

Epidemiological Research

Directly Observed Treatment-Short course (DOTS), a global strategy for control of TB is being implemented in India in a phased manner since 1997. The epidemiological impact of this strategy in high burden countries is not known. To understand this, TRC is undertaking an epidemiological impact study in 5 blocks of Tiruvallur district, Tamil Nadu. This is the same area where the BCG trial was done and epidemiological data on TB is available in this area prior to DOTS implementation. Tamil Nadu government is implementing the programme and TRC is monitoring the same. The project has technical support from World Health Organization (WHO) and financial support from United States Agency for International Development (USAID). The project was started in May 1999. TRC is involved in:

- a) Training
- b) Monitoring of the programme
- c) Epidemiological survey of tuberculous disease and infection
- d) Bacteriological and molecular epidemiological studies and
- e) Operational research

a. Training

Good quality training is essential for successful implementation of the programme. TRC has been identified as a nodal centre for training in RNTCP and during the period, we have trained 45 Medical Officers (Tr) in 4 batches, 7 Medical Officers in 1 batch, 63 Senior Treatment Supervisors (STS) in 4 batches, 39 Senior Tuberculosis Laboratory Supervisors (STLS) in 6

batches, 14 Lab Technicians (LTs) in 3 batches and 49 field workers in 4 batches.

b. Monitoring of the programme

During the reporting period, monitoring of the programme by TRC was done only upto December 2004. Six hundred and forty seven patients have been initiated on treatment for TB in the project area covering a population of 580,000. Of these, 336 were new sputum smear positive, 171 new smear negative, 83 extra pulmonary and 109 retreatment patients. Annualised case detection rate for new smear positive patients during the period was 92%.

c. Epidemiological Impact Study – Community survey of TB infection and disease

To assess the epidemiological impact of DOTS implementation, second resurvey to estimate the prevalence of TB disease and the third tuberculin survey to estimate the prevalence of infection in the project area were started.

Background:

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 to estimate the prevalence of TB infection and disease in Tiruvallur district, covering a population of 580,000.

Aim:

The aim of these surveys is to study the trends over time for both infection and disease and thereby to measure the epidemiological impact of DOTS implementation in this region.

Methods:

All adults ≥ 15 years included for the disease surveys 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 symptomatics and/or x-ray abnormalities suggestive of TB. These specimens were processed for smear and culture and those who became bacteriologically positive were referred for anti-TB treatment, if they satisfied the RNTCP guidelines. All children included in the tuberculin survey were tested with PPD 1TU RT23 and the reaction sizes were read after 72-96 hrs.

Results:

Two surveys have been completed and the second resurvey is in progress since January 2004. Of 41,670 subjects targeted for the disease survey, 38,225 (92%) were covered for symptom screening and 37,676 (90%) for x-ray screening. The coverage for sputum collection was 4645 (92%) of the 5027 that were eligible. For the tuberculin survey, of 17,306 subjects targeted the coverage was 16,647 (96%).

Conclusion:

The prevalence of disease from the two completed surveys have shown a decline of 9% among smear positive cases and 11.3% among culture positive cases, demonstrating that DOTS implementation was associated with a more rapid reduction in the prevalence of disease compared to that in the pre-DOTS period.

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d. Molecular Epidemiology

Laboratory analysis of *M. tuberculosis* isolates by Restriction Fragment Length Polymorphism (RFLP):

Background:

TRC is undertaking RFLP studies on positive cultures obtained from patients. Sputum specimens have been collected from patients who were started on treatment and they have been set up for culture and sensitivity. RFLP typing is done on the positive cultures to understand the cluster effect.

Aim:

To understand the molecular epidemiology in the area and to monitor the same over time.

Methods:

M. tuberculosis clinical isolates were cultivated on LJ medium, harvested and killed by heating at 80°C for 30 min. Genomic DNA was isolated and Pvu II-IS6110 RFLP analysis was performed according to standardized methods using a 245 bp right sided probe. In parallel, Alu I cleaved genomic DNA was probed with repetitive DR element isolated from *M. bovis* BCG according to standard protocols.

Results:

The RFLP analysis of *M. tuberculosis* isolates revealed that 40% of the TB isolates showed a single copy of IS6110. To overcome this limitation an additional probe Direct Repeat (DR) was used to fingerprint the isolates. During the last year, the same isolates were subjected to an additional typing method by using a third probe, called polymorphic GC repeat sequence (PGRS). Two thousand five hundred *M. tuberculosis* isolates from the MDP area have been processed for DNA extraction and RFLP has been performed on 1500 samples till June 2004. The PGRS typing has been completed for 500 IS6110 low copy isolates.

The DR probe RFLP has been replaced by an easier PCR based method called 'spoligotyping' and this newer method has been standardized and performed on 500 isolates upto December 2004.

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