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A STUDY ON DRUG RESISTANT *MYCOBACTERIUM TUBERCULOSIS* BY ABSOLUTE CONCENTRATION METHOD

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ABSTRACT

Tuberculosis is a disease that has ben taunting human health since ancient times and now it is again on the rise owing to the rise in Multiple Drug Reistance i.e. in this case resistance to the two major first line drugs used for treatment namely Rifampicin and Isoniazid. This study aims at finding out the incidence of MDR- TB from the collected 100 sputum samples of the patients. Here, out of the 75% giving positive for TB, 13% was resistant to both the drugs while 84% showed sensitivity.

KEYWORDS: Rifampicin and Isoniazid.

INTRODUCTION

Tuberculosis or TB as it is commonly called is a major cause of death worldwide and is caused by a single pathogen, *Mycobacterium tuberculosis* and account for 26% of all preventable adult death globally. According to the WHO, 8 million cases occur each year, resulting in 3million deaths (Xiao- Yong *et al*., 2003). After HIV, TB is the second common cause of death due to infectious diseases and the current trend suggests that TB will still be among the 10 leading causes of global disease burden in the year 2020 (Murray, 1998). The increasing worldwide prevalence of multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis* represents a major threat to tuberculosis (TB) control programs (Espinal *et al*., 2001).

The *Mycobacterium tuberculosis* infecting the lungs is known as pulmonary tuberculosis. These are categorized like pleural effusion, pericardial effusions, military tuberculosis, meningeal tuberculosis, genital tuberculosis, tuberculosis of lymph nodes etc. (Horne, 1992). Tuberculosis infection is said to have started when the bacterium reach the pulmonary alveoli, where they invade and replicate within the macrophages in the alveoli (Houban *et al.,* 2006). All parts of the body may be affected by tuberculosis although infection in the skeletal muscles, pancreas, thyroid and heart are rare (Agarwal *et al*., 2005).

Isoniazid and Rifampicin are among the major first lne drugs used for treating TB. Izoniazid has high tissue penetratin and is given once a day orally. It remains the single most inexpensive drug for treatment of TB. Rifampicin, along with destroying the bacterium, also eliminates the dormant organisms in macrophages, which may later cause a relapse of the disease (Tellenti *et al*., 1993). However, due to the development of resistance to these drugs, an array of second line drugs are now being used. These include Streptomycin, Kanamycin, Amicacin, Capriomycin etc. (Raviglione. M.C and R.J.O' Brien., 2005). Resistance of *Mycobacterium tuberculosis* to both Rifampicin and Isoniazid is called as Multiple Drug Resistance (Rajendra Prasad., 2005).

MATERIALS AND METHODS

Specimen Collection

Early morning sputum samples were collected in sterile, wide- mouthed containers from 100 patients in PSG hospital, Coimbatore. Three subsequent samples were collected from each patient.

Ziel – Neelson Staining

The collected sputum samples were smeared on grease free glass slides, heat fixed and subjected to Ziel – Neelson Staining recommended by World Health Organization (1998).

Modified Petroff's Method (NaOH method)

Sputum samples were taken in sterile screw capped tube and mixed with equal volume of 4% NaOH. The mixture was incubated for 25min for the complete digestion of the mucopurulent mixture and further centrifuged at 3000 rpm for 15 min and the supernatant was discarded. The pellet was diluted with HCl for neutralization and centrifuged at 3000 rpm for 20 min and the supernatant was discarded. Sterile distilled water was added and centrifuged at 3000 rpm for 20 min and supernatant was discarded. 100 ml of the pellet was then inoculated into

Lowenstein Jenson medium and incubated at 37^{O}C for 4-6 weeks (Mukesh Sharma *et al*., 2012).

Preparation of Bacterial Suspension

A suspension was prepared by adding approximately 4 mg of the bacterial mass visualized as 2/3 loopful of 3 mm internal diameter 24 SWG wire loop into 0.2 ml of sterile distilled water in a 7 ml bijou bottle containing 2-3 mm glass beads. This was vortexed for 30 sec to produce a uniform suspension. To this, 3.8ml of sterile distilled water was added to produce a suspension containing approximately 1mg/ml (S_1) and kept undisturbed to let the course particles settle down. From this suspension, a 10 fold dilution was made by adding 0.2 to 1.8 ml sterile distilled water (S_2, 10^{-1}). Two more dilutions were made in the same manner ($S_3 10^{-2}$ and $S_4 10^{-3}$).

Lowenstein Jenson Medium (LJ medium)

About 37.24 g of LJ medium was suspended in 600 ml distilled water containing 12 ml glycerol, heated to dissolve it in the medium completely and autoclaved at 15lb pressure at 121 ^{O}C for 15 min. 1000ml of whole egg emulsion was prepared and added into the medium and mixed well. The medium was distributed in screw capped bottles, kept in slanting position and inspissatted at 85^{O}C for 45 min (Beveja, 2006).

Biochemical Tests

Biochemical tests like Niacin test, Nitrate reduction test, Catalase and Urease tests were performed.

Drug Susceptibility Test

Drug Susceptibility tests were done by absolute concentration method for Isoniazid and Rifampicin. This method uses standardized inoculums grown on drug free media and the media containing graded concentration of the drugs to be tested. Three concentrations of each drug were tested and resistance was expressed in terms of the lowest concentration of the drug that inhibits the growth i.e. MIC. Viability of the organism and inoculums size greatly affects the method (TB Manual, Ministry of Health and Family Welfare, New Delhi, 2000).

Preparation of the Drug Containing Media

The complete LJ medium was prepared in a sterile 700 ml flask and distributed in 7 sterile flasks as described earlier. A stock solution of antibiotic drugs for Isoniazid (10µ/ml) was prepared in sterile distilled water and for Rifampicin in 1 N HCl stored in refrigerator. The stock solution of antibiotic were further diluted with distilled water which contained 20, 100 and 500 µg/ ml for Isoniazid and 3200, 6400 and 12800 µg/ ml for Rifampicin. Each diluted antibiotic solution was added into separate LJ medium flask, mixed thoroughly and the specific concentrations were labeled. Approximately 10 ml of LJ medium containing antibiotic solution was distributed into each screw capped bottle. The final concentration of Isoniazid antibiotic concentration in the LJ medium was made upto 0.2, 1.0 and 5.0 µg/ ml and for Rifampicin 32, 64 and 128 µg/ ml.

One LJ medium slope was kept as control, without the addition of antibiotic. The bottle was placed in slanting position in the inspissator and the medium coagulated for 50min at 85^{O}C to heat the medium (Xia Yu *et al.*, 2011).

Inoculation

One standard loop (3 mm diameter, 27SWG) of the standard strain *M.tuberculosis* H37 RV was inoculated onto all the LJ medium slopes, including the control and incubated at 37^{O} C for 4-6 weeks.

RESULTS AND DISCUSSION

Ziel – Neelson Staining

Ziel Neelson staining showed slightly curved, slender pink rods against a blue background in 13% samples.

Modified Petroff's Method (NaOH method)

After concentrating the samples by modified Petroff's concentration method, 62% samples showed slender pink color rods on staining.

Growth on LJ medium

After 4-6 weeks of inoculation at 37 ^{O}C, 75% samples showed non- pigmented, rough yellowish wrinkled colonies on LJ medium (Koneman, 2006).

Biochemical Tests

The organism was positive for Nitrate reduction test, Urease production and also in Niacin test but did not produce Catalase.

Drug Susceptibility Test

Rich growth was seen in the drug- free control. Growth in medium containing 1.0 µg/ ml Izoniazid and 60 µg/ ml Rifampicin were considered as resistant strains. As such, 13% showed Multiple Drug Resistance, 1.6% showed resistance only to Rifampicin, 1.4% showed resistance only to Isoniazid while 84% were sensitive to both the drugs marked by absence of growth.

CONCLUSION

Mycobacterium tuberculosis, the single causative agent of tuberculosis has claimed the lives of a number of people and now, with the emergence of Muliple Drug Resistance, it poses as a big treat to the society. From all the sputum samples collected, it was seen that the 13% samples showed Multiple Drug Resistance, meaning that the use of the first line drugs to TB namely Isoniazid and Rifampicin are useless against such strains. This is a proof towards the evolving scenario of drug resistance in *Mycobacterium tuberculosis.*

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