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Prevalence of Multidrug-resistant Tuberculosis Among Newly Diagnosed Cases of Sputum-positive Pulmonary Tuberculosis from MGM Hospital Warangal

M. Jojula[1]*, M. V. Ramanama[2] and S.R. Reddy[3]

[1]Department of Pharm. Microbiology, Sri Shivani College of Pharmacy, Warangal, India.
[2]Department of Microbiology, Kakatiya Medical College, Warangal, India.
[3]Department of Microbiology, Kakatiya University, Warangal, India.

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In spite of availability of effective chemotherapy and Bacille-Calmette-Guerian (BCG) vaccine, tuberculosis remains a leading infectious killer world- wide. Tuberculosis (TB) is presently recognized as one of the most common opportunistic infections seen in HIV seropositive patients, mostly presenting in the form of pulmonary and extrapulmonary infections. The prevalence of multidrug-resistant tuberculosis (MDR-TB) is increasing throughout the world. Although previous treatment for TB is the most important risk factor for development of MDR-TB, A total of 100 clinically diagnosed and radiologically evident cases suggestive of pulmonary tuberculosis were selected for study. A total of 27 samples were found to be culture positive and 73 were culture negative, of these 26 stains were identified as *Mycobacterium tuberculosis*, one was identified as *M.avium complex* (MAC). All sputum-positive TB cases were subjected to mycobacterial culture and first-line drug-susceptibility testing (DST). MDR-TB was defined as TB caused by bacilli showing resistance to at least isoniazid and rifampicin. All the 27 isolates were concurrently subjected for minimal inhibitory concentration (MIC), Proportion method and BACTEC. Out of 27 cultures tested by the MIC methods, resistance was observed in 99.9 percent at MIC of 128 mg/l. In proportion method five isolate were shown resistance to Isoniazid (H); Rifampicin (R); but shown sensitive to other drugs Ethambutol (E); Pyrazinamide and Streptomycin.

Key words: In vitro definition, Mycobacterium tuberculosis, rifampicin.

Tuberculosis is among the top ten causes of global mortality[1,2]. It has been estimated that approximately one –third of the world's population is infected with the tuberculosis bacillus. The tuberculosis situation has worsend over the past two decades in Africa owing to the HIV/AIDS epidemic and in Eastern Europe in association with multidrug resistance[3,4]. The emergence and spread of multi-drug resistant tuberculosis (MDR-TB) is threatening to destabilize global tuberculosis control. The prevalence of MDR-TB is increasing throughout the world both among new tuberculosis cases as well as among previously-treated ones Multidrug-resistant strains of Mycobacterium tuberculosis seriously threatens tuberculosis (TB) control and prevention efforts. MDR TB which is an essentially a man made problem and arises as a consequence of incomplete / inadequate treatment, leading to selection of drug resistant strains. Of great concern for the control of the disease is the emergence of drug resistance (DR) since there is no cure for some multidrug-resistant (MDR) strains of M. tuberculosis, and there is concern that they may spread rapidly around the world The standardization of drug susceptibility determinations has become important to formulate treatment policies for patients with drug resistant tuberculosis, especially those with multi-drug

* To whom all correspondence should be addressed.
Mob.: +91-9849630268;
E-mail: jojulamalathi@yahoo.co.in

resistance (MDR-TB). Quantitative evaluation of growth is an essential tool in diagnostic microbiology, to study the pathogenesis of bacterial diseases and also in antimicrobial drug efficacy testing. Conventional procedures based on culture techniques are the most sensitive and specific methods; unfortunately, they do not provide rapid results when applied to quantification of slow-growing bacteria such as *Mycobacterium tuberculosis* (WHO/IUATLD, 2008). There are different methods for detection of TB drug resistance. The proportion method (PM) (S Nalini, 2010), MIC (Jimenez-Arellanes, 2003) and NRT), based on the measurement of growth in culture media containing antibiotics, require several weeks to give results. The BACTEC radiometric system has the advantage of being more rapid (5–10 days), but requires the use of radioisotopes and can be costly to be performed routinely (Lemus *et al.*, 2004; Palomino, 2005).With the development of resistance to isoniazid has been uniform in most of the studies undertaken world-wide, it has not been so in the case of rifampicin resistance. In vitro activity of rifampicin towords Mycobacterium tuberculosis has been consistently using a minimal inhibitory concentration (MIC) of 128 mg/l or more on Lowenstein- jesen (L-J) mediumv (5) by using Drug-susceptibility testing. The activity of rifampicin by the MIC method on L-J medium, Properties method on L- J medium and compared with rapid testing with radioactive method BACTE and undertaken, the results of which are presented.

MATERIALS AND METHODS

A total of 100 clinically suspected causes of tuberculosis were subjected for isolation by using L-J medium. Based on morphology and biochemical test, isolates identified to be Mycobacterium tuberculosis and Mycobacterium avium complex. Out of the 27 isolates, 26 isolates were identified as M.tuberculosis and whereas, one was identified as M.avium complex (MAC).Based on the morphology of the culture isolates on L-J medium 26 isolates where identified as M. tuberculosis, there where showing characteristics of rough, eugonic, buff tint ,and showed production of niacin. Only one isolate were shown morphology, slow rate of growth, inability to produce pigment, hydrolysis of Tween-80 and the ability to producte heat stable catalase as per standard and biochemical tests An attempt was made to test the clinical isolates to determine where they developed drug resistance are not we had compared conventional and rapid methods of drug resistance with same clinical isolates. All the four first line drugs (Isoniazid (H); Rifampicin (R); Ethambutol (E); Pyrazinamide) were used for drug susceptibility testing against the isolates of M.tuberculosis in order to study their sensitivity pattern. The *M. tuberculosis* H37 RV strain was used as a control in every batch of tests as a check on the inoculum size as well as the drug concentrations in the medium. The indirect drug susceptibility test is carried out from a primary isolation or a sub-culture on LJ medium, preferably from a fresh primary isolate as on sub-culturing, especially repeated sub-culturing, some of the bacterial characteristics may change. In any case, try to use an actively growing culture within one to two weeks after the growth appears. If it is pass two weeks from the day positive growth on the medium was observed, subculture again and use the freshly grown subculture**.** The lowest drug concentration which inhibited growth was taken as the MIC[7]. Prepared in the laboratory were used for the study. For the MIC method, a 3mm loopful of the bacterial suspension containing approximately 4 mg/ml was used to inoculate drug free and drug containing slopes, (32 64 128 mg/l). The slopes were incubated at 37°C and read at the end of 4 week. Make serial tenfold dilutions of the standard suspension by diluting sequentially 1.0 ml of the culture suspension in tubes containing 9 ml of sterile distilled water or normal saline (0.9% sodium chloride). Dilutions of 10^{-2} (control 1) and 10^{-4} (control 2) are inoculated on the growth control (L-J without drugs) while the drug containing L-J medium is inoculated with only 10^{-2} dilution. The volume of the inoculum is 0.1 ml. After inoculation, the tubes are incubated at 37 +/- 1 °C in a slanted position. The reading of results is carried out at the 28th and 40th day after inoculation. In Proportion method two dilutions were used to test the drug effect of isolates 10^{-2} and 10^{-4} dilution and sloops were incubated at 37°C for 8 weeks till we get growth on medium. Radiometric method BacT liquid method was used for detection of growth curve by using MIC and Proportion methods.

RESULTUS AND DISCUSSION

The study was carried during the period of 2009 to 2010 and the clinical sputum samples were collected from patients whom were suspected for tuberculosis infections. 100 sputum samples were collected from the out patients those who were attending the DTC and MGM hospital for testing. These sputum samples were used for testing for tuberculosis infection by AFB staining, Culture and DST for confirmation of TB infection. Blood sample collected from the 100 above mentioned patients were used for testing HIV reactivate. Out of 100 clinical samples there were 27 culture positive samples these clinical isolates were subjected for drug resistance and multidrug resistance by using both conventional and rapid drug susceptibility testining. All the 27 isolates were classified as sensitive by the MIC method. The control strain used is H37RV which is sensitive to rifampicin at 0· 32 mg /l. Out of 27 clinical isolates, two isolates were inhibited at MIC 0·64 mg /l, two isolates at 0·8 mg/l, other four isolates were inhibited at lower concentrations that is less than 0.8 mg/l because no growth was observed at lowest MIC concentration. All the isolates were below the drug resistant values (6), so there were no isolates showing resistance to first line drug for the MIC method. Any value of MIC 128 mg/l is interpreted. MIC, Proportion methods, four isolates were inhibited at MIC 64mg/l, four isolates at 8mg/l, six isolates at 32mg/l, therten isolates at lower concentrations that is less than 8mg/l. All the isolates were below the drug resistant values, so there were no isolates showing resistance to drug rifampicin for the MIC method. Sensitivity and resistance standrization of antituberculosis drugs varies between laboratories and also between various methods. The MIC method is known to be affected by inoculum size bases both on the bacterial content in the suspension as well as the volume used to inoculum the slopes (8. Paramasivan et al. (2001), concluded that out of the 598 clinical isolates tested by the MIC and proportion methods, 572 (95.6%) were classified as sensitive by the MIC method while 26 (4.3%) were resistant. By proportion method we observed that 5 isolate was showing resistance to two drugs Isoniazid (H); Rifampicin (R) but for other two drugs shown sensitive. By the proportion method 570 (95.3%) were classified as sensitive and 28 (4.7%) as resistant.

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