Chapter - 6

Summary
Present study was carried out to find out the prevalence of Pulmonary Tuberculosis in south gujarat region and to study the drug resistance pattern of M.tuberculosis, in terms of MDR & XDR TB cases. For early diagnosis of infection with multi-drug resistant strains of M. tuberculosis rapid diagnostic tests such as MTT, NRA and MGIT were done. In addition to this the effect of herbal drug on MDR strains of M.tuberculosis was also carried out during the tenure of this study total 230 numbers of sputum samples were collected from the suspected TB patients by standard method. From each patient two sputum samples were collected i.e. spot and morning according to the RNTCP guidelines (N Selvakumar et al 2002.)

All the sputum samples were not cultured in media without screening. The screening for the bacilli of M.tuberculosis was done by acid fast staining technique. Results were recorded as per RNTCP Grading system. The sample which was showing 1-10 bacilli per field were further studied for the sputum culture. For the target isolation of Mycobacterium species, digestion and decontamination was done by N-Acetyl-L-Cysteine sodium hydroxide method as mentioned in the standard book Henry D. Isenberg (2004).After decontamination, deposits were inoculated on Lowenstein-Jensen (LJ) slopes along with Middle Brook 7H9 broth (Anargyros et al.1990) for solid culture and liquid culture techniques. These media were then incubated aerobically at 37 °c for 48-72 hours after inoculation to detect gross contaminants. Thereafter cultures were examined weekly, up to 8 weeks. Culture reports were noted as qualitatively (positive or negative) and quantitatively (number of colonies isolated) as per RNTCP manuals. The positive cultures were identified by special tests such as Niacin production, Nitrate reduction, semi quantitative Catalase production test, sensitivity to PNB (para-nitrobenzoic acid) test, 5 % NaCl tolerance test and Pyrazinamidase (CDC 1981). In the present study total 230 samples of sputum were analyzed. Among them 18% were from rural area, where as majority of them were from urban area 82%. The new cases screened from rural and urban area were 54%, while pretreated cases were 46%. In our study maximum numbers of patient were diamond workers (25%). We have screened a samples for age group from 10 years to >70 years and from both the gender. We found highest (45.21) % in case of 10-30 years and the disease was found prevalent in male (73.91%) compared to
female (26.08%). In the present study maximum numbers of patient belongs to 30-45 kg weight group (54.34%).

We have screened out sputum samples by Acid Fast staining technique. Among them 39.56% were 3+, 29.56% and 30.86% were 1+. The major biochemical characteristics shows that 95.21% strains were \textit{M.tuberculosis} and 4.79% strains were MOTT.

The culture positivity was checked with two different methods, proportion method and MGIT method. By proportion method 98.41% \textit{M.tuberculosis} were found positive after 28 days where as in case of another method i.e. MGIT the maximum culture positivity (97.96 %) was found only after 15 days.

The identified \textit{Mycobacterium tuberculosis} were subjected to drug susceptibility testing using first line drugs viz. isoniazid (INH), rifampicin (RIF), ethambutol (EMB), streptomycin (SM) and screened for MDR strains, which were subjected to second line drugs, viz. kanamycin (KA), ethionamide (ETH) and Ofloxacin (ZN) by proportion method as per standard procedure (Kent et al. 1985).

The drug susceptibility testing was performed by Gold Standard Method (Lall et al.2001) for the detection of resistant strains (MDR & XDR). Resistance was confirmed by rapid method known as MGIT (Ashok et al. 2006). Two reductase tests were also performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and Nitrate reductase assay an important reducing agents (Palomino et al., 2002).

The organisms showing growth while testing drug susceptibility against first line and second line drugs were interpreted as a MDR or XDR strains. These MDR strains were selected for the further studies and tested to study effects of herbal extracts from Garlic (Allium sativum) bulb and Turmeric (Curcuma longa) root along with \textit{M.tuberculosis} H37Rv. Their effective concentration for both herbal extracts was determined by MGIT to find out minimum inhibitory concentration (MIC) of herbal compound.

First and second line drug susceptibility testing of isolates of \textit{M.tuberculosis} were analyzed by the NRA, MTT, MGIT and Proportion methods.
First-line drug susceptibility testing of isolates of *M.tuberculosis* by the nitrate reductase assay & the proportion method, by the MTT test & the proportion method and by the MGIT test & the proportion method using LJ as the gold standard shows 98.8%, 99% and 97.3% concordance respectively.

The present study shows the prevalence of MDR-TB among pulmonary TB patients as **20.86%**, where as other than MDR 18.69%, Mono drug resistant 16.95%, Resistant to all drugs 10% and 43.47% were sensitive to all drugs.

The present study demonstrate the prevalence of MDR-TB among new TB cases and previously treated TB cases as 2.1% and 16.1%.

MDR strains of *M. tuberculosis* shows 20.8 % resistance to rifampicin and isoniazid only; 16.7 % resistance to streptomycin (in addition to rifampicin, isoniazid) ; 14.6 % resistance to ethambutol  (in addition to rifampicin, isoniazid) and 47.9 % resistance to all four first line drugs.

Among total culture positive strains, 39 strains were Mono drug resistance strains in which resistance to isoniazid was highest (30.8%) followed by streptomycin (28.2%), rifampicin (25.6%) and ethambutol (15.4%).

Other than MDR pattern suggests higher % of  HS resistance (i.e. 23.2%), followed by equal % of  ER & SE resistance (i.e. 16.3%) than with SR (14.0%), SER (11.7%), HE & SHE (9.3%).

The present study shows that higher number of MDR *M. tuberculosis* strains were resistance to ethionamide (68.7%), while lower number of strains were resistance to ofloxacin (14.6%) and kanamycin (6.2%) and no one MDR strain was found to be resistant to both ofloxacin and kanamycin therefore the percentage of XDR-TB was 0%.

Second-line drug susceptibility testing of isolates of *M.tuberculosis* by the nitrate reductase assay & the proportion method, by the MTT test & the proportion method and by the MGIT test & the proportion method using LJ as the gold standard shows 98.7%, 88.0% and 96.1% concordance respectively.
Antimycobacterial activities of synthesized (1, 2, 4-triazoles with benzothiazoles) compounds on MDR *M.tuberculosis* shows that 6b and 6h containing 6-bromo and 4-nitro showed good activity (62.5 µg/ml) where as compound 6j containing 4-chloro substituent on benzothiazol ring showed better activity (50 µg/ml).

Garlic and turmeric plant extracts were evaluated for their activity against drug resistant variants of *M.tuberculosis*. The results of inhibitory effect of both plant extracts showed that different isolates of MDR were inhibited at different concentrations of extracts ranging from 1.0-2.0 mg/ml.