

## EPIDEMIOLOGICAL & OPERATIONAL RESEARCH

### Epidemiological impact study: Disease survey

#### Background

Directly observed treatment short-course (DOTS) was implemented in Tiruvallur district of Tamil Nadu in May, 1999. To assess the epidemiological impact of DOTS strategy, TRC is carrying out a series of sample surveys with 2½ years duration between surveys to estimate the prevalence of disease in this district, covering a population of 5,80,000.

#### Aim

- To study the trends over time for disease and thereby to measure the impact of DOTS strategy in this region

#### Methods

All adults aged  $\geq 15$  years included for the disease survey were screened by two screening methods namely, elicitation of symptoms and X-ray examination. Two samples of sputum specimens were collected from those who were either symptomatic and/or having abnormal X-ray suggestive of TB. These specimens were processed for smear and culture and those who became bacteriologically positive were referred for ATT if they satisfied the RNTCP guidelines.

#### Results

Three serial disease prevalence surveys have already been completed. The fourth survey was started in June, 2006 and is in progress. Coverage in the survey was above 90% for all examinations, namely, symptoms, X-ray and sputum examination. The coverage upto March, 2008 is shown in table 7.

**Table 7:** Coverage for examinations – Fourth survey (till March, 2008)

Activities	Fourth survey
Enumeration	69133
Symptom screening	64076 (93%)
X-ray screening	62918 (91%)
Sputum eligible	8085
Sputum collection	7818 (95%)

A total of 266 individuals were identified as cases through examination either by smear, culture or both.

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## **External quality assessments for sputum smear microscopy in the laboratory network**

### **Background**

RNTCP is a DOTS based programme and sputum smear microscopy is the diagnostic technique used. The quality of services in the microscopy centres need to be constantly evaluated and assured. The External Quality Assurance (EQA) protocol is implemented throughout the country which includes onsite evaluation, panel testing and random blinded cross-checking that are done to evaluate the performance.

### **Aims**

- To review the Intermediate Reference Laboratory (IRL)-EQA activities in 19 districts in India
- To conduct National Reference Laboratory-Onsite evaluation (NRL-OSE) visits focused on identifying the operational and technical problems in smear microscopy

### **Methods**

#### **NRL-Onsite evaluation visits**

TRC conducted NRL-OSE visits in 9 states during the year 2007. It reviewed the problems that were identified and corrective actions were suggested. Some of the corrective suggestions made for IRL and districts during the year are given below:

#### **IRLs**

- All the IRLs have started functioning well. But all the equipments supplied for culture and drug sensitivity in some IRLs need replacement or repair

- In IRL Goa, appointment of a microbiologist and 2 laboratory technicians should be done and trained in RNTCP, EQA as well as in culture and DST procedures
- Record maintenance for internal quality control at State TB Training and Demonstration Centre, Gujarat should be done properly
- Guidelines on fluorescence microscopy recently published by Central TB Division should be followed for grading smears and for internal quality control

### **District**

Most of the operational and technical problems were identified at the district level. The following suggestions were made for districts:

- Proper glassware should be made available at the District TB Centres, for bulk preparation of reagents wherever required.
- Random blinded rechecking (RBRC), coding and reporting should be carried out under the supervision of District TB Officer.
- All the newly appointed technicians should be trained in RNTCP-EQA activities.
- Disposal pits were posing a problem during rainy seasons, which needs to be addressed.
- District TB Officer should be motivated to take interest in the programme and supervise the activities.

More emphasis is given on the RBRC data and correcting the system whenever there are errors in the RBRC. At the end of OSE visits, the summary reports were made based on observations. This report was submitted to the IRL Director and State TB Officer for taking corrective actions. The action taken reports were obtained from the states within one month after the visit. Of the 131 suggestions provided to nine IRLs for corrective action, 104 (79%) were implemented within a month.

## Panel testing

For the year 2007, 5 (9.4%) out of 53 persons from 7 states made 6 errors (includes Microbiologist-1, Laboratory Technician-1 and Senior TB Laboratory Supervisor -3) in panel testing.

## RBRC activities at state level

RBRC data available from 8 states under TRC indicated that some of the districts were completely devoid of errors while some districts reported high and low false errors (table 8).

**Table 8:** State-wise RBRC activities

S.no	State	Districts	DMC	Sample size	HFP	HFN	LFP	LFN	QE	Total Error
1	Andhra Pradesh	24	893	123234	62	133	34	65	127	<b>421</b>
2	Goa	2	18	3047	2	5	0	2	4	<b>13</b>
3	Gujarat	29	694	75812	29	125	29	138	77	<b>398</b>
4	Kerala	14	457	101307	32	87	86	89	38	<b>332</b>
5	Punjab	20	285	3484	5	43	21	26	103	<b>198</b>
6	Sikkim	4	20	2972	1	3	1	1	4	<b>10</b>
7	Tamil Nadu	30	782	27751	24	57	8	33	41	<b>163</b>
8	UP-Lucknow	31	717	78171	135	198	131	138	367	<b>969</b>
9	UP-Agra	35	711	84939	169	265	101	119	262	<b>916</b>
<b>Total</b>		<b>189</b>	<b>4577</b>	<b>500717</b>	<b>459</b>	<b>916</b>	<b>411</b>	<b>611</b>	<b>1023</b>	<b>3420</b>

## Conclusion

Onsite evaluation is important to ensure the quality of sputum microscopy. NRL suggestions resulted in immediate corrective actions and had a sustained impact. OSE and RBRC supervision at the district level needed regular monitoring for improvement and sustenance of the program.

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## **The impact of HIV infection on recurrence of TB**

### **Background**

Molecular typing of the strains of tubercle bacilli is a reliable method available today to determine whether the patient's relapse is due to exogenous re-infection or endogenous reactivation. For decades, the issue of the role of exogenous re-infection versus endogenous reactivation has been debated. We have earlier shown from studies conducted in TB patients not infected with HIV that endogenous reactivation predominates. However there is no data with regard to HIV-TB patients from India. Specific phenomena such as emergence of HIV related TB co-infection could modify the evolution of TB transmission. Hence in this study we have compared the molecular epidemiology of HIV-TB co-infection with non HIV-TB using multiple tools.

### **Aim**

- To study the rate of exogenous re-infection and endogenous reactivation in HIV-TB patients compared with non HIV-TB patients by directly comparing the fingerprints of pre and post treatment isolates using three genotyping markers

### **Methods**

Twenty five HIV-TB patients and 23 non HIV-TB patients were included in this study. Three sputum samples each at the start of ATT, during treatment and 2 sputum samples during the follow up period were collected. Smear and culture was done on the LJ medium. Deoxy ribonucleic acid (DNA) from *M. tuberculosis* cultures was extracted by standard cetyl trimethyl ammonium bromide-sodium chloride extraction method.

Molecular typing was done by the following three methods:

- IS6110 RFLP
- Spoligotyping
- Mycobacterial interspersed repeat unit-Variable number of tandem repeat (MIRU-VNTR)

Clustering by the different typing methods was analyzed by using Bionumerics (Applied Maths).

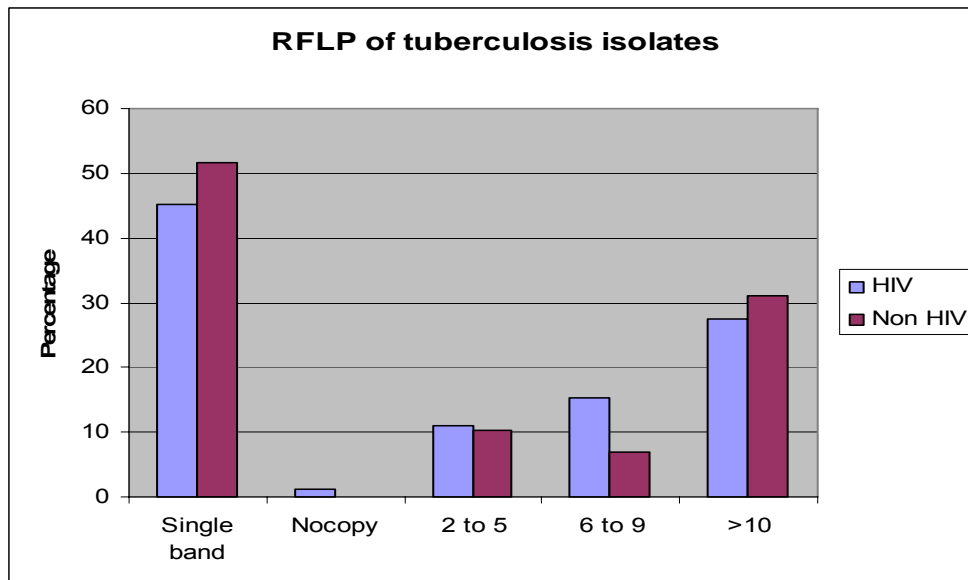
## Results

The major finding of the study was that exogenous re-infection occurred in a higher percentage of HIV-TB patients who relapsed compared to non HIV-TB patients who relapsed due to endogenous reactivation. IS6110 RFLP data revealed that both HIV patients and non HIV patients were infected by higher percentage (45.1 and 46.9% respectively) of single copy *M. tuberculosis* isolates. Spoligotyping data of HIV-TB and non HIV-TB patients in whom TB reoccurred showed that *M. tuberculosis* isolates of EAI3 Clade predominantly infected HIV-TB patients (HIV-TB 48.9% and non HIV-TB 38%), whereas *M. tuberculosis* isolates of EAI5 Clade predominantly infected non HIV-TB patients (HIV-TB 17.8% and non HIV-TB 26%) (Fig.1). Beijing strains infected HIV-TB patients to a higher degree than non HIV-TB patients.

## Conclusion

The high recurrence rate of TB in HIV-positive patients could be reduced by intensification of therapy. Since TB infection in an HIV-positive individual does not seem to confer immunity to individual, continuous prophylaxis is a better choice. Conversely, since the risk of infection is low in non HIV-TB patients the prospect of vaccine in these patients is promising.

**Fig.1:** RFLP of HIV and non-HIV tuberculosis isolates



X-axis denotes the strains harboring various numbers of IS6110 elements

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