Banurekha; Dr. Dina Nair (email:sbabu@nirt.res.in; pavankumarn@nirt.res in; banurekha@nirt.res.in; dinanair@nirt.res.in)
Source of funding	:	ICER
Collaborators	:	Dr. Thomas Nutman (NIH)
Study Period	:	2015-2017

Background*:* Although IL-10 is known to be an important cytokine in the immune response to TB, very little is known about the role of IL-20 subfamily of cytokines in the host response to TB.

Aims/Methodology*:* To identify the role of $CD4^+$T and $CD8^+$ T-cells producing IL-20 subfamily of cytokines in human TB, we enumerated the frequencies of IL-10, IL-19 and IL-24 expressing $CD4^+$ and $CD8^+$ T-cells following Mtb-specific antigen(s) stimulation of cells from individuals with PTB and LTB.

Results*:* We first demonstrated that Mtb-specific antigen(s) induce an expansion of $CD4^+$ and $CD8^+$ T-cells expressing IL-10, IL-19 and IL-24 in PTB and LTB individuals, with frequencies being significantly higher in the PTB. Next, we demonstrated that IL-10, IL-19 and IL-24 play an important role in the regulation of $CD4^+$ and $CD8^+$ T-cells expressing Th1/Tc1 and Th17/Tc17 cytokines in PTB but not LTB individuals.

Conclusion*:* Active PTB is characterized by an IL-10, IL-19 and IL-24 mediated down modulation of Th1/Tc1 and/or Th17/Tc17 cytokines in $CD4^+$ and $CD8^+$ T-cell subsets. This suggests that the IL-20 subfamily of cytokines, similar to IL-10 might play a potentially crucial role in the modulation of T-cell responses in active TB disease.

III. Immunology of extra-pulmonary TB: A. Tuberculous lymphadenitis is associated with altered levels of circulating angiogenic factors

Principal Investigators	:	Dr. Subash Babu; Dr. D. Bhaskaran (email: sbabu@nirt.res.in; baskar.d@nirt.res.in)
Source of funding	:	ICER
Collaborators	:	Dr. R. Sridhar (Stanley Hospital)
Study Period	:	2012-2018

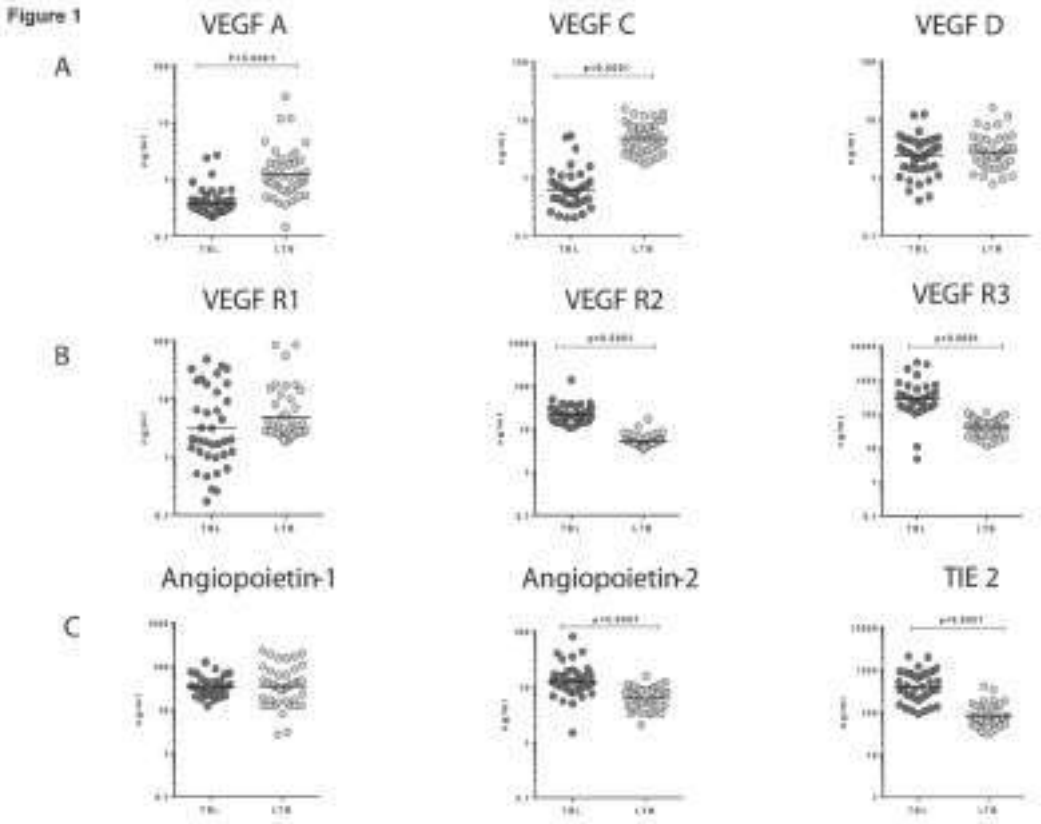
Background*:* Angiogenic factors like vascular endothelial growth factor (VEGF) and angiopoietin (Ang) are important in granuloma formation and serve as biomarkers in pulmonary tuberculosis. The association of these angiogenic factors in tuberculous lymphadenitis (TBL) has not been explored.

Aims/Methodology*:* This study examined the association of VEGF and Ang family molecules in TBL. We measured the systemic levels of VEGF-A, VEGF-C, VEGF-D, VEGF-R1, VEGF-R2, VEGF-R3, Ang-1, Ang-2 and TIE2 levels in TBL and latent TB individuals (LTB). Similarly, we also examined VEGF-A, VEGF-C and Ang-2 levels in the lymph node (LN) culture supernatants of the same TBL individuals.

Results*:* The circulating levels of VEGF-A and VEGF-C were significantly diminished, whereas VEGF-R2, R3, Ang-2 and TIE2 levels were significantly elevated in TBL (Fig. 41). VEGF-A, VEGF-C and Ang-2 levels were significantly elevated in LN supernatants when compared to plasma levels in TBL individuals. ROC analysis revealed VEGF-C and VEGF-R2 markers clearly distinguished TBL from LTB. Following chemotherapy, VEGF-C and Ang-1 levels were significantly altered. No association was observed for angiogenic factors with culture grade and lymph node (LN) size, except for VEGF-A. TBL VEGF-A levels were significantly decreased in multiple LN when compared with single LN.

Conclusion*:* Our data reveals that altered levels of circulating angiogenic factors in TBL might reflect underlying vasculo-endothelial dysfunction. Reversal of angiogenic markers after anti-TB treatment reveals that these angiogenic markers may serve as biomarkers of disease severity or response to chemotherapy in TBL.

<u>Fig. 41:</u> Circulating angiogenic factors in TBL individuals are associated with decreased systemic levels of VEGFs and enhanced VEGF, TIE2 receptors and Ang levels



The plasma levels of (A) vascular endothelial growth factors (VEGF-A, C and D), (B) receptors (VEGF-R1, R2 and R3) and (C) Ang-1, 2 and Tie2 were measured in TBL (n = 44), and LTB (n = 44) individuals. The data are shown as scatter plots with each circle representing a single individual-GM depicted with bar. P values were calculated using the Mann-Whitney *U* test.

III. Immunology of extra-pulmonary TB: B. Diminished circulating plasma and elevated lymph node culture supernatant levels of IL-10 family cytokines in tuberculous lymphadenitis

Principal Investigators	:	Dr. Subash Babu; Dr. D. Bhaskaran (email: sbabu@nirt.res.in; baskar.d@nirt.res.in)
Collaborators	:	Dr. R. Sridhar (GHTMI);
Source of funding	:	ICER
Study Period	:	2012-2018

Background*:* IL-10 family cytokines are associated with the host immune response to PTB, but their association with host response in tuberculous lymphadenitis (TBL) is not known.

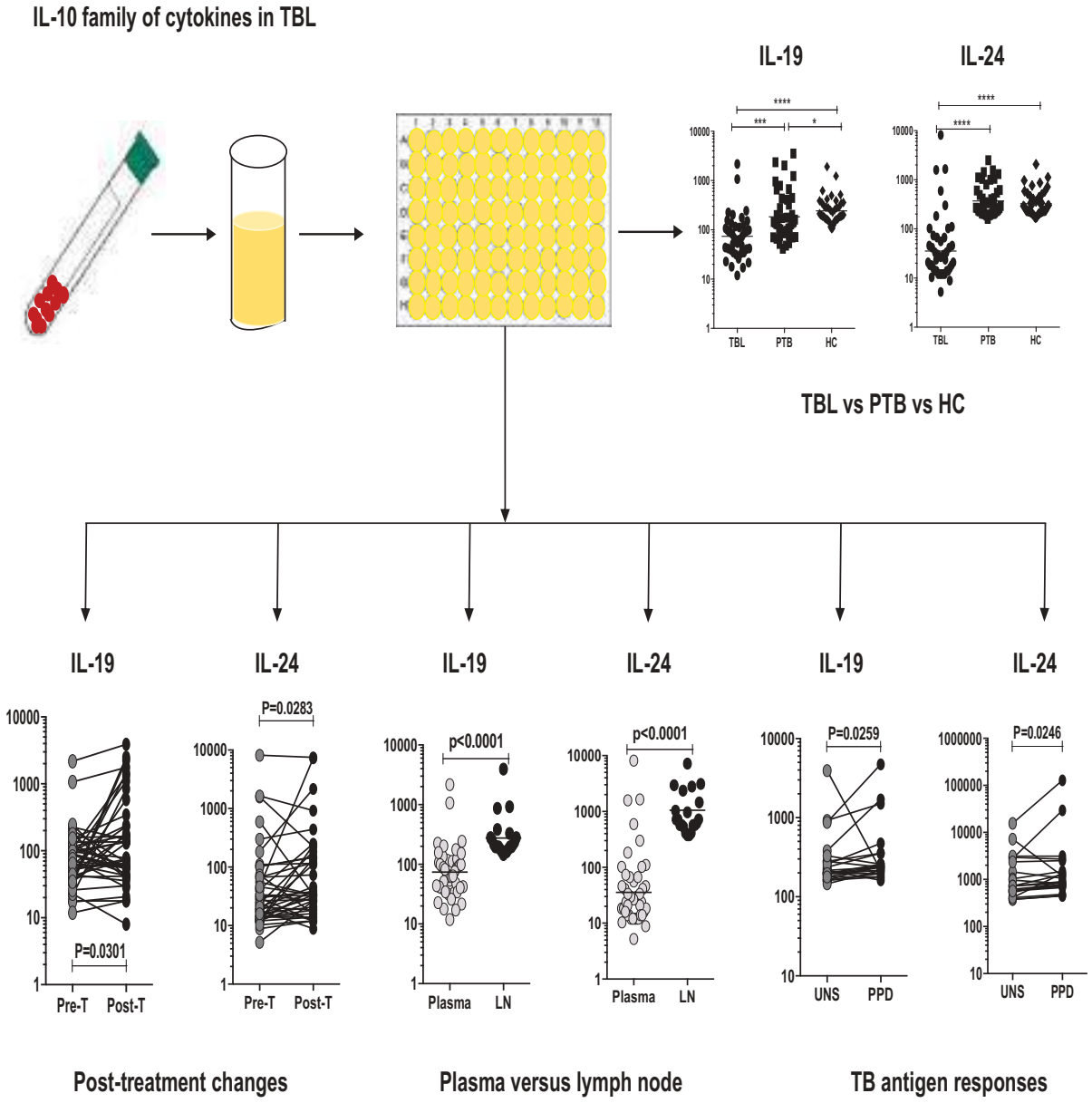
Aims/Methodology*:* We examined the circulating levels of the whole panel of IL-10 family cytokines in TBL (n=44) and compared them to the levels in PTB (n=44) and healthy control (HC, n=44) individuals. We also assessed the pre and post-treatment cytokine levels in TBL individuals following the completion of ATT. Next, we also compared the levels of IL-10 family cytokine in circulation versus lymph node (LN) culture supernatants in a subset of TBL individuals (n=22). Finally, we also measured the levels of IL-10 family cytokines in TB antigen (purified protein derivative, PPD) stimulated and unstimulated LN culture supernatants

Results*:* TBL individuals exhibited significantly decreased levels of IL-10, IL-19, IL-20, IL-24, IL-28B and IL-29 in the circulation when compared to PTB (except IL-10) and HC (except IL-20 and IL-28B) and significantly increased levels of IL-22 when compared to PTB individuals. Following ATT, TBL individuals exhibited significantly elevated levels of IL-10, IL-19, IL-20, IL-24, IL-28B and IL-29 and significantly diminished levels of IL-26. Similarly, TBL individuals also exhibited significantly increased levels of IL-10, IL-19, IL-20, IL-24, IL-28A and IL-29 in LN culture supernatants compared to plasma and significantly decreased levels of IL-22. This was associated with enhanced levels of IL-19, IL-20, IL-24, IL-28B and IL-29 upon PPD stimulation of LN cultures (Fig. 42).

Conclusion*:* We demonstrate that TBL is associated with significantly

diminished plasma and elevated LN culture supernatant levels of most of the IL-10 family cytokines. This to our knowledge is the first comprehensive examination of IL-10 family cytokines in TBL.

Fig. 42: IL-10 family of cytokines in TBL



IV. Immunology of diabetes-TB: A. Heightened circulating levels of antimicrobial peptides in TB – diabetes co-morbidity and reversal upon treatment

Principal Investigators	:	Dr. Subash Babu; Dr. Pradeep Menon (email: sbabu@nirt.res.in; menonpa@nirt.res.in)
Collaborators	:	Dr. Vijay Viswanathan (MV Diabetes); Hardy Kornfeld (UMass)
Source of funding	:	DBT and NIAID
Study period	:	5 years (2014-2019)

Background*:* The association of antimicrobial peptides (AMPs) with TB – diabetes comorbidity (PTB-DM) is not well understood.

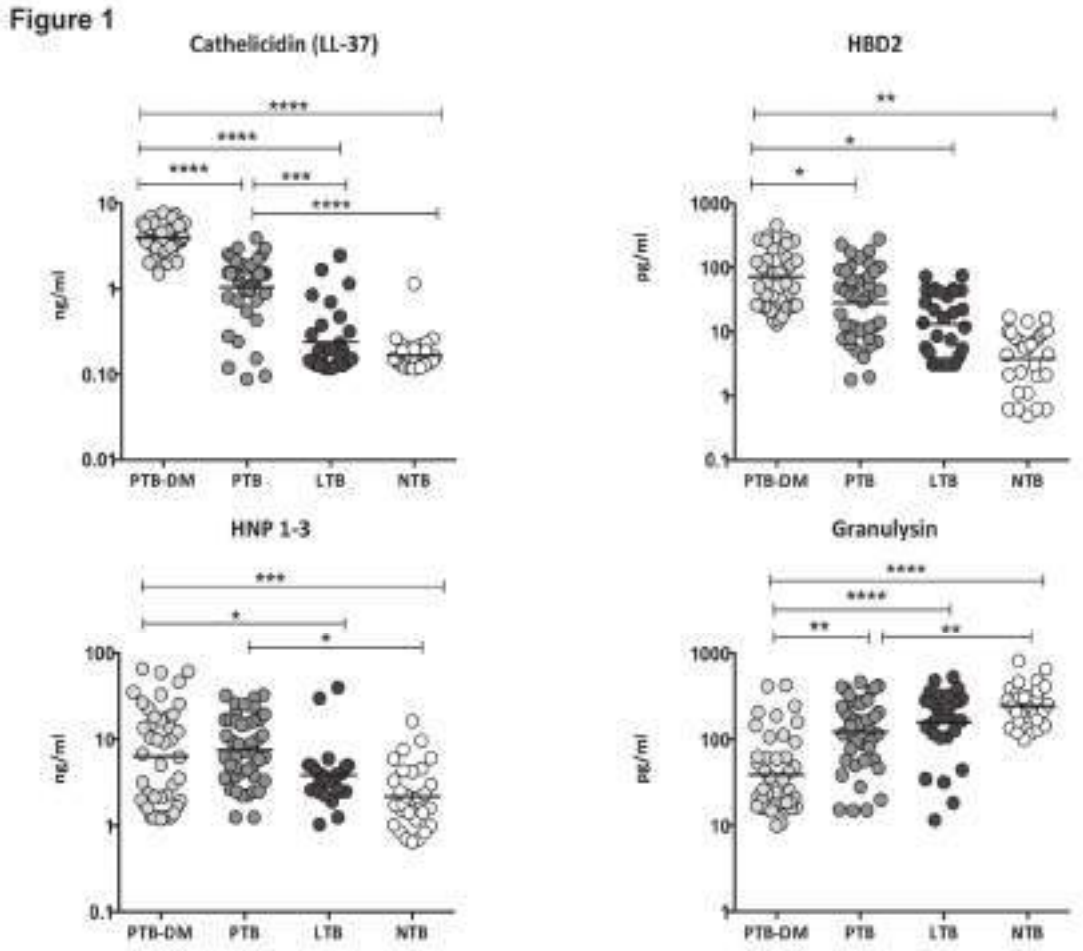
Aims/Methodology*:*

(i) To study the association of AMPs with PTB-DM, we examined the systemic levels of cathelicidin (LL37), human beta defensin – 2 (HBD2), human neutrophil peptides 1 – 3, (HNP1-3) and granulysin in individuals with either PTB-DM, PTB, latent TB (LTB) or no TB infection (NTB)

Results*:* Circulating levels of cathelicidin and HBD2 were significantly higher and granulysin levels were significantly lower in PTB-DM compared to PTB, LTB or NTB, while the levels of HNP1-3 were significantly higher in PTB-DM compared to LTB or NTB individuals (Fig. 43). Moreover, the levels of cathelicidin and/or HBD2 were significantly higher in PTB-DM or PTB individuals with bilateral and cavitary disease and also exhibited a significant positive relationship with bacterial burden. Cathelicidin, HBD2 and HNP1-3 levels exhibited a positive relationship with HbA1c and/or fasting blood glucose levels. Finally, anti-TB therapy resulted in significantly diminished levels of cathelicidin, HBD2, granulysin and significantly enhanced levels of HNP1-3 and granulysin in PTB-DM and/or PTB individuals.

Conclusion*:* Our data demonstrate that PTB-DM is associated with markedly enhanced levels of AMPs and diminished levels of granulysin.

<u>Fig. 43:</u> Elevated circulating levels of AMPs in PTB-DM and PTB individuals



The plasma levels of cathelicidin (LL37), HBD2, HNP1-3 and granulysin were measured in PTB-DM (n=44), PTB (n=44), LTB (n=30) and NTB (n=30) individuals. The data are presented as scatter plots with each circle representing a single individual. P values were calculated using the Kruskal – Wallis test with Dunn's post – hoc for multiple comparisons.

IV. Immunology of diabetes-TB: B. Systems Immunology of Diabetes-TB co-morbidity reveals molecular signatures associated with disease complications

Principal Investigators	:	Dr. Subash Babu; Dr. Pradeep Menon (email: sbabu@nirt.res.in; menonpa@nirt.res.in)
Source of funding	:	DBT and NIAID
Collaborators	:	Dr. Vijay Viswanathan (MV Diabetes); Dr. Hardy Kornfeld (UMass)
Study period	:	5 years (2014-2019)

Background*:* Diabetes mellitus (DM) is associated with increased (TB) risk and adverse TB outcomes, but the pathological interactions between DM and TB remain incompletely understood.

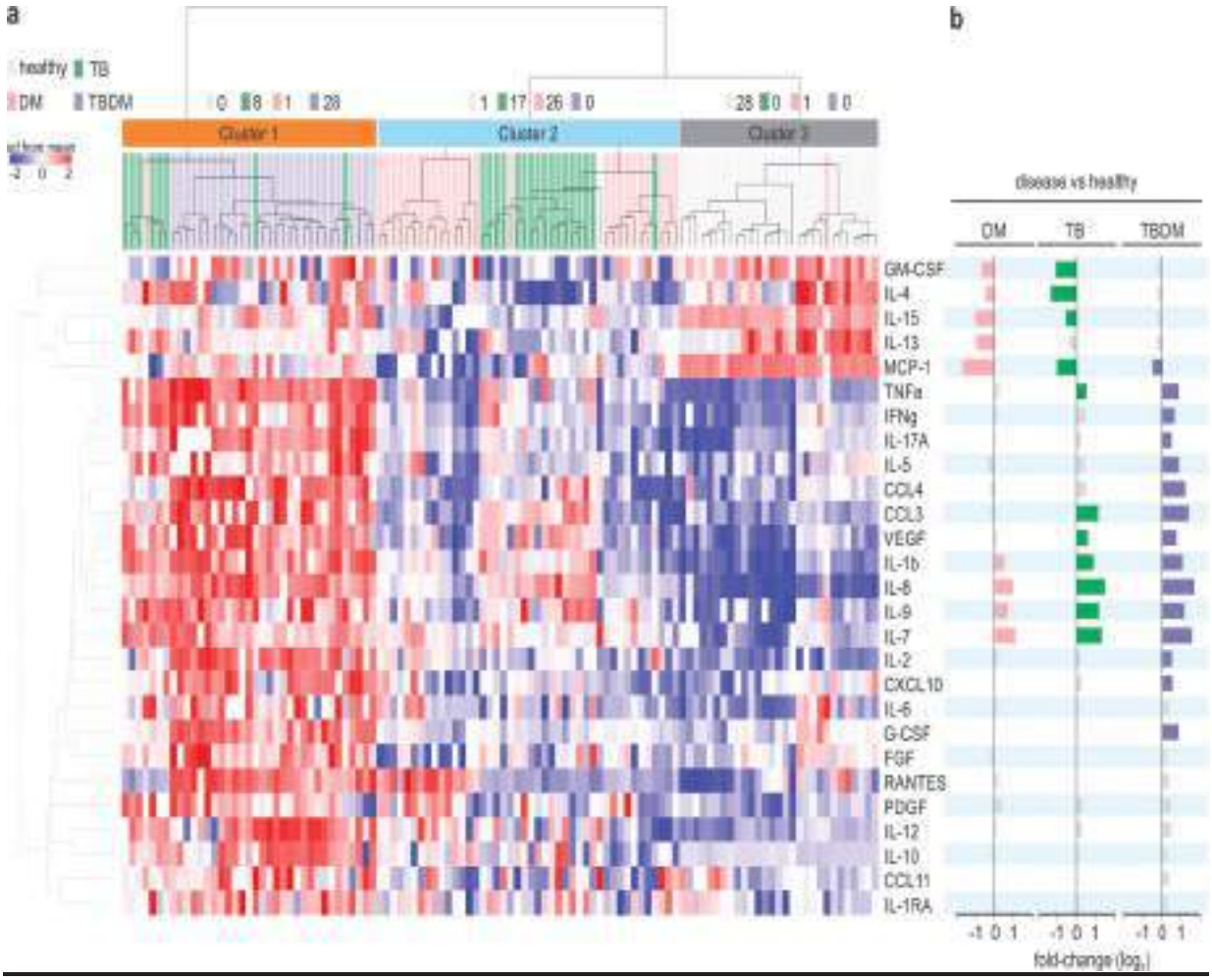
Aims/Methodology*:* We performed an integrative analysis of whole blood gene expression and serum and plasma analytes, comparing TB patients with and without DM to diabetic and non-diabetic controls without TB. Clinical data and blood samples were obtained at TB diagnosis (baseline) in 30 diabetic and 30 normoglycemic participants. Thirty diabetic and 30 normoglycemic controls without TB were recruited from the same community in Chennai, India. Blood transcriptional profiles, serum lipids, plasma cytokines, cell counts and chest radiographs were analyzed using multidimensional statistical approaches applied to systems immunology.

Results*:* Dual TB and DM burden (TBDM) was associated with higher neutrophil count and neutrophil: lymphocyte and monocyte : HDL ratios. Luminex assay of plasma cytokines and growth factors delineated a distinct signature in TBDM (Fig. 44). Transcriptional profiling revealed elements in common with published TB signatures from cohorts where DM was an exclusion criterion (Fig. 45). Neutrophil count was strongly correlated with the molecular degree of perturbation, especially in TBDM patients. Pathways implicated in diabetic complications were activated in TBDM above levels observed with DM alone.

Conclusion*:* Our data provide a rationale for trials of host-directed therapies in TBDM, targeting neutrophilic inflammation and diabetic complication pathways to address the greater morbidity and mortality

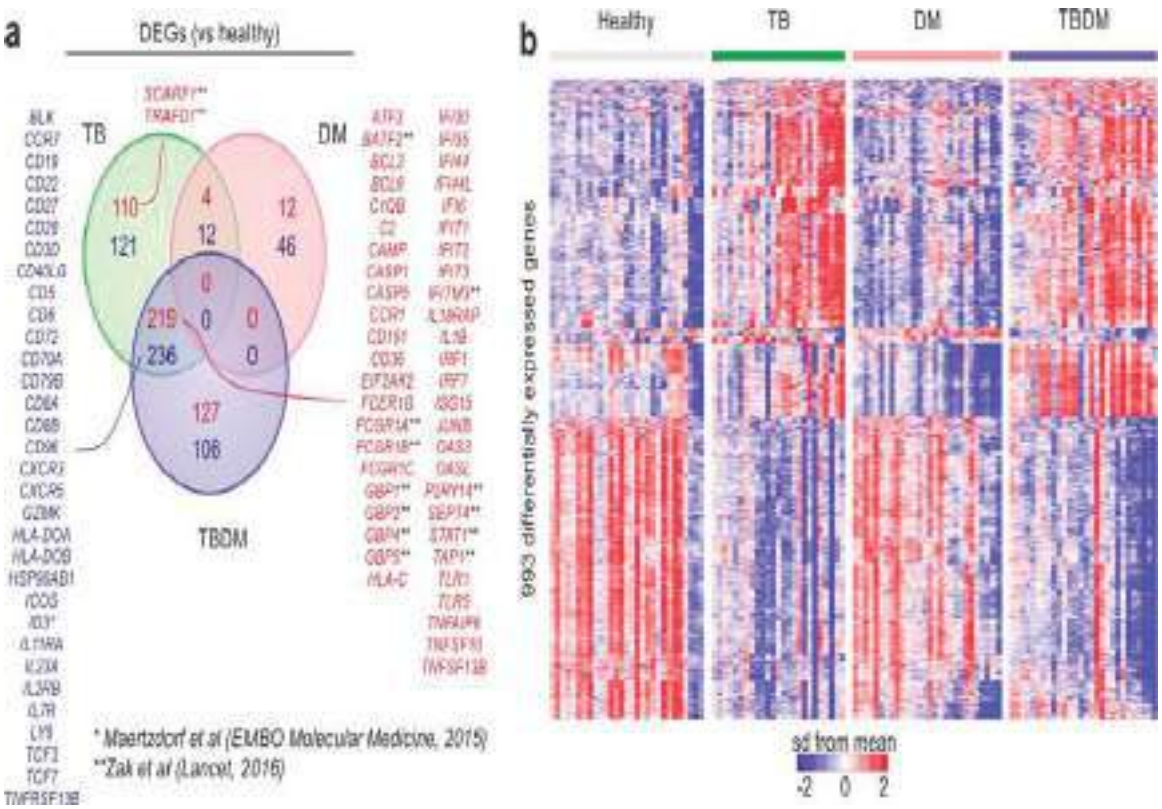
associated with this increasingly and non-communicable diseases. prevalent dual burden of communicable

Fig. 44: Cytokine profiling in plasma of patients with TB and/or DM



Cytokine concentrations were z-score normalized across all subjects (**A**). Each column represents one patient. The cytokine names are shown to the right of the heat map. Cytokine profiles were ordered by hierarchical clustering (Euclidean distance and clustered with ward method). The condition tree at the top shows 3 main clusters. The number of subjects from each clinical phenotype (class) on each cluster is indicated. Differences in cytokine levels for each disease compared to healthy subjects (**B**). Differences which did not reach statistical significance (Adjusted $P < 0.05$, fold-change > 1.4) are represented as grey bars.

<u>Fig. 45:</u> Transcriptomic changes with TB and/or diabetes compared to healthy subjects



(A) Differentially expressed genes (DEGs) in patients with TB and/or DM compared to healthy subjects (adjusted $P < 0.05$, fold-change > 1.4). The Venn diagram shows the number of DEGs in common to two or more clinical phenotypes or unique to only one disease. (B) Expression patterns of the DEGs from (A). Cohort subgroups are shown by the colored bars. Each column represents one subject. The genes (rows) were normalized by z-score across all samples.

STUDIES IN PROGRESS:

ICER-1: Characterization of immune responses in helminth-TB co-infection

Principal Investigators:		Dr. Subash Babu; Dr.P. Paul Kumaran (email: sbabu@nirt.res.in; pkumaran@nirt.res.in)
Collaborators	:	Dr. Thomas Nutman (NIH)
Source of funding	:	ICER
Study Period	:	2012-2019

We are studying the influence of helminth infection on the immunological responses to TB antigens in LTB infected individuals. This study is being conducted as a prospective case-control study in Kanchipuram district, Tamil Nadu. We will be comparing immune responses to mycobacterial antigens between individuals with LTB and helminth co-infection and individuals with LTB alone. The expected sample size is 150 per group. We have recruited 110 individuals in each group thus far.

ICER-3: Host immune responses in lymphatic filariasis and Strongyloidiasis

Principal Investigators	:	Dr. Subash Babu; Dr.P. Paul Kumaran (email: sbabu@nirt.res.in;ppkumaran@nirt.res.in)
Collaborators	:	Dr. Thomas Nutman (NIH); Dr.R. Nandhini (GGH); Dr.V. Lakshmi (CDH)
Source of funding	:	ICER
Study Period	:	2012-2019

This study is designed to determine the presence of and the immune response to filarial and Strongyloides infections in an area endemic for lymphatic filariasis and Strongyloidiasis in south India. This study aims to examine the presence of filarial infection at a community level as well as in hospital settings. We will compare immune responses between filarial infected, filarial diseased and control patients. Similarly, we will compare immune responses between Strongyloides infected and uninfected individuals. In addition, we will examine the immune responses following anti-helminthic therapy. Patient recruitment is ongoing.

ICER-4: Host immune responses TB lymphadenitis

Principal Investigators	:	Dr. Subash Babu; Dr.D. Baskaran (email: sbabu@nirt.res.in; baskar.d@nirt.res.in)
Collaborators	:	Dr.R. Sridhar (GHTM, Tambaram)
Source of funding	:	ICER
Study Period	:	2012-2018

Tuberculous (TB) lymphadenitis is the most common presentation of extra-PTB, accounting for 30–40% of cases. It constitutes a significant disease burden and differs from other forms of TB in that patients have large tuberculin reactions and there is a strong female preponderance. The immune responses in TB lymphadenitis are poorly understood. In addition, no study till date has evaluated the immune responses pre- and post treatment in TB lymphadenitis. We are comparing immune responses in lymphnode versus peripheral blood in these individuals. We will also compare immune responses between pre- and post-treatment time points in the same individuals in peripheral blood. Recruitment is completed and immunological studies are ongoing.

ICER-5: Effect of diabetes on the immune responses inTB

Principal Investigators	:	Dr. Subash Babu; Dr. Pradeep Menon (email: sbabu@nirt.res.in; menonpa@nirt.res.in)
Collaborators	:	Dr. Vijay Viswanathan (MV Diabetes); Dr. Hardy Kornfeld (UMass)
Source of funding	:	DBT and NIAID
Study Period	:	2014-2019

This study will be utilizing samples collected in the Indo-US VAP funded study called the Effects of Diabetes on TB Severity (EDOTS). The aim of the EDOTS study is to develop a cohort for prospective investigation of the impact of diabetes on the presentation, treatment response and outcomes of PTB. The study will recruit a cohort of patients newly diagnosed with PTB, equally divided between those with and without diabetes. These patients will be followed longitudinally in a clinical study aiming to establish whether diabetes alters the clinical presentation and treatment outcome of TB disease in an Indian population. Our study aims to primarily examine the immune responses to TB in this cohort of individuals. Using approaches including flow cytometry and gene expression profiling, we will conduct fundamental studies in this cohort, comparing selected features of the immune response to TB in subjects with or without diabetes. We have obtained samples from 420 patients thus far out of a sample size of 450.

CONTRIBUTION TO THE NATIONAL PROGRAMMES

HIV Laboratory testing services to National AIDS Control Programmes (NACO)

(Contact person: Dr. Luke Elizabeth Hanna; email: hanna@nirt.res.in)

During the reporting year, the HIV lab has continued its support for the NACO's Early infant diagnosis testing under the PPTCT Programme. The program aims to screen the HIV exposed infants/children aged between 6 weeks to 18 months for HIV-1 positivity. Under this programme, dried blood spot (DBS) samples collected from different districts of Tamilnadu, Kerala, Pondicherry and Andhra Pradesh were referred for HIV-1 testing by DNA PCR assay by this Laboratory.

The Molecular HIV testing section of the laboratory also continues to extend its HIV-1 Viral load testing services to the NACO Programme for the Virological Monitoring of Patients with HIV-1 on first and second line ART. A summary of testing services provided for the NACO programmes is shown in Tables 74 & 75.

Table 74: Summary of HIV-1 DNA PCR testing services provided for the NACO-EID program during the period April 2017 - March 2018

S.No	Month/Year	Samples Received	Samples Tested	Screening Detected	Confirmatory Detected
1	Apr-2017	138	442	19	10
2	May-2017	220	207	6	6
3	Jun-2017	199	212	6	5
4	Jul-2017	217	246	20	6
5	Aug-2017	182	149	3	5
6	Sep-2017	143	87	1	3
7	Oct-2017	187	183	8	1
8	Nov-2017	207	222	14	0
9	Dec-2017	230	118	4	0
10	Jan-2018	205	122	4	0
11	Feb-2018	185	416	17	7
12	Mar-2018	151	115	5	5
		2264	2519	107	48

Table 75: HIV-1 virological monitoring services provided for the NACO Program during the period April 2017 - March 2018

S.No	Month/Year	Samples Received	Samples Tested
1	Apr-2017	198	343 *
2	May-2017	311	170
3	Jun-2017	257	285
4	Jul-2017	263	0
5	Aug-2017	272	134
6	Sep-2017	231	174
7	Oct-2017	203	553
8	Nov-2017	334	625
9	Dec-2017	300	335
10	Jan-2018	248	264
11	Feb-2018	240	213
12	Mar-2018	200	219
		3057	3315

* samples received in the preceding reporting year and tested during the current reporting year

PARTICIPATION IN EXTERNAL QUALITY ASSURANCE PROGRAMMES

The HIV Lab has continued to participate successfully in various External quality assurance programmes and achieved good scores and met the QA body's criteria in Molecular Diagnostic testing. A Summary of the EQA related activities for the current year is provided in Table 76.

Table 76: Summary of EQA activities during the period April 2017 - March 2018

Programme	Name of the Assay	EQA/Accreditating Body	Number of Panels tested	Results Received	Results
PPTCT-Early Infant Diagnosis	HIV DNA PCR	VQA	2	2	Certified
Virological monitoring of patients on ART	HIV Viral load test	VQA RCPA	5 2	5 2	Certified

CONTRIBUTION TO PATIENT CARE

HIV Laboratory testing services to support clinical studies

(Contact person: Dr. Sudha Subramanyam; email:sudhas@nirt.res.in)

The HIV Department offers laboratory support for the ongoing clinical studies in terms of HIV testing, screening for Hepatitis B and C, complete blood count (CBC) testing, CD4/CD8 cell count estimation, HIV-1 viral load testing and HIV drug resistance testing. A summary of the testing services provided during the period April 2017 – March 2018 is provided in Tables. 77 & 78.

Table 77: Summary of testing services towards patient care provided during the period April 2017-March 2018 for various clinical trials:

CBC	CD4	HIV	HBV	HCV	CSF	Blood Grouping	*C. trachomatis*	*N. gonorrhoea*	Syphilis
2656	250	739	227	193	82	90	927	927	127

Table 78: Summary of testing services towards patient care provided during the period April 2017-March 2018 for various clinical trials:

CBC	CD4	HIV	HBV	HCV	CSF	Blood Grouping	*C. trachomatis*	*N. gonorrhoea*	Syphilis
2656	250	739	227	193	82	90	927	927	127

III. Bacteriology Lab services:

Update on MDR-TB diagnosis by Line Probe assay and Gene Xpert for the period between 1st April, 2017 and 31st March 2018

Department of Bacteriology is offering services for rapid diagnosis of multi drug resistant TB (MDR-TB) under the programmatic management of drug resistant TB (PMDT) of the RNTCP of India. These services are provided for three districts of Chennai and the District of Kanchipuram. Diagnosis of MDR-TB by Line Probe Assay using Genotype MTBDRplus (Hain's Life Sciences, Nehren, Germany) was done for 2897 smear positive patients during the period between 1st April, 2017 and 31st March 2018. Of them, 173 were found to have DRTB, (42 were mono resistant to RMP and 131 were resistant to both RMP and INH), 345 were mono resistant to INH and 2299 were susceptible to both INH and RMP. Among 1424 sputum smear negative patients, the samples were tested by Gene Xpert system. Among them, 29 were found to be RMP resistant, 411 were RMP susceptible and the remaining 1001 were negative for MTB. In addition, 911 follow-up samples were received during the year and processed for culture by MGIT960 system.

RNTCP ACTIVITIES IN NATIONAL REFERENCE LABORATORY, NIRT, CHENNAI (2018-19)

Contact person : Dr. Srikanth Prasad Tripathy
(email: srikanth.p@nirt.res.in)
Source of Funding : Ministry of Health and Family Welfare, Central TB Division, New Delhi

The National Institute for Research in Tuberculosis (NIRT) is one of the National Reference Laboratories (NRL) which closely monitors RNTCP activities in five states (Andhra Pradesh, Gujarat, Kerala, Tamil Nadu and Telangana state) and five Union territories (Andaman & Nicobar, Puducherry, Lakshadweep, Daman & Diu and Dadra & Nagar Haveli). The NRL Microbiologists visit each state at least once a year for 3 to 5 days. An onsite evaluation (OSE) of EQA activities of smear microscopy, culture and DST by both phenotypic and genotypic methods as per the RNTCP protocol is undertaken. NIRT is also monitoring EQA in sputum smear microscopy of 3501 DMCs and DR-TB diagnosis of 107 CBNAAT sites, 16 IRLs/Medical colleges/private labs. During the OSE visit, the NRL microbiologists provide technical support for establishing quality assured smear microscopy, C & DST services, including facility design for the introduction of newer diagnostic tools (liquid culture and molecular tests) for the rapid diagnosis of MDR/XDR TB. NRL also undertakes annual proficiency testing of IRLs/ C & DST labs as part of the certification process under RNTCP. Till date, 8 IRLs and 7 C & DST labs have been certified for diagnosis of DR-TB patients from the respective states. Uring 2017-18, the Institute conducted the ninth round of proficiency tesing of 20 labs including 3 NRLs with a panel of 30 cultures for both first and second line susceptibility testing of anti-TB drugs. Retesting process has also been completed for 4 IRLs and one C & DST laboratory in a Medical College for certification by liquid culture. As the NRL, NIRT is supporting second line DST for presumptive XDR-TB patients, and a total of 359 cultures were received from different states and processed. BDQ conditional access programme (BDQ CAP) has been implemented in Tamil Nadu and a total of 460 and 850 patients' samples were received for extended SL-DST and follow-up culture respectively from the state. NRL Microbiologists have visited

four states (Tamil Nadu, Andhra Pradesh, Telangana State and Puducherry) for OSE of sputum microscopy and 305 panel slides were used to assess 61 laboratory personnel, Onsite training has been facilitated for extended SL-DST by MGIT for laboratory personnel of IRL in Telangana state and refresher training on EQA of smear micrsocopy has been conducted for 104 STLS of Andhra Pradesh.

TRAINING:

Onsite training was facilitated for Extended SL-DST by MGIT for laboratory personnel of IRL, Telangana state and refresher training on EQA of smear microscopy has been conducted for 104 STLS of entire Andhra Pradesh.

As part of Supra National Reference Laboratory (SNRL) activity of NIRT, Department of Bacteriology provided training facility for students of various educational and/or research institutions in the country. Between April 2017 and March 2018, a total of 6 M. Sc. Microbiology, 60 Medical Lab Technology, 23 MD Microbiology and 12 PG medical students from GHTM were trained in the Department of Bacteriology on mycobacterialogical procedures. In addition, 45 students underwent summer internship training for mycobacterial culture and DST. During the period, 14 students of different disciplines from different institutions (7 B. Tech Biotechnology, 3 M. Sc Biotechnology, 1 M. Sc Biochemistry, 1 M. Sc., Applied Microbiology, 1 M. Sc., Medical Lab Technology and 1 M. Sc. Biomedical Science) successfully completed their final year dissertation work under the guidance of faculties in the department.

LIBRARY AND INFORMATION CENTRE

Library and Information Centre

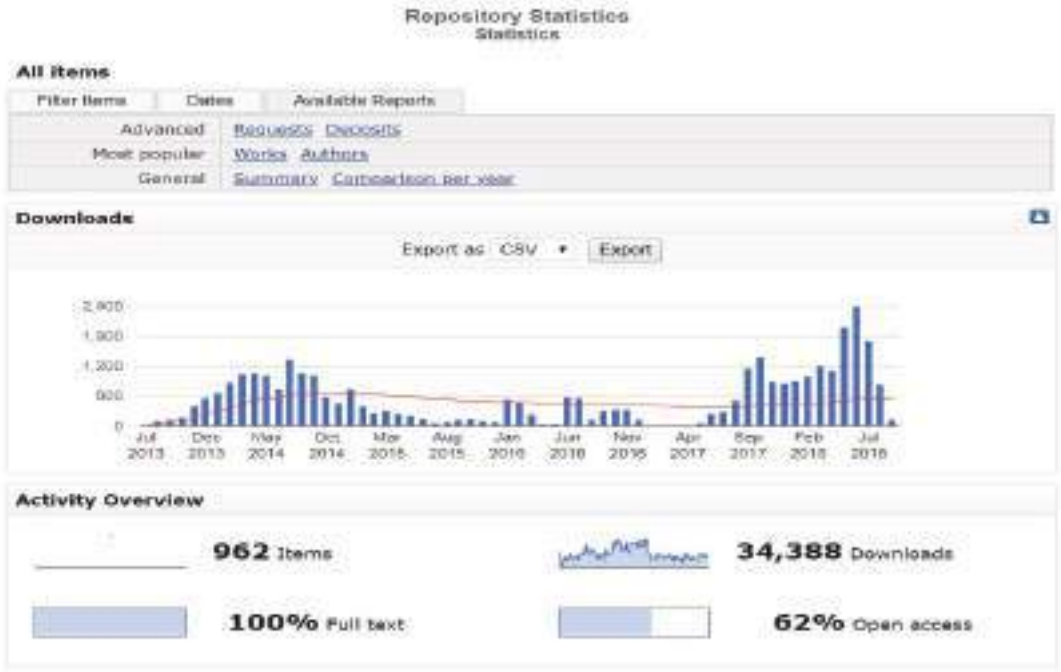
(Contact person: Dr. R Rathinasabapati; email: rrathinasabapati@nirt.res.in)

During the last fifteen years, the Library has made significant investments in acquiring e-resources, i.e., e-Journals, e-Books and e-archives. For providing access to these e-resources, the customised **Digital Library** portal has been updated frequently. It also provides a gateway to ICMR-Consortium; ICMR Resource sharing portal viz., 'J-Gate@ICMR'; ERMED (**E**lectronic **R**esource **MED**icine) Consortium viz., 'J-Gate@ERMED', Specialized health science databases, Open Access resources and link to 'Cochrane Library', which has National Provision License (NPL) supported by ICMR. It enhances a simplified integrated 24hours access facility (intranet) to our patrons; all it needs are a few mouse clicks.

Institutional Repository

NIRT Institutional Repository –NIRTIR (https://eprints.nirt.res.in) functions as a two-in-one repository, i.e., institute cum specific subject repository. This repository will facilitate long-term preservation of our research output and provide easy access to our publications. Also, the repository is enhancing the visibility and status of our institute. Fig. 46 shows its visibility at the global level:

Fig. 46: Institutional Repository



Publications

As part of our **V**alue **A**dded **S**ervices, in addition to our existing 'TB Alert', a monthly publication viz., "**HIV Monitor**" has been introduced in January 2017 and being circulated to all ICMR institutes. Since the volume of articles is more, it has been converted into 'Fortnightly' since January 2018. To take into consideration of press clipping service, a new weekly publication "**News Bulletin**" is introduced from July 2018; it comprises of message pertaining to tuberculosis from daily newspapers, online, media etc.

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APPENDICES

SUMMARY SHEET OF PUBLICATIONS FROM NIRT (2017-2018)
(SORTED AS PER TOTAL IMPACT FACTOR ORDER)

Sl.No.	Publishing journals	No. of papers	Impact factor	Total Impact Factor
1	**IMMUNOLOGY**			
	European Respiratory Journal	1	10.569	10.569
	Clinical Infectious Diseases	2	8.216	16.432
	Trends in Analytic Chem	1	7.034	7.034
	Journal of Leukocyte Biology	1	4.018	4.018
	Immunology	1	3.701	3.701
	International Journal of Medical Microbiology	1	3.391	3.391
	Clinical Therapeutics	1	2.947	2.947
	Tropical Medicine and International Health	1	2.850	2.850
	Biochem Biophys Res Comm	2	2.559	5.118
	Human Immunology	1	2.311	2.311
	Journal of Parasitology Research	1	2.202	2.202
	Curr HIV Research	1	1.612	1.612
	Lymphology	1	1.079	1.079
	Int J Current Microbiology and Applied Sciences	1		
	TOTAL	**16**	**52.489**	**63.264**
2	**ICER**			
	Frontiers in Immunology	1	6.429	6.429
	Frontiers in Cellular and Infection Microbiol	1	4.300	4.300
	Science Report	1	4.259	4.259
	PLOS Neglected Trop Dis	1	3.834	3.834
	Immunology	1	3.701	3.701
	Infection and Immunity	1	3.593	3.593
	Tuberculosis	1	2.873	2.873
	Tropical Medicine and International Health	1	2.850	2.850
	PLOS One	3	2.806	8.418
	Clinical Vaccine Immunology	1	2.425	2.425
	Microbial Pathogenesis	1	2.009	2.009
	Biomedicine	1		
	TOTAL	**14**	**39.079**	**44.691**
3	**CLINIC**			
	Frontiers in Immunology	1	6.429	6.429
	Journal of Proteome Research	1	3.950	3.950
	Expert Opin Pharmacotherapy	1	3.894	3.894
	Expert Rev Anti Infect Ther	1	3.139	3.139
	Int J Tuberculosis and Lung Disease	1	2.468	2.468
	Pediatric Infectious Disease Journal	1	2.305	2.305
	Journal of Neurology Science	1	2.295	2.295
	Indian Journal of Medical Research	2	1.532	3.064
	The Natlonal Medical Journal of India	2	1.412	2.824

	Public Health Action	2		
	Journal of Epidemiology Global Health	1		
	Indian Journal of Community Medicine	2		
	Journal of Association of Physicians of India	1		
	Total	**17**	**27.424**	**30.368**
4	**HIV**			
	Frontiers Immunology	2	6.429	12.858
	Science Report	1	4.259	4.259
	Proteins	1	2.274	2.274
	AIDS Res Hum Retroviruses	2	2.095	4.190
	TOTAL	**6**	**15.057**	**23.581**
5	**BACTERIOLOGY**			
	Polymer Chemistry	1	4.927	4.927
	Antimicrobial Agents Chemotherapy	1	4.302	4.302
	PLOS One	2	2.806	5.612
	Annals of Clinical Microbiol & Antimicrobiol	1	2.376	2.376
	Microbial Pathogenesis	1	2.009	2.009
	International Journal of Mycobacteriology	1		
	TOTAL	**7**	**16.420**	**19.226**
6	**DSBR**			
	AIDS and Behavior	1	3.017	3.017
	PLOS One	1	2.806	2.806
	BMC Infectious Disease	1	2.768	2.768
	Arch Sex Behav	1	2.720	2.72
	Int J Tuberculosis and Lung Disease	1	2.468	2.468
	Indian Journal of Medical Research	1	1.532	1.532
	Indian Journal of Tuberculosis	1		
	TOTAL	**7**	**15.311**	**15.311**
7	**CLINICAL PHARMACOLOGY**			
	Antimicrobial Agents Chemotherapy	1	4.302	4.302
	PLOS One	2	2.806	5.612
	Indian J Med Res	2	1.532	3.064
	TOTAL	**5**	**8.640**	**12.978**
8	**BIC**			
	Vaccine	1	3.235	3.235
	Tuberculosis	2	2.873	5.746
	Journal of Theoretical Biology	1	2.113	2.113
	J Glob Antimicrob Resist	1	1.276	1.276
	Tuberc Respir Dis	1		
	Meidcal Hypothesis	1		
	TOTAL	**7**	**9.497**	**12.370**

9	**STATISTICS**			
	Surgery	1	3.904	3.904
	Inter J Res Pharmaceutical Science	1		
	IOSR Journal of Dental and Medical Sciences	3		
	Indian J App Res	1		
	TOTAL	**6**	**3.904**	**3.904**
10	**EPIDEMIOLOGY**			
	Trans R Soc Trop Med Hyg	1	2.279	2.279
	PLOS One	1	2.806	2.806
	TOTAL	**2**	**5.085**	**5.085**
	Grand Total	**87**	**192.906**	**230.778**

LIST OF PUBLICATIONS

Publications in Journals	**:**	**87**		
Accepted	**:**	**18**		
Published	i) International	:	75	
	ii) National	:	12	
Chapters in Books		:	4	
Accepted	i) International	:	17	
	ii) National	:	1	

International:

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Chapter:

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Accepted:

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4. Ashokkumar M, Aralaguppe SG, Tripathy SP, Hanna LE, Neogi U. Unique phenotypic characteristics of recently transmitted HIV-1 subtype C envelope glycoprotein gp120: Use of CXCR6 co-receptor by transmitted founder viruses. Journal of Virology.

5. Daskapan Alper, Rismon Lusiana, Postma Maarten, Wilffert Bob, Kosterink Jos, Stienstra Ymkje, Touw Daniel, Andersen Åse, Bekker Adrie, Denti Paolo, Hemanth Kumar AK, Jeremiah Kidola, Kwara Awewura, McIlleron Helen, Meintjes G, van Oosterhout Joep, Ramachandran Geetha, Rockwood Neesha, Wilkinson Robert, van der Werf Tjip, Alffenaar Jan-Willem. A systematic review on the effect of HIV infection on the pharmacokinetics of first-line tuberculosis drugs. J Antimicrob Chemother.

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10. Guiastrennec B, Ramachandran G, Karlsson MO, Kumar AKH, Bhavani PK, Gangadevi NP, Swaminathan S, Gupta A, Dooley KE, Savic RM. Suboptimal anti-tuberculosis drug concentrations and outcomes in small and HIV

coinfected children in India: Recommendations for dose modifications. Clinical Pharmacology & Therapeutics

11. Hassan S, Sanjay KS. Yuvaraj C, Thangam M, Muthukumar S, Gayathri Devi PK, Hanna LE. Exploring the conformational landscapes of HIV protease structural ensembles using principal component analysis. Proteins: Strcuture, Function and Bioinformatics.

12. Hemanth Kumar AK, Polisetty AK, Sudha V, Vijayakumar A, Ramachandran G. A selective and sensitive high performance liquid chromatography assay for the determination of cycloserine in human plasma. Indian Journal of Tuberculosis.

13. Hemanth Kumar AK, Kumar A, Kannan T, Bhatia R, Agarwal D, Kumar S, Dayal R, Singh SP, Ramachandran G. Pharmacokinetics of second-line anti-tuberculosis drugs in children with multidrug-resistant tuberculosis in India. Antimicrobial Agents and Chemotherapy.

14. Kadar M, Kumar NP, Nair D, Banurekha VV, Ramalingam B, Babu S. Heightened systemic levels of neutrophil and eosinophil granular proteins in pulmonary tuberculosis and reversal following treatment. Infection and Immunity.

15. Kumar NP, Moideen K, Banurekha VV, Nair D, Babu S. Modulation of Th1/Tc1 and Th17/Tc17 responses in pulmonary tuberculosis by IL-20 subfamily of cytokines. Cytokine.

16. Madhumitha H, Mohan V, Babu S, Aravindhan V. TLR-induced secretion of novel cytokine IL-27 is defective in newly diagnosed type-2 diabetic subjects. Cytokine.

17. Rao VG, Bhat J, Yadav R, Sharma R, Muniyandi M. A comparative study of the socio-economic risk factors for pulmonary tuberculosis in Saharia tribe of Madhya Pradesh, India. Transactions of the Royal Society of Tropical Medicine and Hygiene.

18. Shivakoti R, Gupte N, Kumar NP, Kulkarni V, Balasubramanian U, Bhosale R, Sambrey P, Kinikar A, Bharadwaj R, Patil S, Inamdar S, Suryavanshi N, Babu S, Bollinger RC, Gupta A. Intestinal barrier dysfunction and microbial translocation in HIV-infected pregnant women is associated with preterm birth. Clinical Infectious Diseases.

National:

1. Hemanth Kumar AK, Chandrasekaran V, Kannan T, Lavanya J, Soumya Swaminathan, Geetha Ramachandran. Intra-patient variability in plasma rifampicin and isoniazid in tuberculosis patients. Indian Journal of Medical Research.

Awards/Honours

- Mr.M. Ashok Kumar - Awarded HIV Research Trust Scholarship Award, UK, 2017 – HIV & Hepatitis Nordic conference, Hotel Hilton, Stockholm, Sweden – Sept. 2729, 2017.

- Dr.S. Ramesh Kumar: Pursued and completed the Masters in Public Health (MPH) course in May 2017 at SRM School of Public Health, SRM University, Kattankulathur, Kancheepuram Dt. Received Gold medal for 1st rank holder in the MPH course (Clinical trials) from SRM Unviesity from the Honourable Vice-President of India, Shri Venkaiah Naidu during convocation ceremony.

ADVOCACY PROGRAMME

Training Programmes / Courses :

The following staff members completed Masters in Public Health (MPH)

1. Dr. V.V. Banurekha
2. Dr. P.K. Bhavani
3. Dr. S. Ramesh Kumar

Special Assignments

Dr. Geetha Ramachandran

(i) Nodal Officer, Performance Evaluation Committee, ICMR
(ii) Member, Board of Studies, Dept. of Biochemistry, Ethiraj College for Women, Chennai

Dr.N. Saravanan

- Nominated by CPCSEA to conduct inspection at the following sites:

(i) Frontier Lifeline Pvt Ltd., Chennai
(ii) GLR Laboratories P Ltd., Madhavaram, Chennai
(iii) Chettinad Hospitals & Research Centre, Chennai
(iv) Bioscience Research Foundation, Chennai
(v) Green Signal Biopharma P Ltd., Chennai
(vi) Animal House Faciality, Govt. Stanley Medical College, Chennai
(vii) HLL Laboratories, Chengalpattu
(viii) Micro Therapeutics Lab, Chennai
(ix) Sathyabama Inst of Science & Technology, Chennai
(x) Central Leather Research Institute, Chennai

Dr.C. Padmapriyadarsini:

- Member of National Expert Committee on 'Diagnosis and Management of Tuberculosis under RNTCP" Central TB Division, MOH & FW : July 2016 (for 3 years).
- Member of Sginal Review Panel of Indian Pharmacopeia, Pharmacovigilance Programme of India : 2017 (for 3 years)
- Member of National Technical Working Group (NTWG0 for HIV/TB meeting, NACO, MoHFW : Aug. 2016.
- Member of Core Committee of Therapeutics Group for the India TB Research Consortium, ICMR & GOI.

Dr.S. Ramesh Kumar:

- Enrolled as Life Member in Indian Medcial Association
- Member in Editorial board of International Journal titled "Journal of Repsiratory Research"

Dr.R. Radhakrishnan:

- External facilitator for On-site training on culture and DST of both FL & SL-DST by liquid (MGIT) for IRL, Chattisgarh.

Dr. Beena E. Thomas, Scientist 'E'
Membership in Expert Committees & Special Assignments

- Member of the Scientific Working Group (SWG) WHO-TDR for research capacity strengthening and knowledge management unit (RCS/KM)
- Member of Board of Studies – Institute of Public Health SRM University
- Member of National Review Meeting of Drug Resistant TB Counselors
- Editorial Board Member for Journal of Health Sciences
- Member of National Advisory Committee on Advocacy Communication Social Mobilization (ACSM)
- Member of International Association of Schools of Social Work (IASSW)
- Member of International Federation for Social Workers (IFSW)
- Member of the Institutional Review Board – GHTM (Govt. Hospital of Thoracic Medicine)
- Member of the Board of studies – University of Madras – Social work

Mr. Senthil Sellappan

- Life member of Professional Social Worker's Association

Mr. P. Murugesan

- Life member of Professional Social Worker's Association

Dr. E. Thiruvalluvan

- Member of FERCAP

Dr. M. Muniyandi

- Health Economics subject for building a research analytic initiative

Dr.Subash Babu:

- Member of the Scientific Program Committee of the American Society of Tropical Medicine and Hygiene 2017.
- Member of the Young Investigator Award Committee of the American Society of Tropical Medicine and Hygiene 2017.

Training programmes

- Every 3 months DSBR provides internship placement for the students of Loyola College, Madras Christina College and Madras Social of Social Work, Chennai
- Training programme to community forum members in Madurai on Leadership skills, Communication, Problem identification and problem solving by Dr. E. Thiruvalluvan
- Two days training program for the project staff of the study titled "Evaluating 99DOTS novel strategy for monitoring adherence for TB medication" from 31st August to 01st September, 2017 at NIRT, Chennai.
- Training on "Power Point Presentation" organized by Excel Prodigy on 15th February and 28th March, 2018 at National Institute for Research in Tuberculosis.
- Field based training in North Chennai slum for field supervisors on selection of community health volunteers
- In-house journal club of DSBR by project staff of DSBR on:-
    i. The challenges and legal implications
    ii. Challenges due to disability and way forward
- Presentation on the topic of "Sexual harassment of women in the workplace" by Mr. Murugesan on 26th March, 2018
- Training of community Health volunteers and field supervisors of greater Chennai Corporation on community preparedness activities for TB Free Chennai initiative at NIRT, ICMR

Participation in Conferences / Seminars / Workshops

INTERNATIONAL

1. Workshop on "Tuberculosis Meningitis: Advancing Immuno-pathogenesis, Diagnosis and Treatment" at Rockville, Maryland, USA from 22nd May, 2017 – **Dr.D. Bella Devaleenal**

2. Participation in the WHO Initiative for Vaccine Research (IVR) Consultation on Tuberculosis Vaccine Development at Geneva, WHO Headquarters, Switzerland on 24 May, 2017 – **Dr.C. Padmapriyadarsini**

3. Oral abstract presentation on "Mobile phone based intervention for high-risk Indian MSM sex workers: Could this be an effective tool for improvements in communication towards sexual risk reduction" at "Indernational Federation Social Work (IFSW) European Conference 2017" at Reykjavik, Iceland on 26th May – 1 st June 2017 – **Dr. Beena E. Thomas**

4. Faculty Speaker for the training course on Paediatric Treatment of HIV-AIDS "Tr@inforPed HIV Scientific Workshop 2017" at Shymkent, Kazakhstan on 14th - 16th June 2017 – **Dr. Syed Hissar**

5. Participation in the 3rd Annual Regional Prospective Observational Research for Tuberculosis (RePORT) International Meeting at Rio de Janeiro, Brazil on 12th - 13th September 2017 – **Dr.C. Padmapriyadarsini**

6. Participation in the 1st BRICS TB Research Networking Meeting at Rio de Janeiro, Brazil on 14th - 15th September 2017 - **Dr.C. Padmapriyadarsini**

7. Workshops on "Building consensus around performance indicators for TB programs" and "TB Infection: Building a Framework for Eradication" at Dubai, United Arab Emirates on 26th - 28th September, 2017 - **Dr.V.V. Banu Rekha**

8. Workshops entitled "Building consensus around performance indicators for TB programs" and "TB Infection: Building a Framework for Eradication" at Dubai, United Arab Emirates on 26th - 28th September, 2017 - **Dr. Sriram Selvaraju**

9. Participation in the Relational Sequencing TB Expert Panel meeting and the workshop on Standard language for reporting Next Generation Sequencing and Molecular Drug Sensitivity Tests at Canary Wharf, London on 26th - 28th September, 2017 – **Dr.K.R. Uma Devi**

10. Workshop on TB Infection: Building a Framework for Eradication at Dubai, United Arab Emirates on 26th - 28th September 2017 – **Dr. Beena E. Thomas**

11. Participation in the WHO Initiative for Vaccine Research (IVR) & Global Tuberculosis Program Consultation on Tuberculosis Vaccine Research and Development at World Health Organization Headquarters, Geneva, Switzerland on 3rd - 4th October 2017 - **Dr.C. Padmapriyadarsini**

12. Oral presentation on “Advancing HIV prevention among Men who have sex with men in India: Cultural approaches, contextual challenges and new strategies” at Harvard Medical School in Boston, USA from 9th - 13th October 2017 - **Dr. Beena E. Thomas**

13. Participation in the “WHO Third Consultation on Bio-banking: access to storage and use of samples during a public health emergency” at Geneva, Switzerland from 24th - 25th October 2017 - **Dr. Luke Elizabeth Hanna**

14. Participation in the “Inaugural Centre Partnership meeting” of the MRC-DBT grant Cambridge-Chennai Centre Partnership on Antimicrobial Resistant Tuberculosis at Cambridge, UK on 29th October - 1st November, 2017 – **Dr. Dina Nair**

15. Participation in the “Inaugural Centre Partnership meeting” of the MRC-DBT grant Cambridge-Chennai Centre Partnership on Antimicrobial Resistant Tuberculosis at Cambridge, UK on 29th October - 1st November 2017 - **Dr. Mohan Natrajan**

16. Participation in the “Inaugural Centre Partnership meeting” of the MRC-DBT grant Cambridge-Chennai Centre Partnership on Antimicrobial Resistant Tuberculosis on 29th - 31st October 2017 and attended training program in DNA library preparation, sequencing, sequence data QC and data analysis at Cambridge, UK on 1st – 17th November 2017 - **Dr. K.R. Uma Devi**

17. Participation in the “Inaugural Centre Partnership meeting” of the MRC-DBT grant Cambridge-Chennai Centre Partnership on Antimicrobial Resistant Tuberculosis at UK on 30th October - 1st November 2017 - **Dr.S. Siva Kumar**

18. Participation in the “DR-TB Symposium focussing on Clinical and Programmatic Practices that have improved the management of DR-TB in South Africa” at Johannesburg, South Africa on 30th - 31st October, 2017 - **Dr.P.K. Bhavani**

19. Participation in the Trial Management Group (TMG) and Trial Steering Committee of the SHINE trial at Kampala, Uganda on 14th - 16th November 2017 - **Dr.S. Syed Hissar**

20. Awarded “Australia Awards Fellowship” entitled “Metagenomics guided management of drug resistant tuberculosis and HIV: Advances in Diagnostics” to be hosted by the University of Sydney at Sydney, Australia on 19th November - 2nd December, 2017 - **Dr. Luke Elizabeth Hanna**

21. Awarded “Australia Awards Fellowship” entitled “Metagenomics guided management of drug resistant tuberculosis and HIV: Advances in Diagnostics” to be hosted by the University of Sydney at Sydney, Australia on 23rd November – 5th December, 2017 - **Dr.K.R. Uma Devi**

22. Awarded “Australia Awards Fellowship” entitled “Metagenomics guided management of drug resistant tuberculosis and HIV: Advances in Diagnostics” to be hosted by the University of Sydney at Sydney, Australia on 19th November - 2nd December, 2017 - **Dr.S. Sivakumar**

23. Participation in the “Meeting of the Scientific Working Group for Research Capacity Strengthening and Knowledge Management (RCS-KM)” at Geneva, Switzerland on 27th - 29th November 2017 - **Dr. Beena E. Thomas**

24. Participation in the 5th Nikkei Asian Conference on Communicable Diseases at Okinawa, Japan on 2nd - 3rd February 2018 - **Dr. Sriram Selvaraju**

25. International workshop on "The use of oral BCG in animals and humans" at Jerusalem, Israel on 28th February - 5th March, 2018 - **Dr.P. Kannan**

26. Participation in the WHO/CPTR Workshop on Advances in Clinical Trial Design for Development of New TB Treatments at Glion, Switzerland on 14th - 16th March 2018 - **Dr.C. Padmapriyadarsini**

27. Participation in the Asian Tuberculosis Research and Clinical Trials Integrated Organisational Network (A-TRACTION) at University of Singapore, Singapore on 19th - 20th March, 2018 - **Dr.C. Padmapriyadarsini**

NATIONAL

DIRECTOR-IN-CHARGE

1. Participation in the India-Africa Health Sciences Collaboration at ICMR HQ, New Delhi on 06th April 2017 – **Dr. Srikanth Tripathy**

2. Participation in the Union TB meeting at CTD, New Delhi on 07th – 08th April 2017 – **Dr. Srikanth Tripathy**

3. Participation in the Expert committee on TB diagnostics at ICMR HQ, New Delhi on 18th April 2017 **– Dr. Srikanth Tripathy**

4. Participation in the viva-voce examination of Ph.D. scholar at NARI, Pune on 17th May 2017 – **Dr. Srikanth Tripathy**

5. Participation in the consultative meeting with partners on implementation feasibility of Rifapentine-Isoniazid based TB preventive therapy at NACO, New Delhi on 18th May 2017 – **Dr. Srikanth Tripathy**

6. Participation in the development on UNITAID proposal at ICMR HQ, New Delhi on 20th May 2017 – **Dr. Srikanth Tripathy**

7. Participation in the MBAPS committee at ICMR HQ, New Delhi on 24th May 2017 – **Dr. Srikanth Tripathy**

8. Participation in the GHSA Quarterly Review meeting at NIMHANS, Bengaluru on 29th May 2017 – **Dr. Srikanth Tripathy**

9. Participation in the expert committee to review the receptol study protocol at NARI, Pune on 06th June 2017 – **Dr. Srikanth Tripathy**

10. Participation in the National Technical Working Group for HIV/TB at NACO, New Delhi on 19th June 2017 – **Dr. Srikanth Tripathy**

11. Participation in the first expert group meeting to discuss proposal of BSL-3 at ICMR HQ, New Delhi on 04th July 2017 – **Dr. Srikanth Tripathy**

12. Participation in the ICMR-SAG 2017 meeting at ICMR HQ, New Delhi on 19th – 20th July 2017 – **Dr. Srikanth Tripathy**

13. Participation in the high level committee to discuss the development and upgradation of regional SRL in STAC, Kathmandu on 25th August 2017 – **Dr. Srikanth Tripathy**

14. Participation in the CTD-CDC TB meeting with partners at Nirman Bhawan, New Delhi on 28th September 2017 – **Dr. Srikanth Tripathy**

15. Participation in the 2nd International SAG meeting at ICMR, New Delhi on 31st October 2017 – **Dr. Srikanth Tripathy**

16. Participation in the India State Level Disease Burden Initiative – Dissemination of findings at Shangri-la Eros hotel in New Delhi on 14th November 2017 – **Dr. Srikanth Tripathy**

17. Participation in the expert committee on regulation of anti-TB drugs in India at New Delhi on 21st September 2017 – **Dr. Srikanth Tripathy**

18. Participation in the 2nd India US Health Dialogue at Nirman Bhawan, New Delhi on 26th September 2017 - **Dr. Srikanth Tripathy**

19. Participation in the conferment of IAL president appreciation gold plaque 2017 at Hotel Nest, Shankarpur, Digha, West Bengal on 2nd November 2017 – **Dr. Srikanth Tripathy**

20. Participation in the expert consultation on finalizing strategic priorities for evidence generation towards achieving 90-90-90 at The Gateway Taj Hotel, Pune on 12th December 2017 – **Dr. Srikanth Tripathy**

21. Participation in the media training at Mumbai on 15th December 2017 – **Dr. Srikanth Tripathy**

22. Participation in the 7th annual REPORT India Joint leadership meeting at ICGEB, New Delhi on 17th February 2018 – **Dr. Srikanth Tripathy**

23. Participation in the HIV cases in Unnao, UP at NACO, New Delhi on 21st February 2018 – **Dr. Srikanth Tripathy**

24. Participation in the project review committee at ICMR HQ, New Delhi on 05th March 2018 - **Dr. Srikanth Tripathy**

DEPARTMENT OF CLINICAL RESEARCH:

1. Participation in the Induction training on research methodology & research administration for ICMR Scientists at NIE, Chennai on 03rd – 07th April 2017 – **Dr. Prathiksha. G**

2. Participation in the site visit & training of study staff “Nutritional supplementation of adult with pulmonary TB in Odisha” at RMRC, Bhubaneswar on 11th – 13th April 2017 – **Dr. Padmapriyadarsini**

3. Guest lecture on key issues in research initiatives of indigenous medical systems and probable means of overcoming at University of Pondicherry on 21st April 2017 – **Dr. Syed Hissar**

4. Participation in the systematic review-writing workshop at Hotel Crowne plaza, New Delhi on 23rd – 28th April 2017 – **Dr. C. Padmapriyadarsini**

5. Participation in the training program for ECHO platform at New Delhi on 02nd – 04th May 2017 – **Dr. V. V. Banu Rekha**

6. Participation in the course on advances in biology of communicable diseases at NIRRH, Mumbai on 08th May – 02nd June 2017 – **Dr. Dina Nair**

7. Participation in Bedaquiline clinical trial discussion – J & J at ICMR HQ, New Delhi on 11th May 2017 – **Dr. C. Padmapriyadarsini**

8. Participation in delamanid discussion – WHO/CTD at ICMR HQ, New Delhi on 11th May 2017 – **Dr. C. Padmapriyadarsini**

9. Participation in Investigators meeting of Bedaquiline clinical trial at ICMR HQ, New Delhi on 11th May 2017 – **Dr. P. K. Bhavani**

10. Participation in the meeting with Otsuka on delamanid introduction in India at Nirman Bhavan, New Delhi on 11th May 2017 – **Dr. Dina Nair**

11. Participation in site selection for ITRL trials at ICMR HQ, New Delhi on 12th May 2017 – **Dr. C. Padmapriyadarsini**

12. Participation in Rifapentine meeting – NACO at ICMR HQ, New Delhi on 12th May 2017 – **Dr. C. Padmapriyadarsini**

13. Participation in ITRF meeting for site selection at ICMR HQ, New Delhi on 12th May 2017 – **Dr. P. K. Bhavani**

14. Participation in X signal review panel meeting, PvPI at CDSCO, New Delhi on 16th May 2017 – **Dr. C. Padmapriyadarsini**

15. Participation in meeting to finalize clinical trial protocols at ICMR HQ, New Delhi on 29th May 2017 – **Dr. C. Padmapriyadarsini**

16. Participation in the ITRC – Expert & Working group meeting to finalize clinical trial protocols at ICMR HQ, New Delhi on 29th May 2017 – **Dr. P.K. Bhavani**

17. Participation in ICMR strategic plan : Core group meeting at NIE, Chennai on 05th -06th June 2017 - **Dr. C. Padmapriyadarsini**

18. Participation in the meeting of Himachal Pradesh State Health Commission at National Health Commission, Himachal Pradesh on 05th – 06th June 2017 – **Dr. V. V. Banu Rekha**

19. Guest lecture on Analysis on ADR/AE & deficiencies in data entry in the Bedaquiline CAP review meeting at CTD, Guwahati on 06th – 09th June 2017 – **Dr. C. Padmapriyadarsini**

20. Participation in the Velos software installation & initiation at NIRT in ICMR HQ, New Delhi on 13th – 16th June 2017 – **Dr. G. Narendran**

21. Workshop on Vellos software application for data management protocol development and CR7 at ICMR HQ, New Delhi on 14th – 16th June 2017 – **Dr. Bhavani.P.K.**

22. Invited talk on the pediatric treatment of HIV/ADS 'Tr@inforPedHIV Scientific Workshop 2017' at Shymkent, Kazakhstan on 14th – 16th June 2017 – **Dr. S. Syed Hissar**

23. Participation in the data management of clinical trials at NIMS, New Delhi on 14th – 16th June 2017 – **Dr. C. Padmapriyadarsini**

24. Participation in the End TB workshop-clinical management of XDR-TB at MSF Office, Mumbai on 18th -23rd June 2017 – **Dr. Shrinivasa.B.M**

25. Participation in the End TB workshop-clinical management of XDR-TB at MSF Office, Mumbai on 18th -23rd June 2017 – **Dr. C. Padmapriyadarsini**

26. Participation in the NACO TWG meeting at NACO Office, New Delhi on 19th June 2017 – **Dr. P.K. Bhavani**

27. Participation in the MSF training on clinical management of XDR TB with new TB drugs at ITRF and MSF Mumbai on 22nd – 23rd June 2017 – **Dr. Dina Nair**

28. Participation in the MSF training on clinical management of XDR-TB with new TB drugs at ITRF and MSF Mumbai on 22nd – 23rd June 2017 – **Dr. V. V. Banu Rekha**

29. Participation in the 4th summit on good and replicable practices and innovation in public health care system at Indore on 06th – 08th July 2017 – **Dr. Dina Nair**

30. Participation in the 4th summit on good and replicable practices and innovation in public health care system at Indore on 06th – 07th July 2017 – **Dr. Syed Hissar**

31. Guest lecture on XDR-TB protocol/High dose RMP/Metformin protocol in consultative meeting on OR under medical college at New Delhi on 06th July 2017 – **Dr. C. Padmapriyadarsini**

32. Participation in the induction training program for ICMR scientists("Research methods and research administration) at NIE, Chennai on 10th – 14th July 2017 – **Dr. B. M. Shrinivasa**

33. Participation in the BDQ CAP review meeting at Claridges Hotel, New Delhi on 24th – 25th July 2017 – **Dr. C. Padmapriyadarsini**

34. Workshop on policy brief at NIMR, New Delhi on 24th – 25th July 2017 – **Dr. G. Narendran**

35. Participation in the national TB HIV coordination meeting (NTCC) for HIV/TB at Nirman Bhavan, New Delhi on 31st July 2017 – **Dr. C. Padmapriyadarsini**

36. Oral presentation on consolidated pharmacovigilance data of Bdq-CAP at CTD, Mumbai on 17th – 18th August 2017 – **Dr. Padmapriyadarsini**

37. Poster presentation of phase III multicentre open label RCT to asses efficacy and safety of delamanid in combination with other drugs in ITRC Therapeutics at ICMR HQ, New Delhi on 24th August 2017 – **Dr. B. M. Shrinivasa**

38. Participation in the Vaccine trial POD protocol development workshop at ITRC, New Delhi on 28th – 30th August 2017 – **Dr. V.V. Banu Rekha**

39. Participation in the workshop on research methods at CMC, Vellore on 28th – 30th August 2017 – **Dr. B. M. Shrinivasa**

40. Participation in the core-group meeting for preparing common submission form for ECS in country at NCDIR, Bangalore on 06th September 2017 – **Dr. V.V. Banu Rekha**

41. Poster presentation of Phase II clinical trial of verapamil in the meeting of SEC (Antimicrobial & Antiviral) at FDA Bhavan, New Delhi on 22nd September 2017 – **Dr. C. Padmapriyadarsini**

42. Participation in the therapeutic group meeting of ITRC at ICMR HQ, New Delhi on 27th – 28th September 2017 – **Dr. Bhavani .P.K**

43. Participation in the international course in health research methods & evidence based medicine at St. Johns Research Institute, Bangalore on 09th – 13th October 2017 – **Dr. Devarajulu Reddy**

44. Participation in the expert committee meeting to set-up CCRS-research centre, Tirupati at Chennai on 11th October 2017 – **Dr. Syed Hissar**

45. Poster presentation on weekly high dose INH & Rifapentine for TB prophyasis at FDA Bhavan, New Delhi on 12th October 2017 – **Dr. Dina Nair**

46. Participation in the India International Science Festival 2017 – Young Scientsits conclave at Anna University, Chennai on 13th – 16th October 2017 – **Dr. S. Syed Hissar**

47. Poster presentation of TB Therapeutics & future plan of II international scientific advisory group meeting at ICMR HQ, New Delhi on 31st October – 02nd November 2017 – **Dr. C. Padmapriyadarsini**

48. Participation in the International scientific advisory group meeting at ICMR HQ, New Delhi on 31st October – 01st November 2017 – **Dr. V. V. Banu Rekha**

49. Guest lecture on the workshop on basics of bio-medical research at Captain Srinivasa Murthy Regional Ayurveda Drug Development Institute, Chennai on 07th November 2017- **Dr. Syed Hissar**

50. Rapporteur on the national workshop to identify strategic research priorities for HIV/AIDS at NACO on 09th – 10th November 2017 – **Dr. C. Padmapriyadarsini**

51. Guest lecture on the Medanta TB conclave 2017 at Medanta hospital, Gurgaon on 09th – 10th November 2017 – **Dr. C. Padmapriyadarsini**

52. Participation on the building a research analytic Initiative at NARI, Pune on 13th – 15th November 2017 – **Dr. V. V. Banu Rekha**

53. Poster presentation on shorter regimen for MDR-TB in NAPCON 2017: Tuberculosis workshop at Kolkata Science City on 16th November 2017 – **Dr. C. Padmapriyadarsini**

54. Presentation of TB therapeutics in the expert group meeting of Therapeutics group at India TB Research Consortium, New Delhi on 05th December 2017 – **Dr. C. Padmapriyadarsini**

55. Participation in the 9th Bengaluru India Nano at Lalit Ashok, Bengaluru on 07th – 08th December 2017 – **Dr. B. M. Shrinivasa**

56. Poster presentation on NTM disease in HIV patients in diagnostic challenges in opportunistic infections at PGI, Chandigarh on 07th – 09th December 2017 – **Dr. C. Padmapriyadarsini**

57. Poster presentation of Indo-US symposium on HIV-TB at PGIMER, Chandigarh on 07th – 09th December 2017 – **Dr. G. Narendran**

58. Participation of media training at Mumbai on 15th December 2017 – **Dr. D. Bella Devaleenal**

59. Participation in the 72nd national conference of TB and chest diseases at KIMS, Andhra Pradesh on 15th – 17th December 2017 – **Dr. Devarajulu Reddy**

60. Oral presentation on SAE monitoring & CEM at State Level training on revised PMDT Guidelines 2017 at Mumbai on 15th – 16th January 2018 – **Dr. C Padmapriyadarsini**

61. Guest lecture on "ADSM(CEM) & monitoring systems the RNTCP" in Capacity building workshop on Delamaind at JW Marriott Aerocity, New Delhi on 31st January – 01st February 2018 – **Dr. C. Padmapriyadarsini**

62. Guest lecture on HIV Mysore ART Update 2018 at Ashakirana, Mysore on 02nd – 04th February 2018 – **Dr. G. Narendran**

63. Poster presentation on Ëffect of co-administration of Unani pharmacopoeias formulations with ATT drugs in adult wistar albino rats in International Conference on Unani medicine at ICAR Conference hall, New Delhi on 10th – 11th February 2018 – **Dr. Syed Hissar**

64. Participation on State level training on revised PMDT guidelines at State TB Officer, Chennai on 13th – 15th February 2018 – **Dr. Syed Hissar**

65. Poster presentation on "C-TRIUMPH & substitutes" on REPORT Annual conference meeting at REPORT India consortium, New Delhi on 15th – 16th February 2018 – **Dr. C. Padmapriyadarsini**

66. Participation on International Conference on anti-microbial resistance at NICED, Kolkata on 16th – 17th February 2018 – **Dr. Syed Hissar**

67. Participation on REPORT annual conference meeting at REPORT India Consortium, New Delhi on 16th – 18th February 2018 – **Dr. B. M. Shrinivasa**

68. Participation on REPORT India 7th Annual meeting at International centre for Genetic Engineering and Biotechnology, New Delhi on 17th February 2018 – **Dr. V. V. Banu Rekha, Dr Dina Nair**

69. Participation on ISAG meeting & BRICS meeting at ICMR HQ, New Delhi on 19th February 2018 – **Dr. C. Padmapriyadarsini**

70. Guest lecture on "how to write an original article" in workshop on scientific writing at Dr. MGR Medical University, Chennai on 22nd February 2018 – **Dr. Syed Hissar**

71. Workshop on research methods at CMC, Vellore on 05th – 07th March 2018 - **Dr. N. Poorana Ganga Devi**

72. Participation on evidence based medicine of clinical research conference (EBCON 2018) at SRM Medical College Hospital & Research Centre, Chennai on 10th March 2018 – **Dr. Syed Hissar**

73. Participation on Delhi – End TB Summit at Vigyan Bhavan, New Delhi on 12th – 13th March 2018 – **Dr. C. Padmapriyadarsini**

74. Participation on 27th Asia pacific association for the study of liver (APASL 2018) at New Delhi on 14th -18th March 2018 – **Dr. Syed Hissar**

75. Workshop on research methodology at Chennai Medical College Hospital and Research Centre, Trichy on 22nd – 24th March 2018 – **Dr. N. Poorana Ganga Devi**

76. Guest lecture on "Framing of research question" in research methods at Anna Hospital, Chennai on 22nd March 2018 – **Dr. Syed Hissar**

77. Guest lecture on **"**TB Update" in World TB Day observation at Saveetha Dental College on 24th March 2018 – **Dr. D. Bella Devaleenal**

78. Poster presentation on "IPT in PLHIV" in NACO at New Delhi on 27th March 2018 – **Dr. C. Padmapriyadarsini**

79. Poster presentation on "MDR-TB→Drug procurement" in ITRC meeting at ICMR, New Delhi on 27th March 2018 - **Dr. C. Padmapriyadarsini**

DEPARTMENT OF SOCIAL BEHAVIORAL RESEARCH:

1. Participation in the site visit & training of study staff "Nutritional supplementation of adult with pulmonary TB in Odisha" at RMRC, Bhubaneswar on 11th – 13th April 2017 – **Dr. Beena E. Thomas**

2. Participation in the Training programme at Sree Chitra Tirunal Institute for Medical Sciences & Technology(SCTIMST), Kerala on 08th – 13th May 2017 – **Dr. Beena E Thomas, Dr.M. Muniyandi, Dr.N.Karikalan**

3. Participation in the induction training program for ICMR scientists("Research methods and research administration) at NIE, Chennai on 10th – 14th July 2017 – **Dr. N. Karikalan**

4. Participation in the expert committee meeting-India TB research consortium at ICMR, New Delhi on 26 & 27 July 2017 – **Dr. Beena E.Thomas**

5. Participation in the 99 DOTS meeting with Microsoft team at BMGF, Bangalore on 3 & 4 August 2017 – **Dr. Beena E. Thomas**

6. Participation in the THRF meeting at NIRTH, Jabalpur on 16 & 17 August 2017 – **Dr. Beena E. Thomas**

7. Participation in the 2nd ISAG meeting at ICMR, New Delhi during 30 October – 1 November 2017 – **Dr. Beena E. Thomas**

8. Participation on the workshop of developing strategies for health promotion and care awareness at Tata Memorial Hospital, Mumbai on 8 & 9 December 2017 – **Dr. Beena E. Thomas**

9. Participation on the adherence all hands meeting at BMGF, New Delhi on 18 & 19 December 2017 – **Dr. Beena E. Thomas**

10. Participated on the 7th International Fellowship on Health Technology Assessment Workshop and 2nd National Conference on Health Technology Assessment at Chandigarh, organized by School of Public Health, PGIMER, Chandigarh during Feb. 19-25, 2018 – **Dr. Beena E. Thomas**

11. Participation on training on Network analysis at NIRRH, Mumbai during 26 – 28 February 2018 – **Dr. N. Karikalan**

12. Oral presentation on emerging trends in Health Economics in India –Opportunities for academics and research at Popes's College, Thoothukudi on 5th March 2018 – **Dr. M. Muniyandi**

13. Participation on Delhi – End TB Summit at Vigyan Bhavan, New Delhi during 12 – 14 March 2018 – **Dr. Beena Thomas**

DEPARTMENT OF BACTERIOLOGY

1. Guest Lecture on global challenges in latent tuberculosis at Sathyabama University, Chennai on 7 April 2017 – **Dr. V. N. Azger Dusthackeer**

2. Participation in the training of LT, STLS, DTO, IRL microbiologists for TRUNAT Study at Karnataka during 9 – 11 April 2017 – **Dr. N.S. Gomathi**

3. Guest Lecture on recent trends in antimicrobial screening at Madurai Kamarajar University, Madurai on 12 & 13 April 2017 – **Dr. V. N. Azger Dusthackeer**

4. Participation in the ICMR & DBT expert committee meeting at ICMR HQ on 18 & 19 April 2017 – **Dr. N.S. Gomathi**

5. Participation in the ECHO training programme at New Delhi during 1 – 5 May 2017 – **Dr. R. Priya**

6. Participation in the preparatory activities for NABL accreditation at NITRD, New Delhi during 23 – 27 May 2017 – **Dr. V. N. Azger Dusthackeer**

7. Participation in the internal auditors and QMS training at NITRD, New Delhi during 24 – 27 May 2017 – **Dr. S. Siva Kumar**

8. Participation in the Genetics in public health at IIPH, Gurgaon during 29 May – 2 June 2017 – **Dr. V. N. Azger Dusthackeer**

9. Participation in the discussion with CTD on GHSA meeting at Nirman Bhawan, New Delhi on 05th June 2017 – **Dr. Siva Kumar**

10. Participation in the 1st mentoring workshop scheduled for preparatory activities towards NABL accreditation at RMRC Bhubaneshwar during 27 June – 1 July 2017 – **Dr. V. N. Azger Dusthackeer**

11. Participation to facilitate 2nd line LPA onsite training for lab staff at IRLS and C&DST labs at IRL-Kerala during 5 – 7 July 2017 – **Dr. N. S. Gomathi**

12. Participation to facilitate 2nd line LPA onsite training for lab staff at IRLS and C&DST labs at IRL-Ahmedabad during 16 – 18 July, 2017 – **Dr. N. S. Gomathi**

13. Participation to facilitate 2nd line LPA onsite training for lab staff at IRLS and C&DST labs at IRL-Jamnagar during 19 – 22, July 2017 – **Dr. N. S. Gomathi**

14. Participation in the training programme of SLD-LPA training at IRL Trivandrum during 31 July – 1 August 2017 – **Dr. N. S. Gomathi**

15. Guest lecture on the current concepts and diagnostic challenges in clinical microbiology at Government Medical College, Kozhikode on 4 & 5 August 2017 – **Dr. N. S. Gomathi**

16. Participation in the expert committee meeting: TB diagnostics at ICMR HQ, New Delhi on 18th September 2017 – **Dr. N. S. Gomathi**

17. Participation in the India International Science Festival-2017 at Anna University, Chennai on 13th October 2017 – **Dr. N. S. Gomathi.**

18. Conducted onsite evaluation (OSE) of two states (Tamil Nadu and Andhra Pradesh) in 2017 for monitoring EQA of smear microscopy and culture and DST by both phenotypic and genotypic methods for RNTCP programme – **Dr. Prabu Seenivasan.**

19. Facilitated onsite training on culture & extended second line DST by MGIT at IRL, Telangana state during 28 Feb – 2 March, 2018 - **Dr. Prabu Seenivasan.**

20. Facilitated one day workshop for updating on Recording and reporting of BEDAQUILINE CAP for staff of DR TB Centers on 25th May 2017 at GHTM Tambaram. Chennai, Tamil Nadu - **Dr. Prabu Seenivasan.**

21. Facilitated the training on Revised PMDT Guidelines to District DRTB Coordinators and paramedical Staff on 01.02.2018 at Health & Family Welfare Training Centre, Egmore, Chennai – 600008 - **Dr. Prabu Seenivasan.**

22. Facilitated refresher training on EQA of smear microscopy for STLS of entire Andhra Pradesh at STDC, Vishakhapatnam, Andhra Pradesh from 19-22 March 2018 - **Dr. Prabu Seenivasan.**

23. Guest lecture given on “Air pollution and Tuberculosis” presented in a National seminar on "Environmental toxicology and its impact on Biodiversity" organised by Department of Biotechnology, KRMM College, Chennnai on 1st September 2017- **Dr. Prabu Seenivasan**

24. Guest lecture given on "Diagnosis of drug resistant tuberculosis by MGIT" presented in CME jointly organized by Department of Thoracic medicine and Department of Microbiology Govt. Stanly Medical College, Chennai on 3&4 November 2017- **Dr. Prabu Seenivasan**

25. Guest lecture given on "Tuberculosis, Dengue and Malaria" as part of swatchta Abiyan awareness programme conducted by NCC Navel Wing, Pachaiyappas' College, Chennai on 7th September 2017- **Dr. Prabu Seenivasan**

26. Conducted onsite evaluation of two states (Telangana state and Puducherry) in 2017 for Monitoring EQA of smear Microscopy and Culture and DST by Both Phenotypic and Genotypic methods for RNTCP programme - **Dr.R. Radhakrishnan.**

DEPARTMENT OF IMMUNOLOGY

1. Participation in the Induction training on research methodology & research administration for ICMR Scientists at NIE, Chennai on 03rd-07th April 2017 – **Dr. K. R. Uma Devi**

2. Participation in the TAG meeting organized by REACH, Chennai at Hotel Reganta Central Deccan, Royapettah on 05th April 2017 – **Dr. K. R. Uma Devi**

3. Participation in the second quarterly meeting to review the progress of implementation of GHSA projects in India at DGHS, New Delhi on 29th May 2017 – **Dr. K. R. Uma Devi**

4. Participation in the second medical technology steering committee meeting at Tamilnadu Medical Services Corporation Limited, Chennai on 01st June 2017 – **Dr. K. R. Uma Devi**

5. Participation in the discussion with CTD on GHSA meeting at Nirman Bhawan, New Delhi on 05th June 2017 – **Dr. K. R. Uma Devi**

6. Workshop to attend doctoral committee meeting for pre-confirmation of PhD degree at Sri Venkateswara College of Engineering, Sri Perumbudur on 14th June 2017 – **Dr. K. R. Uma Devi**

7. Invited as resource person for the ICMR sponsored two day national level workshop on awareness about safety of human health amongst natural environment at Paavai Engineering College, Namakkal on 22nd – 23rd June 2017 – **Dr. K. R. Uma Devi**

8. Participation in the induction training program for ICMR scientists("Research methods and research administration) at NIE, Chennai on 10th – 14th July 2017 – **Dr. Himanshu Singh Chandel**

9. Participation in the networking meeting at Hotel Le Meridian, Chennai on 28th August 2017 – **Dr. K. R. Uma Devi**

10. Participation to attend the TAG meeting organized by REACH at Hotel Reganta Central Deccan, Royapettah, Chennai on 15th September 2017 – **Dr. K. R. Uma Devi**

11. Participation on the TAG meeting organized by REACH at Hotel Reganta Central Deccan, Royapettah on 21st December 2017 – **Dr. K. R. Uma Devi**

12. Participation on the foundation for Medical Research discussion meeting at Mumbai on 12th January 2018 – **Dr. K. R. Uma Devi**

13. Guest lecture on REACH dissemination meeting – project EQUIP and pharmacy initiative at Hotel GRT Convention Centre, Chennai on 21st February 2018 – Dr. K. R. Uma Devi

14. Workshop on research methodology at NIE, Chennai on 20th – 22nd March 2018 – **Dr. K. R. Uma Devi**

DEPARTMENT OF STATISTICS

1. Workshop on research methods at CMC, Vellore on 05th – 07th March 2018 – **Dr. R. Mahalakshmi & Dr. Muthu Vijayalakshmi**

2. Participated 12th Capacity building programme for Technical personnel held at IIPA, New Delhi during March 5-16, 2018.

3. Workshop on Public Health Dynamics at NIMS, New Delhi on 05th – 09th March 2018 – **Dr. C. Ponnuraja**

4. Participated in East Asia Regional conference of International Biometric Society during March 5-7, 2018 – **Dr.C. Ponnuraja**

5. Participated Evidence Based Medicine and Clinical Research Conference (EBCCON 2018) at SRM Medical College, Kattankulathur, on March 9 & 10, 2018 – **Ms.V. Mythily**.

6. Participated as a Resource person in workshop on Univariate and Multivariate analysis with R-Programming and Time to event data analysis with practical session using R. Conducted at VIT, on March 1, 2018 **– Dr.C. Ponnuraja.**

7. Participation in the training programme for the data management of clinical trials under TB consortium at ICMR, New Delhi on 14th – 16th June 2017 – **Dr. C.Ponnuraja**

8. Participation in the BDQ CAP review meeting at New Delhi on 24th – 25th July 2017 – **Dr. C. Ponnuraja**

9. Participation in the DSMC meeting at Mumbai on 17th August 2017 – **Dr. C. Ponnuraja**

10. Participation in vaccine trial protocol development workshop at ICMR HQ, New Delhi on 28th – 29th August 2017 – **Dr. C. Ponnuraja**

11. Participation in the workshop on research methods at CMC Vellore on 28th – 30th August 2017 – **Dr. C. Ponnuraja**

12. Presented a paper on “Identification of tuberculosis cluster using spatial scan statistic and model selection using lof likelihood ratio” in the XXXV Annual Conference of Indian Society for Medical Statistics (ISMSCON-2017), Lucknow, during Nov. 2-4, 2017 – **Dr.K. Chandrasekaran.**

DEPARTMENT OF EPIDEMIOLOGY STATISTICS

1. Participation in the training programme for the data management of clinical trial conducted under TB consortium at NIMS, New Delhi on 14th – 16th June 2017 - **Dr. Basilea Watson**

2. Participation on diagnostic and screening test evaluation: Imperfect Gold Standard, Meta Analyses and application of latent class models workshop at CMC, Vellore on 09th – 11th October 2017 – **Dr. K. Rajendran**

DEPARTMENT OF HIV/AIDS

1. Participation in the meeting of expert committee of the ICMR Biomedical Informatics project at ICMR HQ, New Delhi on 05th April 2017 – **Dr. Luke Elizabeth Hanna**

2. Participation in the seminar on bio-medical waste management at The Tamil Nadu Dr. MGR Medical University, Chennai on 28th June 2017 – **Dr. Luke Elizabeth Hanna**

3. Participation in the doctoral committee meeting at Sathyabama University, Chennai on 30th June 2017 – **Dr. Luke Elizabeth Hanna**

4. Participation on the National HIV Cohort program at NIMS, New Delhi on 09th January 2018 – **Dr. Luke Elizabeth Hanna**

5. Participation on REPORT India annual conference at REPORT India consortium, New Delhi on 05th February 2018 **– Dr. Luke Elizabeth Hanna**.

6. Participated in the "International symposium on Application of AMD and Public Health Partners in India" at NIRT. Aug 18-19, 2017 – **Dr. Luke Elizabeth Hanna**.

7. Awarded Australia Awards Fellowship to attend the training program on "Metagenomics guided management of drug resistant tuberculosis and HIV: advances in diagnostics" at University of Sydney, Australia. Nov 19 - Dec 2, 2017 - **Dr. Luke Elizabeth Hanna**.

8. Attended the "Third Consultation on Biobanking: access to storage and use of samples during a public health emergency" organized by WHO at Geneva, Switzerland. Oct 24-25, 2017 - **Dr. Luke Elizabeth Hanna.**

9. Invited talk on "Current Trends in HIV Research" at the National Conference on "Emerging trends in viral diseases" at the Post graduate Institute of Basic Medical Sciences, Madras University. Feb 14, 2018 - **Dr. Luke Elizabeth Hanna.**

10. Attended the "RePORT India Annual meeting" at ICGEB, New Delhi. Feb 15-17, 2018 - **Dr. Luke Elizabeth Hanna.**

11. Poster presentation at HIV & Hepatitis Nordic conference, Stockholm, Sweden during Sept. 27-29, 2017 - **Mr. Ashok Kumar M.**

12. Underwent training programme on viral inhibition assay at Human Immunology Laboratory, IAVI, Imperial College of London, London – during Sept. 10-21, 2017 - **Mr.M.P. Sivasankaran.**

13. Poster presentation at International Vaccinology Conference at ICGEB, New Delhi during Nov. 27 & 28, 2017 - **Mr.M.P. Sivasankaran**.

14. Attended a workshop on 'Quality management system and internal audit' at Apollo Speciality Hospital, Chennai during Dec. 18-21, 2017 – **Ms. Lucia Precilla.**

15. Attended a workshop on 'Quality management system and internal audit' at Apollo Speciality Hospital, Chennai during Jan. 9-12, 2018 – **Mr. Saravanan**.

16. Underwent training programme at Vaccinology @ Institute, Institute Paasteur, Paris, France during Feb 8 – March 11, 2018 - **Mr.M.P. Sivasankaran**.

DEPARTMENT OF NIH-ICER

1. Keystone Symposium in Tuberculosis, Whistler, Canada 2018 attended by – **Dr. Subash Babu, Dr N Pavan Kumar**

2. Annual meeting of the American Society of Tropical Medicine and Hygiene, Baltimore, MD, USA 2017 attended by **Dr. Subash Babu, Dr R Anuradha**

3. Participation on the 7th annual report India joint leadership meeting at International Centre for Genetic Engineering and Biotechnology (ICGEB) on 15th – 17th February 2018 – **Dr. Subash Babu, Dr N Pavan Kumar, Mr Kadar Moideen**

4. Global conference on TB Vaccines, New Delhi, India 2018 attended by **Dr. Subash Babu, Dr N Pavan Kumar, Dr R Anuradha, Dr K Gokul Raj, Mr Kadar Moideen**

5. Workshop on Advanced Techniques in Genomics, Madras Diabetes Research Foundation, Chennai – **Dr M Saravanan**

6. National level advanced workshop on Effective strategies in research project proposal writing and linking funding agencies, LIFE – Loyola College – **Dr N Pavan Kumar, Dr R Anuradha, Dr M Saravanan, Mr Kadar Moideen, Ms Yukti Bootra**

DEPARTMENT OF CLINICAL BIOCHEMISTRY

1. Participation in the training course on advances in Biology of communicable diseases at NIRRH, Mumbai on 08th May - 02nd June 2017 – **Dr. N. Saravanan**

2. Participation in the media training at NIN, Hyderabad on 14th – 16th November 2017 – **Dr. Geetha Ramachandran**

3. Participation in the ISO 15189:2012 IA & QMS Certificate course at Apollo Hospitals, Chennai on 20th – 23rd November 2017 – **Dr. A.K. Hemanth Kumar**

4. Participation in the 72nd national conference on TB and chest diseases (NATCON 2017) at Rajahmundry, Andhra Pradesh on 14th – 17th December 2017 – **Dr. A. K. Hemanth Kumar**

5. Participation in the media training at NIRRH, Mumbai on 15th December 2017 – **Dr. Geetha Ramachandran**

6. Workshop on National Conference on Interdisciplinary Research and Innovations in Biosciences, NATCON – 2018 at Mohamed Sathak College of Arts & Science, Chennai on 25th January 2018 – **Dr. Geetha Ramachandran**

7. Invited speaker at the Dept. of Pharmacology, Madurai Medical College, Madurai during January 2018 – **Dr. Geetha Ramachandran**

8. Workshop on Dissemination program on ICMR National Ethical Guidelines organised by Sri Ramachandra Medcial College and Reseqrch Institute, Chenani during Feb. 2018 – **Dr. Geetha Ramachandran, Dr.A.K. Hemanth Kumar, Dr.N. Saravanan**

DEPARTMENT OF EPIDEMIOLOGY

1. Participation in the RNTCP/CTD meeting at CTD, New Delhi on 11th – 12th April 2017 – **Dr. Pradeep Aravindan Menon**

2. Participation in RNTCP-DDG-TB-Vaccine trial unit at Serum Institute of India, New Delhi on 24th – 25th May 2017 – **Dr. Pradeep Aravindan Menon**

3. Participation in the RNTCP/CTD meeting at CTD, New Delhi on 11th – 12th April 2017 – **Dr. Pradeep Aravindan Menon**

4. Participation in the policy brief workshop at NIMR, New Delhi on 24th -25th July 2017 – **Dr. Pradeep Aravindan Menon**

5. Participation in the protocol development workshop at ICMR HQ, New Delhi on 27th – 30th August 2017 – **Dr. Sriram Selvaraju**

6. Participation in Building consensus around performance indicator for TB programs TB infection, building a framework for eradication at Harvard Medical School, Dubai on 25th – 29th September 2017 – **Dr. Sriram Selvaraj**

7. Poster presentation of cohort study in 5th global forum on TB vaccines at Taj Hotel, New Delhi on 20th – 23rd December 2017 – **Dr. Sriram Selvaraju**

8. Training programme on MECOR – 2018 program at Hotel Chancery, Bangalore on 15th – 20th January 2018 – **Dr. Pradeep Aravindan Menon**

9. Workshop on MECOR-2018 at Hotel Chancery, Bangalore on 15th – 20th January 2018 – **Dr. Sriram Selvaraju**

10. Participation on National OR committee meeting at Nirman Bhavan, New Delhi on 05th February 2018 – **Dr. Sriram Selvaraju**

11. Participation in Report leadership conference meeting at International centre for genetic engineering and biotechnology, New Delhi on 17th February 2018 – **Dr. Sriram Selvaraju**

12. Poster presentation on 5th global forum on TB vaccines at New Delhi on 20th – 23rd February 2018 - **Dr.C.K.Dolla**

14. Participation on public health dynamics workshop at ICMR HQ, New Delhi on 05th – 09th March 2018 – **Dr. Sriram Selvaraj**

15. Participation in ECO-India Implementation training at the Surya Hotel, New Delhi on 14th – 16th March 2018 – **Dr. Pradeep A Menon**

DEPARTMENT OF LIBRARY AND INFORMATION CENTRE

1. Oral presentation at Sixth International Library and Inforamtion Professional Summit (LIPS 2017) at Indian Institute of Science Education and Research (IISER), Punjab on 06th – 08th April 2017 – **Mr. R. Rathinasabapati**

2. Participation on the national workshop on koha: An Open source integrated library management software at NITTR, Chennai on 14th – 18th August 2017 – **Dr. R. Rathinasabapati**

3. Participation on the National workshop on Copyright consideration for Digital Libraries at IIT Kharagpur, West Bengal on 08th – 10th February 2018 – **Dr. R. Rathinasabapati**

Workshop(s) / Symposia/ Other Events

1) RePORT India PBMC training
Date: May 5-7, 2017

A training workshop for isolation and cryopreservation of peripheral blood mononuclear cells was conducted for the RePORT India CRUs. The program covered all aspects of PBMC isolation and cryopreservation in three days.

2) Report on the Workshop on "Socio Behavioural Interventions – Changing Trends" Date: June 15th, 2017

The workshop "Socio Behavioural Interventions – Changing Trends" was held on June 15th 2017. The scope of the workshop was presented by Dr Beena Thomas, Head of the Department of Social and Behavioural Research (DSBR). The workshop primarily aimed at bringing social and behavioural professionals from diverse disciplines including academicians, practitioners and researchers on a common platform. This gathering sought to enable sharing and learning from manifold experiences that addressed social and behavioural issues as a part of development in the various areas they worked with. The workshop was well attended with ninety participants.

The chief guest was Dr.Upendra Choudhary, Director, ICSSR. He emphasized the importance of social and behavioural research and appreciated the efforts taken by NIRT in organising a workshop of this nature bringing together professionals working in diverse areas.

He outlined the various opportunities ICSSR offered by way of grants for fellowships, courses, workshops, conference presentations and encouraged participants to make use of this opportunity. He said little was known of the work done in the southern areas and most applicants for ICSSR grants came from the North and Central regions.

The agenda included various eminent speakers – Dr Raja, Principal of Madras School of Social work, Dr Shuba Kumar, Director – SAMARTH, Dr. Gladston Xavier, Head of the Department, Social Work, Loyola College, Dr Shanmugavelayutham, Mr. Thaddeus Alfonso, Associate Director, Niraivagam, Don Bosco Institute of Psychological Services, Dr. Shymala Natarajan, Director, SIAAP, Mr. Manikandan and Ms. Preenu from NPS (Network of Professional Social Workers). The topics covered were on Human Rights (refugees and migrants), socio-behavioural research in education, qualitative research as an integral part of social and behavioural research, ethics in social and behavioural research, socio-behavioural research in Mental Health, use of social media in networking which is an important intervention tool, psychosocial intervention studies of NIRT and community outreach programs to reach the unreached.

The panel discussion brought together social workers, academicians and practitioners to present their contributions and focus on areas that need to be addressed in social and behavioural research. The panel included Dr. Miriam Samuel who heads the department of Social Work in Madras Christian College (MCC), Chennai, Mrs. Sudha Ganapathy, retired staff of NIRT and former HOD of Social and Behavioural Research, NIRT, Ms. Hydwick Rosy from the Tamilnadu Slum Clearance Board (TNSCB), Ms. Aspy, founder of Aruwee, an NGO for Elderly

in Chennai, Dr. Shanmugavelayutham, TN-Forces, Ms. Girija Krishnan from Indian Council for Child Welfare (ICCW), Tamil Nadu who work for the cause of juvenile delinquents, Ms. Rohini Krishnan from Government Stanely Hospital, Chennai, Dr Thilakavathy, Social Scientist, National Institute of Epidemiology (NIE), Chennai, and Dr. Geetha Shanmugam, senior Social Worker and former HOD of Social and Behavioural Research in NIRT.

The need for promoting social and behavioural research in the many unexplored areas as defined by the panel and presentations was widely felt. This included among the youth in juvenile homes, the elderly, Dalits, migrants, need for a gender perspective in understanding many issues such as issues around caste, disasters, among urban youth, the need for understanding reasons for sexual abuse, violence and crime. There is urgent need to work on interventions that are feasible and scalable apart from research that only highlights problems. The consensus which evolved from the workshop was the creation of a Socio-behavioural Research Forum. The proposed activities of the Social and Behavioural Research Forum was presented by Dr Karikalan, Social Scientist, NIRT. This included:-

• Acting as a platform for information exchange and dissemination of practises and other experiences in socio-behavioural research

• Identification of thematic research areas and forming core groups to pursue research in that thematic area

• Promoting the need for ethics to be a crucial component of all research activities

• Developing multi-disciplinary research proposals in socio-behavioural research with involvement of diverse pool of researchers academics and implementers

• Developing repository for socio-behavioural research data and documents derived from various sources

• Identifying socio-behavioural researchers and practitioners for best practise and encouraging them through awards annually

• Conducting advocacy among stakeholders for key socio-behavioural issues

• For initiating an official newsletter of the forum

• Providing training and capacity building in socio-behavioural research that include qualitative research

• Membership into the forum could be from multi-disciplines as any social development activity towards change would require the inputs from other related disciplines.


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WHO Collaborating Centre for Tuberculosis Research


MAJOR OUTCOMES

3) **Yoga Day Celebrations**
Date: June 21st, 2017

The rich cultural heritage of Mother India and the spirituality of its soul were revisited by the reverberation of the Yoga day celebrations at NIRT, fulfilling the vision of the senior leadership of our country.

The Yoga day started with the invocation to almighty by Dr.N.S. Gomathi, followed by recital of Adi-Sankara's Guru Ashtakam reiterating the greatness of the Guru by Dr.G. Narendran, the convenor of this programme. "Speak only when your words are more beautiful than silence" is the rule we followed, starting the session with the meditation.

This was followed by the Welcome address by Director –In-Charge, Dr. Srikanth Tripathy, who stressed the need for regular practice of yoga, not only in the office but also carry home the message of the eternal love, benefitting the society. He added that Yoga has the dual advantage of increasing concentration and reducing stress simultaneously, enhancing the efficiency of the working environment.

This was followed by the YOGA demonstration by the Simplified Kundalini Yoga (SKY) group members trained by the Sky instructors, Mr. Sri Ramachandran and Mrs. Girijalakshmi. Mrs. Girija Lakshmi was the recipient of the SKY award recently for her contribution to Yoga. This Yoga teaching initiative was started under the leadership of Dr. Soumya Swaminathan who is currently the Director General ICMR and Health Secretary DHR. These yoga classes have been successfully continuing for the last 6 years. This demonstration was attended by around 80 staff with 15 of them performing various "Asanas", led by Mr. Sri Ramachandran.

Prof. Ganesan, the Chief Guest, who was one of the direct disciples of Shri Vedathri Maharishi himself, provided practical solutions and salvations from day to day problems through the practice of yoga. He said that the life of the human beings has become mechanical and a revolution needs to take place where the individual starts allocating time to understand his health, body and mind rather than spending more time and money when he becomes ill and his homeostasis gets disturbed. He suggested simple life modifications based on ancient practices which could benefit the human body. His speech was interspersed with anecdotes that were filled with humour and humanity.

Mr.T.N. Venkatesan, Yoga therapist cum instructor from the prestigious Krishmacharya Yoga Mandiram, talked about the significant contributions of his eminent institution in the field of Yoga and the use of Yoga for remedial measures. As his interactive session commenced, he impressed the entire audience by demonstrating simple exercises which could be routinely practised by merely sitting in a chair. He explained the importance of synchronising respiratory movements with the body and the soul.

Dr.G. Narendran explained the scientific principles behind some of these exercises performed during the Asanas, integrating science and religion.

Quoting that life is a journey between human being and being human, he laid emphasis on the need for spirituality in life. He reckoned the efforts of the great sons of our “Mathru-bhumi” like Swami Vivekananda and Shri Sankaracharya who were active in spreading Yogic practices.

Dr. Mohan Natrajan, HOD Clinic, shared similar views and remarked that a tiny individual contribution finally could make a mass appeal. He confessed that he could perceive the internal peace in his mind and soul, with just 10 minutes of participating in this programme.

Mr. Jagdish Rajesh, Sr.Administrative Officer delivered the vote of thanks and appreciated the staff for the active and zealous participation. He said that in the world of stressful life, yoga helps in overcoming these problems providing us with a balanced mind to solve our problems.

The programme ended with the National Anthem, with all the staff singing in unison, the glory of our motherland,-

JAI HIND.

4) Dissemination Meeting on the study 'Investigating Pre-treatment Loss to Follow-up (PTLFU) of smear positive Tuberculosis patients in the RNTCP in Chennai and Tiruvallur districts, Tamilnadu'
Date: June 23rd, 2017

A dissemination meeting was held by the Department of Social and Behavioral Research (DSBR) on 23rd June 2017 to share the findings of the study titled "Investigating pre-treatment loss to follow-up of smear positive TB patients in RNTCP in Chennai and Tiruvallur districts, Tamil Nadu." The objective of the meeting was not only to communicate the major findings from the study but also to express our gratitude to all the stakeholders and health care providers for their support in carrying out this important study.

Around 130 members attended the workshop that included DTOs, HVs, LTs, STS from the Revised National Tuberculosis Control Programme (RNTCP) from Chennai, Tiruvallur, Villupuram and Tiruvanamalai districts as well as representatives from the institute.

The findings of the study were presented by Dr.Beena Thomas and Mr. Senthil Sellapan. The findings highlighted the prevalence of PTLFU rates among smear positive TB patients in 22 DMCs in Chennai and Tiruvallur districts, which is 22.1% and 3.4% respectively. The presentations also pointed out the factors associated with PTLFU such as older age, history of prior TB, absence of legible patient contact information (phone and address) and having residence outside Chennai. The qualitative findings determined the reasons for PTLFU such as multiple referrals, alcohol and substance use, not patient-choice based referral, poor record keeping, improper direction and guidance for treatment, etc. Case studies were discussed in which the healthcare workers shared their experiences about the reasons for 'missing cases' and 'default'. Dr.J. Lavanya, DTO, RNTCP Chennai gave a presentation on the ongoing study, which is a multi-component intervention study to reduce PTLFU among smear positive TB patients in Chennai. She explained about the intervention tools that would help to track the loss to follow-up patients and result to reductions in PTLFU rates among TB patients.

The meeting concluded with the need to reduce PTLFU among TB patients and improve linkage to care and the call for health system strengthening interventions with a goal of preventing PTLFU and providing a patients centered referral services in all RNTCP centres.

5) A Technical consultation on Latent Tuberculosis Infection in India
Date: June 29th – 30th, 2017

National Institute for Research in TB (NIRT) in collaboration with the National TB Programme, International Union Against Tuberculosis and Lung Disease (The Union) South East Asia office and ICMR, supported by USAID, organised a Technical Consultation on June 29-30, 2017 at NIRT Chennai. The participants for this consultation were national and international technical experts on latent TB infection, national, state and district level TB programme

(RNTCP) managers, technical partners of RNTCP, researchers, donor agencies, academic institutions and non-governmental organisations.

The objective of the consultation was to deliberate discussions on current situation of LTBI in India, understand global responses to LTBI, update on the current guidelines, identify modalities to implement and scale up of LTBI treatment, to design locally appropriate interventions to combat LTBI and to address the policy - practice gap in implementation in the country. A literature review on LTBI in India for the last 20 years had been undertaken and a summary document is proposed.

The key recommendation from the 2-day consultation was to set up a national working group on LTBI by the RNTCP or India TB Research Consortium. Three major thematic areas diagnosis, treatment and research need to be taken forward by the working group. The summary document of literature review to be completed and shared with the working group. The document would guide in LTBI agenda setting.

Discussions and Recommendations:
The presentations by the experts were insightful with stimulating discussions. This is the first national level consultation on LTBI. In a time when India is grappling with the active TB disease, LTBI is often put aside due to various reasons. But the expert group as well as the national TB program leadership agreed that it's time for India to take on LTBI. And in fact for this reason, the national strategic plan for the next five years includes LTBI in its action plan. It was highly recommended by this consultation that a national working group on LTBI should be constituted and supported by RNTCP or ICMR coordinated India TB Research Consortium. The working group should have experts from key thematic areas identified in the consultation and representing various stakeholders that participated in the consultation.

Dr. Sunil Khaparade, DDG TB delivering the opening address

Dr. Sarabjit, presenting the scope and objectives of the consultation

Dr. Ranjani Ramachandran, presenting on LTBI Diagnosis

Group photo of all the participants attending the 1st Technical Consultation on LTBI

6) **A glimpse of the 61st Annual day celebrations**
Date: August 11th, 2017

The 61st Annual Day started with the invocation to God by reciting the Tamizh Thai Vazhthu. This was followed by a welcome speech by Dr. Srikanth Tripathy, MD, Director-In-Charge, NIRT. Two of the eminent Chest physicians in the city, who were also the heads of their respective institutions, Dr. A. Mahilmaran, MD (Chest)-(Director, ITM) and Dr. R. Sridhar, MD (Chest)-(Superintendent, GHTM, Tambaram), appreciated the efforts of NIRT in TB research and commended our Institution and its Directors for the achievements of NIRT. They recalled the goal of starting this institution, with a motto to frame guidelines for the National TB Programme, and also elaborated on their never-ending friendship with the institute and its staff that had been built over decades of effective, fruitful and eminent collaboration. The staff, who had completed 25 years of selfless and memorable service, were felicitated by the dignitaries. This included Mr. C. Gopala Krishnan, Mr. D. Thangaraj, Mr. C. Uthara Bahadur, Mrs. R. Saraladevi, Mr. B. Durairaj and Mrs. A. Gunasundari. The Director-in-charge, in his address, gave a vivid description regarding the role of various staff, both past and present, who had contributed immensely to the progress and scientific development of this prestigious institution. Various achievements and awards that have been obtained by the staff during this year, were enlisted by the head of the institute. The Senior Administrative Officer stressed that each one of the staff should strive to make this institution, a pioneer in TB research .This was followed by the Cultural programme that stole the lime light, lightening the hearts of the entire audience who keenly watched the show and enjoyed the programme. At the end of the day, the members of the Annual Day Committee had done their job efficiently and satisfactorily, to make the entire event a grand success. This was followed by the Vote of thanks by Dr. G. Narendran, DNB (Chest), Sci-'E', NIRT and the programme ended with the National Anthem.


ANNUAL DAY CELEBRATION

7) World Drivers day – 2017
Date: September 17th, 2017

"World Drivers Day 2017" was celebrated on 17th September 2017 that was organized by the drivers of the Institution. Mr.S. Natarajan, Director-in-charge of Tamilnadu State Health Transport Department was the chief guest and Mr.S.R. Muralidharan, Assistant Director of Road Transport was the guest of honour. The Director-in-charge inaugurated the event followed by the address of delegates. All were expressing their appreciation to drivers for their hard work and dedication towards their work, without which the research activities in the Institute may not carried smoothly.

8) **Farewell to Dr. Soumya Swaminathan-DG, ICMR**
Date: October 23rd, 2017

A felicitation cum farewell event was organised for Dr. Soumya Swaminathan, Director General, ICMR on obtaining the post of Deputy Director General, WHO, Geneva. This was attended by the staff of NIRT existing, retired and Scientific Advisory Committee members.

This event was creatively done and stood apart from usual farewell function held in NIRT (ICMR). It started with our Director General been escorted with female staff holding light up Diya on their palm while she entered the gathering seated in the atrium. This entry was accompanied by loud music “waving flag (football anthem)” and an energetic dance from different corners by the staff holding up balloons. After the lighting of the lamp there was a song by the staff with catchy lyrics and own composition.

This was followed by a classic rendition of Dr. Soumya Swaminathan’s favourite song titled “Mahro Pranam Banke Bihari Ji“ by Dr. N.S Gomathi. Tributes were given by Dr. P. R. Narayanan, Dr. Srikanth P. Tripathy, NIRT Welfare Association and Senior Administrative Officer Mr. Jagdish Rajesh.

A surprise item on the agenda was a song sung by our Director General accompanied by few staff of NIRT. This reflected the hidden talents of singing of our Director General who is internationally recognised for our scientific expertise and leadership quality. Following this was a take home message rendered beautifully by our Director General going down memory lane of how she started her career at NIRT and reminding the staff on the need to keep the NIRT flag flying high through commitment to team works, development of scientific research and the need for a holistic multi-disciplinary approach.

We wish our Director General the very best in the future as she takes up a prominent leadership role as Deputy Director General, World Health Organisation, Geneva.

9. Polychromatic Flowcytometry workshop (Basics)
Date: November 2-3, 2017

The last decade has seen many significant advances in flow cytometry instrument design and fluorochrome dye chemistry, bringing 8-18-colour analysis within the easy reach of many. The intent of the workshop was to provide an opportunity to understand the fundamentals of flow cytometry, multicolor panel design, instrument setup, validation, assay standardization, and data analysis. There were 15 participants in the workshop including researchers from Christian Medical College & Hospital (Vellore), Madurai Kamarai University (Madurai), JIPMER (Pondicherry), Institute of Basic Medical Sciences (Madras), Periyar University (Salem), Sathyabama University (Chennai), TANUVAS (Chennai), etc.

10. **Training on "Concepts and applications of Health Economics in research with emphasis to tuberculosis".**
Date: December 18-19, 2017

11. Dissemination workshop on the study "Targeted intervention to expand and strengthened TB control among tribal populating in four districts under RNTCP, Gujarat, India" at State TB Training and Demonstration Centre, BJ Medical College, Ahmedabad, Gujarat
Date: December 29, 2017

12. Research Methodology and Biostatistics workshop for Medical Professionals
Date: January 1st -12th, 2018

ICMR-National Institute for Research in Tuberculosis, Chennai conducted the Research Methodology and Biostatistics workshop for Medical Professionals from 1st - 12th January 2018, which was funded by the Department of Health Research, Ministry of Health and Family Welfare, Government of India. Fifteen participants from various Medical Colleges in India including North East participated in this workshop. The participants included mid-level Medical College Faculty and Post Graduate Students. They were trained on various aspects of research methodology including identifying health problems, framing the right research question and objectives, choosing appropriate methodology, data collection, analysis plan and presentation of data. Hands on exercise was given on doing literature search using Pubmed, reference management using Mendeley and data analysis using Epi-Info. The participants developed and presented a concept note on an identified research topic.


Certificate Course in Research
Methodology and Biostatistics
for Medical Professionals

13. **Dissemination Workshop on the study titled "Strengthening the implementation and operational research under RNTCP in India"**
Date: January 24 -25, 2018

14. **Bioethics Workshop conducted by Clinical data support team from NIH-NIRT-ICER. This workshop covered the topics of Biothermics device overview, monitoring, alarming and reporting**
Date: February 2, 2018

15. Dissemination Program on ICMR National Ethical Guidelines
Date: February 7th, 2018

A Seminar to disseminate the ICMR Guidelines National Ethical Guidelines for Biomedical and health research involving Human participants and involving children, National Guidelines for Stem Cell Research 2017 was conducted by National Centre for Disease Informatics and Research (NCDIR), Indian Council of Medical Research (ICMR).In collaboration with Sri Ramachandra Medical College & Research Institute (DU), National Institute for Research in Tuberculosis.

Inauguration:
Welcome Address: Dr. S.P. Thyagarajan, Prof. of Eminence & Dean (Research)
Inaugural Address: Prof. P.V. Vijayaraghavan, Vice Chancellor
Key Note Address: Dr. Nandini K Kumar, General Sr. Grade (ICMR)
Vote of Thanks: Dr. Srikanth Prasad Tripathy, Director-in- charge, NIRT

Dr. Srikanth Prasad Tripathy, Director - In-Charge, NIRT, Chennai & Dr. Vijayalakshmi Thanasekaraan, Prof. Emeritus, Dept. of Pulmonary Medicine were special guests for the program.

Scientific session: Dr. Roli Mathur, Head, ICMR Bioethics Unit, National Centre for Disease Informatics and Research, Bengaluru – Spoke on Overview of National Ethical Guidelines for Biomedical and health research involving Human participants and involving children. She highlighted the differences between 2006 & 2017 guidelines. The new

chapters that are included in the current guideline are (responsible conduct of research, vulnerability, social and behavioral sciences, biological materials bio-banking & dataset and research during humanitarian emergencies and disaster research). Several chapters have been revised with clearer instructions and explanations.

Dr. Geeta Jotwani, Scientist F, Division of BMS, ICMR, New Delhi – gave an overview of National Guidelines for Stem Cell Research 2017. She spoke on the ethical, legal and social concerns of stem cell research, mechanism of review and oversight of stem cell research and various categories of stem cell research. She stressed the need for institutional ICSCR to be registered with NAC-SCRT.

The presentations were followed by a panel discussion. The panel members were Dr. Roli Mathur, Dr.Nandini K Kumar, Dr.Geeta Jotwani, Dr.Urmila Thatte, Dr.Bikash Medhi, Dr.Paul Kumaran, Dr. Alan Mathew Punnoose and Dr. Nalin Mehta. There was active interaction between participants & panelists.

Approximately 1200 participants attended the meeting.

16. **A training session & demonstration on Fire Safety was conducted by Mr. A.K. Kumar, Fire Safety Officer, Tamil Nadu Fire and Rescue Services 7th March, 2018.**
Date: March 7th, 2018

17. World TB Day Programme
Date: March 23rd, 2018

The World TB Day (March 24) is designed to build awareness on the global epidemic of tuberculosis and on the elimination of TB. It commemorates the date in 1882 when Dr. Robert Koch announced his discovery of Mycobacterium tuberculosis, the bacterium that causes TB. The World TB Day celebrations 2018 at ICMR-National Institute for Research in Tuberculosis (NIRT) was held on 23rd March, 2018. The programme was very much in line with the World TB Day theme "Wanted: Leaders for a TB-Free World". Dr. Srikanth Tripathy, Director-in-charge of ICMR-NIRT welcomed the gathering and stressed on the need to eliminate TB epidemic by 2025, as envisaged by the Hon'ble PM of India.

The cultural programmes emphasised the need for public awareness about the early diagnosis, treatment initiation and continuance and the importance of alcohol and smoking cessation. An interactive session with treated TB patients was also held where the patients shared their experiences. All health care workers were updated on the Latest TB guidelines. A booklet on "Prof. Wallace Fox, a Pioneer in TB research" was released in the gracious presence of Dr. S Radhakrishna, Ex-Director, IRMS, Shri Paul Somasundaram, Dr Santha Devi, Dr M.S. Jawahar, retired senior scientists of our institute. The scientists of NIRT gave an over view of the future projects namely the 'Manage TB" – A Online TB Course for Physicians, TB-Free Chennai initiative and use of Next Generation Sequencing for TB Research.


23.03.2018 11:01


World TB Day - 2018
23.03.2018 11:26

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Ph.D. Scholars

List of staff / students who have obtained their Ph.D. degree (Full time) from University of Madras

Sl. No.	Name of the candidate	Title of the Ph.D. thesis	Part time / Full time	Supervisor/ Guide
1.	Ms.D. Santhi	Novel subunit vaccine targets from *M. tuberculosis*	Full time	Dr. Alamelu Raja
2.	Ms.A.S. Shainaba	Drug target identification for tuberculosis: Mycobacteriophage Che12 based approach	Full time	Dr. Vanaja Kumar
3.	Mr.P. Balaji	Evaluation of antigen specific immune response for identification of *M. tuberculosis* infection	Full time	Dr. Alamelu Raja

List of staff/students who have submitted their Thesis and waiting for their Ph.D. degree from the University of Madras (Full time)

Sl.No.	Name of the candidate	Title of the Ph.D. thesis	Part / Full time	Supervisor/ Guide
1.	Mr.P. Pugazhventhen	Immunoproteomic identification of B-cell antigens of *M. tuberculosis*	Full time	Dr. Alamelu Raja
2.	Mr. Narayanaiah Cheedarla	Identification and characterization of neutralizing antibodies in clade 'C' HIV-1 infected individuals	Full time	Dr. Luke Elizabeth Hanna

List of students who have registered (full-time) for their Ph.D. programme with University of Madras

Sl.No.	Name of the Candidate	Source of Funding	Title of the Ph.D. thesis	Supervisor/Guide
1	Ms.G. Akilandeswari	INSPIRE FELLOW	Biophysical and biochemical characterization of two DNA binding proteins (Rv3716c and Rv3405c) from *M. tb*	Dr.B. Ramalingam
2.	Ms. Vidya Vijayan	INSPIRE FELLOW	Characterizaiton of viral and host factor responsible for clinical differences seen in HIV-1 and 2 infection	Dr. Luke E. Hanna
3.	Ms.B. Hemalatha	CSIR	Understanding pathogenesis of HIV infection	Dr. Luke E. Hanna
4.	Mr. Ashok Kumar	ICMR	Genetic identity and unique biological phenotype of early transmitted/founder viruses in recent HIV-1 infection	Dr. Luke E. Hanna
5.	Mr.S. Sivasankaran	DST	Evaluation of mucosal immune responses to HIV infection in discordant couples: Templates for a vaccine	Dr. Luke E. Hanna
6.	Mr.C. Yuvaraj	INSPIRE Fellow	Characterization of three intermediatory metabolic enzymes (DlaT, Icl-1 and GlnA 1) in the laboratory strain H37Rv and clinical isolates of MDR-TB	Dr.K.R. Uma Devi
7.	Ms. Gunapati Bhargavi	INSPIRE Fellow	Functional characterization of oxidoreductases of *M. tb*	Dr.P. Kannan
8.	Mr. Kadar Moideen A.	ICER	Immune response to TB coincident with diabetes	Dr.B. Ramalignam
9	Ms. Pavithra S.	INSPIRE Fellow	Molecular analysis of monocyte subsets in humans infected with *M. tb*	Dr.B. Ramalignam
10	Mr.S. Deepak	ICMR	Development of lactobacillus strains for the purpose of enhanced DNA stability, mucosal adhesion and delivery of therapeutic proteins	Dr. Luke E. Hanna
11	Mr. Aanand B Sonawane	UGC	Molecular mechanisms of HIV pathogenesis in target cells	Dr.A.R. Anand
12	Ms. Noelin Chinnu Mathew	JRF	Studies on epigenome wide alterations during *M. tb* infection in guinea pig pulmonary tuberculosis model	Dr.P. Kannan

Staff (Part-time) registered for their Ph.D. programme with University of Madras, Chennai

Sl.No.	Name of the staff	Title of the Ph.D. thesis	Supervisor/Guide
1.	Mr. Anbalagan S.	Innate & adaptive immunity in HIV	Dr. Luke Elizabeth Hanna

NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS

OBITUARY

NIRT staff who passed away during the period 2018-2019

Mrs. Parvathy Raghavan, Nursing Officer	-	07.03.2017
Mr.K. Govindaraj, Technical Assistant	-	09.05.2017
Mrs.K. Meenakshi, Technical Assistant	-	30.07.2017
Mr.C.V. Mohan, Technical Assistant	-	27.09.2017
Mrs. Magathalin, Record Sorter	-	03.11.2017
Mr.P.N, Mari, Animal House Keeper	-	01.11.2017
Mr.N.C. Sreedharan, Accounts Officer	-	02.11.2017
Mr.B.N. Gopalan, Technical Officer	-	20.12.2017
Mr.V.A. Sugumar, Sr. Lab. Attendant	-	15.01.2018

NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS
CHETPET, CHENNAI -31
STAFF LIST AS ON 01.04.2018

S.No.	Name	Designation
	SCIENTISTS	
1	Dr Srikanth Prasad Tripathy, MBBS, MD	Scientist G/Director in Charge
2	Dr Mohan Natrajan, MBBS, Ph.D., Dip. in Der	Scientist F
3	Dr P. Paul Kumaran, MBBS, MPH	Scientist E
4	Dr Pradeep Aravindan Menon, MBBS, PGDPM	Scientist E
5	Dr C. Padmapriyadarsini, MBBS, DNB, MS	Scientist E
6	Dr D. Baskaran, MBBS, PGDTCD	Scientist E
7	Dr Sudha Subramanyam, M.Sc., Ph.D.	Scientist E
8	Dr Geetha Ramachandran, M.Sc., Ph.D	Scientist E
9	Dr Beena E Thomas, M.A., Ph.D	Scientist E
10	Dr G. Narendran, MBBS, DTRD, DNB	Scientist E
11	Dr S. Ramesh Kumar, MBBS, MPH	Scientist E
12	Dr Luke Elizabeth Hanna, M.Sc., Ph.D.	Scientist E
13	Dr K. R. Uma Devi, M.Sc., Ph.D.	Scientist D
14	Dr C. Ponnuraja, M.Sc., Ph.D.	Scientist D
15	Dr C.K. Dolla, MBBS, MPH	Scientist D
16	Dr P. Kannan, M.V.Sc., Ph.D.	Scientist D
17	Dr V.V. Banu Rekha, MBBS, PGDPH	Scientist D
18	Dr K. Rajendran, M.Sc., Ph.D.	Scientist D
19	Dr P. K. Bhavani, MBBS, PGDPH	Scientist D
20	Dr A. Sheikh Iliayas, MBBS	Scientist C
21	Dr N. Saravanan, M.Sc., M.Phil., Ph.D.	Scientist C
22	Dr S. Syed Hissar, MD, MPH	Scientist C
23	Dr Dina Nair, MBBS, PGDPH	Scientist C
24	Dr N. Poorana Ganga Devi, MBBS, PGDPH	Scientist C
25	Dr A. K. Hemanth Kumar, M.Sc., Ph.D.	Scientist C
26	Dr M. Makesh Kumar, MBBS	Scientist C
27	Dr D. Bella Devaleenal, MBBS, PGDOL, MPH	Scientist C
28	Dr S. Sriram, MBBS, MPH	Scientist C
29	Dr M. Muniyandi, M.A., M.Phil., MPS, Ph.D.	Scientist C
30	Dr S. Sivakumar, M.Sc., Ph.D.	Scientist C
31	Dr R. Priya, M.Sc., Ph.D.	Scientist B
32	Dr G. Prathiksha, M.B.B.S, MD	Scientist B
33	Dr B. M. Shrinivasa, MBBS, MD, Dip Pub Health	Scientist B
34	Dr Himanshu Singh Chandel, M.Sc., Ph.D.	Scientist B
35	Dr N. Karikalan , B.S.M.S.,MPH	Scientist B
36	Dr Dinesh Kumar, M.Sc., Ph.D.	Scientist B
37	Dr N. Sudhakar, M.Sc., M.Phil., Ph.D.	Scientist B
38	Dr R. Rathinasabapati, MLIS, Ph.D.	Senior Library & Information Officer

	NURSING	
39	Ms. A. Gunasundari, M.Sc.	Senior Technical Officer (3)
40	Ms. G. Mangalambal, M.Sc.	Senior Technical Officer (2)
41	Ms. Valarmathi Nagarajan, M.Sc.	Senior Technical Officer (2)
42	Ms. C. Kavidha, B.Sc.	Senior Technical Officer (2)
43	Ms. S. Chellam	Senior Technical Officer (1)
44	Ms. K. Sureswari	Senior Technical Officer (1)
45	Ms. Shyamala Gopu	Senior Technical Officer (1)
46	Ms. R. Valarmathy	Senior Technical Officer (1)
47	Ms. P. Pandeeswari	Senior Technical Officer (1)
48	Ms. M. Rathinam	Senior Technical Officer (1)
49	Ms. P. Kowsalya	Senior Technical Officer (1)
50	Ms. M. Mohana	Senior Technical Officer (1)
51	Ms. A. Komathi, B.Sc	Nursing Sister
52	Ms. R. Manimegalai	Staff Nurse
53	Ms. V. Farthimunnisa	Staff Nurse
54	Ms. Shakila Shankar	Staff Nurse
55	Ms. K. Porselvi, B.Sc.	Staff Nurse
56	Ms. A. Selvi	Staff Nurse
57	Ms. S. Stella Mary	Staff Nurse
58	Ms. A. Stella Mary	Staff Nurse
59	Ms. R. Saraladevi	Staff Nurse
60	Ms. S. Theensuwai, B.A.	Staff Nurse
61	Ms. R. Vetrichselvi, M.A.	Staff Nurse
62	Ms. C.Hema Giranab	Staff Nurse
63	Ms. A. Seetha	Staff Nurse
64	Ms. S.Gopika	Staff Nurse
65	Ms. Sahaya Mary Lisa B.Sc	Staff Nurse
66	Ms. Mumtas Banu Kottayil	Staff Nurse
67	Mr. Sravan Kumar Adavaith	Staff Nurse
68	Ms. O. R.Vijayalakshmi	Technical Assistant
69	Ms. A.Vijayalakshmi	Technical Assistant
70	Ms. A. Poongkodi	Technical Assistant
71	Ms. S.Vaishnavi, B.Sc.	Technical Assistant
72	Ms. R.Suganthi	Technical Assistant
73	Ms. K. Maheswari, B.Sc.	Technical Assistant
74	Ms. V. Senthamizhselvi	Technical Assistant
75	Ms. V. Shunmugajyothi, M.Sc.	Technical Assistant
76	Mr. Krishna Yadav Kattagoni, B.Sc.	Junior Staff Nurse
77	Ms. J. Jemima, B.Sc.	Junior Staff Nurse
78	Mr. N. Lokeswaran, B.Sc.	Junior Staff Nurse
79	Ms. R. Selvi	Junior Staff Nurse
80	Ms. J. Vanitha	Junior Staff Nurse
81	Ms. R. Supriya, B.Sc.	Junior Staff Nurse
82	Ms. K. Subapriya. B.Sc.	Junior Staff Nurse

	TECHNICAL	
83	Dr L. Sekar, M.Sc., Ph.D.	Principal Technical Officer
84	Dr K. Chandrasekaran, Ph.D.	Principal Technical Officer
85	Dr N. S. Gomathi, M.Sc., Ph.D.	Principal Technical Officer
86	Dr E. Thiruvalluvan, M.A., Ph.D	Senior Technical Officer (3)
87	Mrs. Chandra Suresh, M.A.	Senior Technical Officer (3)
88	Mrs. D. Kalaiselvi, M.A.	Senior Technical Officer (3)
89	Mr. M. Rajasakthivel, M.A.	Senior Technical Officer (3)
90	Mr. P. Murugesan, M.A., PGDC., BDA.	Senior Technical Officer (2)
91	Ms. K. Silambu Chelvi, M.Sc., M.Phil.	Senior Technical Officer (2)
92	Dr K. Ramakrishnan, M.Sc., Ph.D.	Senior Technical Officer (2)
93	Mr. S. Venugopalan, M.Sc.	Senior Technical Officer (2)
94	Dr R. Srinivasan, M.Sc., Ph.D.	Senior Technical Officer (1)
95	Ms. M. Vasantha, M.Sc., M.Phil.	Senior Technical Officer (1)
96	Mr. K. Ramesh, M.Sc.	Senior Technical Officer (1)
97	Dr M. Harishankar, M.Sc., Ph.D.	Senior Technical Officer (1)
98	Mr. S. Anbalagan, M.Sc.	Senior Technical Officer (1)
99	Ms. Lucia Precilla, M.Sc., M.Phil.	Senior Technical Officer (1)
100	Mr. S. Senthil, M.A., M.Phil	Senior Technical Officer (1)
101	Ms. V. M. Girijalakshmi, M.Sc.	Senior Technical Officer (1)
102	Ms. D. Saraswathi, M.Sc., M.Phil.	Senior Technical Officer (1)
103	Ms. K. Devika, M.Sc.	Senior Technical Officer (1)
104	Mr. M. Baskaran, B.Sc.	Senior Technical Officer (1)
105	Mr. S. Rajakumar, M.Sc.	Senior Technical Officer (1)
106	Ms. B. Angayarkanni, M.Sc., M.Phil.	Senior Technical Officer (1)
107	Ms. J. Chitra, M.Sc.	Senior Technical Officer (1)
108	Mr. V. Thiyagarajan, M.Sc., M.Phil.	Senior Technical Officer (1)
109	Mr. D. Ravikumar, M.Sc.	Senior Technical Officer (1)
110	Ms. K. Sumathi, B.Sc.	Senior Technical Officer (1)
111	Mr. S. Govindarajan, M.Sc.	Senior Technical Officer (1)
112	Mr. M. Michel Prem Kumar, M.Sc.	Senior Technical Officer (1)
113	Mr. M. Anandan	Technical Officer - A
114	Ms. A. Deepalakshmi, M.A.	Technical Officer
115	Dr S. Balaji, M.Sc., Ph.D.	Technical Officer
116	Dr D. Anbarasu, M.Sc., M.Phil., Ph.D.	Technical Officer
117	Mr. S. Murugesan, M.Sc.	Technical Officer
118	Mr. M Tamizhselvan, M.Sc. Ph.D.	Technical Officer
119	Mr. M. Asokan	Technical Assistant
120	Mr. D. Thangaraj	Technical Assistant
121	Mr. K. Ramakrishnan	Technical Assistant
122	Ms. B. Pricilla Rebecca, M.A.	Technical Assistant
123	Ms. S. Rani, B.S.W.	Technical Assistant
124	Ms. A. Dhanalakshmi, M.A.	Technical Assistant
125	Ms. Senthanro Ovung, M.S.W.	Technical Assistant
126	Ms. V. Mythily, M.Sc.	Technical Assistant

127	Dr M. Muthu Vijayalakshmi, M.Sc., M.Phil., B.Ed., Ph.D	Technical Assistant
128	Mr. P. Palaniyandi, M.Sc., M.Phil.	Technical Assistant
129	Dr C. Manogaran, M.A., M.Phil., Ph.D.	Technical Assistant
130	Ms. G. Radhika, B.Sc.	Technical Assistant
131	Ms. H. Hemalatha, M.Sc.	Technical Assistant
132	Ms. Devi Sangamithrai, M.Sc.	Technical Assistant
133	Ms. K. Jeyasree, B.Sc.	Technical Assistant
134	Mr. A. Madheswaran, B.Sc.	Technical Assistant
135	Mr. S. Iyyappan	Technical Assistant
136	Mr. R. K. Rajendran	Senior Technician (3)
137	Mrs. V. Shailaja Devi	Senior Technician (2)
138	Mr. E. A. John Washington	Senior Technician (1)
139	Mr. K. S. Venkatesan	Technician (2)
140	Mr. D. Madhavan, M.Sc.	Technician C
141	Mr. R. K Syed Nisar, M.Sc.	Technician C
142	Ms. B. Brindha, M.Sc.	Technician C
143	Mr. P. Nagarajan, M.Sc.	Technician C
144	Mr. P. Sivaraman, M.Sc.	Technician C
145	Ms. V. Sudha, M.Sc., M.Phil.	Technician C
146	Ms. R. Nithya, B.Sc.	Technician C
147	Ms. Rohini Puvaneshwari, B.Sc.	Technician C
148	Mr. R. Rajkumar, B.Sc.	Technician C
149	Mr. D.Srinivasa Raju	Technician C
150	Mr. N. Ramakrishnan	Lab Assistant
151	Mr. P. Chandran	Lab Assistant
152	Mr. K. Ganesan	Lab Assistant
153	Mr. J. Loganathan	Lab Assistant
154	Mr. G. Moshe	Lab Assistant
155	Mr. C. Nagaraju	Lab Assistant
156	Mr. P. Vijayakumar	Lab Assistant
157	Mr. V. Mohan	Lab Assistant
158	Mr. A. Annamalai	Lab Assistant
159	Mr. R. Damodaran	Lab Assistant
160	Mr. D. Bose	Lab Assistant
161	Mr. N. Murali	Lab Assistant
162	Mr. C. K. Chittarasu	Lab Assistant
163	Mr. M. Jayaraj	Lab Assistant
164	Mr. A. Rajavarman	Lab Assistant
165	Mr. J. Venkatesan	Lab Assistant
166	Mr. R. Ankaiah	Lab Assistant
167	Mr. R. Ravichandran	Lab Assistant
168	Mr. V. Athikesavan	Lab Assistant
169	Mr. V. Sundararajan	Lab Assistant
170	Mr. K. Kuttappan	Lab Assistant
171	Mr. J. Selvam	Lab Assistant

172	Mr. G. Easwaran	Lab Assistant
173	Mr. N. Ankaiah	Lab Assistant
174	Mr. Uthra Bahadur	Lab Assistant
175	Mr. Keshabraj Paudel	Lab Assistant
176	Ms. D. Saradha	Lab Assistant
177	Ms. B. Nageswari	Lab Assistant
178	Mr. V. Venkateswaralu	Lab Assistant
179	Ms. J. Neelavathy	Lab Assistant
180	Ms. H. Ponrose	Lab Assistant
181	Mrs. T. Thilakavathy	Lab Assistant
182	Mr. G. Nithyanandam	Lab Assistant
183	Mr. S. Venkatesan	Lab Assistant
184	Mr. R. Mohanraj	Lab Assistant
185	Mr. J. Santhakumar	Lab Assistant
186	Mr. S. Anjaiah	Lab Assistant
187	Mr. P. Senthilvelan	Lab Assistant
188	Mr. P. Kosalaraman	Lab Assistant
189	Mr. S. Nagarajan	Lab Assistant
190	Ms. R. Ankamma	Lab Assistant
191	Mrs. Padmavathy Asaithambi	Lab Assistant
192	Mr. Jayavel Anandan	Lab Assistant
193	Mr. M. Kawaskar, B.Sc	Technician A
194	Mr. Santhana Mahalingam, B.Sc.	Technician A
195	Mr. T. Bharathiraja, B.Sc.	Technician A
196	Mr. S. Mangaiyarkarasi, M.Sc.	Technician A
197	Mr. M. Pandidurai	Technician A
198	Mrs. R. Sathya, B.Sc.	Technician A
199	Mr. A. Vijayakumar, M.Sc.	Technician A
200	Mr. Harihara Ganapathi Subramanian	Technician - 1
201	Mr. T. M. Loganathan, B.Sc	Lab Attendant -2
202	Mr. R. Anbulingam	Lab Attendant -2
203	Mr.Johnson Kennedy	Lab Attendant -2
204	Ms. D. Sundari	Lab Attendant -2
205	Mr. M. Manikandan	Lab Attendant -2
206	Mrs. R. Sakila	Lab Attendant -2
207	Mr. K. N. Thirumalai	Lab Attendant -2
	TRANSPORT	
208	Mr. K. Vadivel	Senior Technician (3)
209	Mr. K. Jayaraman	Senior Technician (3)
210	Mr. P.Anbu	Senior Technician (2)
211	Mr. A.Ravi	Senior Technician (2)
212	Mr. S. Sri Rama Chandran	Senior Technician (1)
213	Mr. A. Elangovan	Senior Technician (1)
214	Mr. I. Seenivasan	Senior Technician (1)
215	Mr. P. Sivakumar	Technician (2)
216	Mr. N. Rajan Babu	Technician (2)

217	Mr. M. Thiyagarajan	Technician (2)
218	Mr. E. Selvaraj	Staff Car Driver (O.G)
219	Mr. M. Sathish Kumar	Staff Car Driver (O.G)
220	Mr. M. S. Mani	Staff Car Driver (O.G)
221	Mr. M. Sekar	Staff Car Driver (O.G)
222	Mr. P. Yuvaraj	Staff Car Driver (O.G)
223	Mr. C. Sivaraman	Staff Car Driver (O.G)
224	Mr. K. Sridhar	Staff Car Driver (O.G)
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225	Mr. Jagdish Rajesh, B.Com	Senior Administrative Officer
226	Mrs. M. Meenal, M.Com	Accounts Officer
227	Mr. M. Mani, B.A.	Administrative Officer
228	Ms. Chithra Sivakumar, B.Sc.	Section Officer
229	Mr. A. Lakshmanan	Section Officer
230	Mr. C. Gopala Krishnan, B.Sc.	Section Officer
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232	Ms. P. S. Shanthi	Private Secretary
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234	Ms. N. Thamilselvi, B.Sc.	Assistant
235	Ms. M. N. Raadha, B.Sc., M.C.S.	Assistant
236	Ms. L. Vijayakumari	Assistant
237	Mr. B. Durai Raj	Assistant
238	Ms. P. Kavitha, MA, MBA	Assistant
239	Mr. H. Krishna Kumar, B.Com., MBA	Personal Assistant
240	Mr. S. Anandaraj	Upper Division Clerk
241	Ms. S. Nirmala, MA	Upper Division Clerk
242	Ms. J. Suguna, B.Com.	Upper Division Clerk
243	Ms. T. Sheela	Upper Division Clerk
244	Mr. A. Gopinathan, BA	Upper Division Clerk
245	Mr. V. Velmurugan	Upper Division Clerk
246	Mr. R. Hariharan, B.Com.	Upper Division Clerk
247	Ms. M. Revathy, M.Com.	Stenographer
248	Ms. P. Anitha, B.Com.	Stenographer
249	Ms. G. H. Jyothipriya, B.E.	Stenographer
250	Mr. S. Sasikumar, BCA	Stenographer
251	Ms. K. Thiriveni, B.Tech., M.Phil.	Stenographer
252	Mrs. K. Sumathi, B.A	Lower Division Clerk
253	Mr. M.Mohan Shankar	Lower Division Clerk
254	Ms. D. Tamilselvi, BBA, MBA	Lower Division Clerk
255	Mr. D. Sukumar	Lower Division Clerk
256	Ms. S. Sundari, M.Sc.	Lower Division Clerk
257	Mr. Durga Mohan Kumar Chenna, B.Com.	Lower Division Clerk
258	Mrs. P.Hemalatha	MTS
259	Mrs. K. Kamatchi	MTS
260	Mr. D. Rajasekaran	MTS
261	Ms. P. Pandiselvi	MTS

262	Ms. V. Amudhavalli	MTS
263	Mr. J. V. Mohanraj	MTS
264	Mr. T. Kumar	MTS
265	Mr. D. Ravichandran	MTS
266	Mr. M. Bel Bhadur	MTS

NATIONAL INSTITUTE FOR RESEARCH IN TUBERCULOSIS
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STAFF LIST AS ON 01.04.2018

S.No.	Name	Designation
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1	Dr V. N. Azger Dusthackeer, M.Sc., Ph.D.	Scientist B
2	Dr S. Devarajulu Reddy, M.B.B.S.	Scientist B
3	Ms. R. Mahalakshmi, M.Sc.	Scientist B
4	Mrs. Basilea Watson, M.Sc.	Scientist B
	TECHNICAL	
5	Mr. G. Komaleeswaran, M.Sc.	Senior Technical Officer (2)
6	Mr. S. Vijayaraj, M.Sc.	Senior Technical Officer (1)
7	Mr. K. Senthil Kumar, M.Sc., M.Phil.	Senior Technical Officer (1)
8	Mr. A. Radhakrishnan, M.Sc.	Senior Technical Officer (1)
9	Mr. N. Ravi, D.E.E.	Technical Officer - B
10	Mr. B. Senthil Kumar, M.Sc.	Technical Officer - A
11	Ms. D. Kalaivani, M.Sc.	Technical Officer - A
12	Mr. V. Partheeban, M.A.	Technical Officer - A
13	Mr. P. Munivardhan, B.Sc.	Technical Officer - A
14	Mr. D. Nithya Kumar, M.Sc.	Technical Officer - A
15	Mr. V. Ramesh Babu, C. R. A.	Technical Officer - A
16	Mr. A. M. Ramesh, M.A.	Technical Officer - A
17	Mr. P. K. Venkataramana, B.Com.	Technical Officer - A
18	Mr. N. Prem Kumar, B.Sc.	Technical Officer - A
19	Mr. S. Venkatesan, M.A., B.Ed.	Technical Officer - A
20	Mr. S. V. Joseph Raj Kumar	Technical Officer - A
21	Mr. T. Thangaraj, M.A., B.Ed.	Technical Officer - A
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23	Mrs. M. Malathi, M.Sc., C.L.T.	Technical Officer
24	Mrs. B. Mahizhaveni, M.Sc., D.M.L.T.	Technical Officer
25	Mrs. G. Vadivu, M.Sc., D.M.L.T.	Technical Officer
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27	Mr. K. Rajaraman, M.Sc., C.L.T.	Technical Officer
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36	Ms. P. Devi Bhagavathy, B.C.A., M.B.A.	Technical Assistant
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38	Mrs. R. Vijayalakshmi, M.Sc.	Technical Assistant

39	Mr. P. Sathyamurthy, M.Sc.	Technical Assistant
40	Mr. M. Kannan, B.Sc.	Technical Assistant
41	Mr. S. Manohar Nesa Kumar, M.Sc., PGDMLT	Technical Assistant
42	Mr. A. Devanathan, M.Com., M.S.W.	Technical Assistant
43	Mr. P. Balaji, M.A., B.Ed.	Technical Assistant
44	Mr. K. Ramesh Kumar, M.Sc., M.Phil.	Technical Assistant
45	Mrs. V. Rani, M.Sc.	Technical Assistant
46	Mr. S. Govindaraj, D.M.L.T., M.Sc.	Technical Assistant
47	Mr. T. K. Bharath, M.Sc.	Technical Assistant
48	Mr. J. Ravi	Senior Technician (2)
49	Mr. S. Nambirajan, M.Sc., M.L.T.	Senior Technician (1)
50	Mr. S. S. Jeganathan, B.Sc.	Senior Technician (1)
51	Mr. C. Saravanan, B.L.I.S., M.A.	Senior Technician (1)
52	Mr. P. Srinivasulu	Senior Technician (1)
53	Mr. P. C. Nagaraja	Senior Technician (1)
54	Mr. R. David	Senior Technician (1)
55	Mr. C. Saravanan	Senior Technician (1)
56	Mr. K. Poongavanam	Senior Technician (1)
57	Mr. L. Venkatesan	Technician (2)
58	Mr. W. Wilkingson Mathew	Technician (2)
59	Mr. Ishwori Dhakal	Lab Assistant
60	Mr. V. Raja	Lab Assistant
61	Mr. R. Krishna Bahadur	Lab Assistant
62	Mr. R. Purushothaman	Lab Assistant
63	Mr. N. Srinivasan	Lab Assistant
64	Mr. C. Ananadan	Lab Assistant
65	Mrs. G. Devaki	Lab Assistant
66	Mr. Til Bahadur	Lab Assistant
67	Mrs. N. Vasantha	Lab Assistant
68	Mr. S. Prakasam	Lab Assistant
69	Mr. K. Vasudevan	Lab Assistant
70	Mr. J. Jeeva	Lab Assistant
71	Mr. F. Albert	Lab Assistant
72	Mr. T. D. Ponnusamy	Lab Assistant
73	Mr. E. Duraivel	Lab Assistant
74	Mr. R. Yuvarajan	Lab Assistant
75	Mr. S. Innamuthan	Lab Assistant
76	Mr. R. Narasimhan	Lab Attendant - 2
77	Mr. S. Karunakaran	Lab Attendant - 2
78	Mr. R. Karunanidhi	Lab Attendant - 2
79	Mr. A. M. Sivakumar	Lab Attendant - 2
80	Mr. D. Sundaramurthy	Lab Attendant - 2
81	Mr. E. Poongavanam	Lab Attendant - 2

	TRANSPORT	
82	Mr. J. Prakash	Senior Technician (3)
83	Mr. P. Soundrarajan	Senior Technician (3)
84	Mr. V. Thanigaivel	Senior Technician (3)
85	Mr. G. Vasu	Senior Technician (2)
86	Mr. B. Vijayakumar	Senior Technician (2)
87	Mr. M. Manogaran	Senior Technician (1)
88	Mr. K. Saravanan	Senior Technician (1)
89	Mr. A. S. Dayalan	Senior Technician (1)
90	Mr. P. Subbaiah	Senior Technician (1)
91	Mr. B. Suresh Kumar	Senior Technician (1)
92	Mr. K. Thulasingam	Senior Technician (1)
93	Mr. K. Jagadeesan	Technician (2)
94	Mr. J. Loganathan	Technician (2)
95	Mr. V. Babu	Technician (2)
96	Mr. V. S. Senthilkumar	Technician (2)
97	Mr. G. Vasu	Technician (2)
98	Mr. S. Dass	Technician (2)
99	Mr. L. Gunalan	Technician (2)
100	Mr. R. Balu	Technician - C
101	Mr. J. Udhaya Kumar	Technician - C
102	Mr. J. Jaya Bharath Veeran	Staff Car Driver (O.G.)
103	Mr. M. N. Balaji	Staff Car Driver (O.G.)
104	Mr. V. Udhayachandran	Staff Car Driver (O.G.)
105	Mr. M. Pushparaj	Staff Car Driver (O.G.)
106	Mr. M. Anbalagan	Staff Car Driver (O.G.)
107	Mr. K. Govindan	Staff Car Driver (O.G.)
108	Mr. U. Murugan	Staff Car Driver (O.G.)
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109	Mrs. D. Devaki, B.A.	Administrative Officer
110	Mrs. D. Vijayakumari, B.A.	Section Officer
111	Mrs. R. Geetha, B.Com.	Assistant
112	Mr. S. Rajendran	Assistant
113	Mrs. R. Latha, B.E., M.B.A.	Assistant
114	Mrs. M. J. Nagalakshmi, M.A.	Assistant
115	Mr. S. N. Babu, B.A., B.L.	Assistant
116	Mr. R. Senthilnathan, DCS & Engg., B.C.S.	UDC
117	Mrs. P. Kowsalya, M.A.	UDC
118	Mr. A. S. Sivaraj, M.A., D.C.A	UDC
119	Mrs. A. Uma, B.Com.	UDC
120	Mrs. K. Kanaga	UDC
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122	Mr. V. Navalan, D.C.A.	LDC
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124	Mrs. J. Supriya, B.Sc., D.C.A.	LDC
125	Mr. Solomon Priya Kumar, M.A.	LDC

126	Mr. P. Madan Kumar	LDC
127	Ms. R. Cathrin Diviya, B.Sc.	LDC
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130	Mr. B. Amavasai	MTS
131	Mrs. J. Rajathi	MTS
132	Mr. K. Selvakumar	MTS
133	Mr. K. Dhamodharan	MTS
134	Mr. S. Kathiravan	MTS
135	Mr. J. Dilavar	MTS
136	Mr. M. Raghunathan	MTS

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