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Original Research Paper

Medical Microbiology

ISOLATION IDENTIFICATION AND PATTERNS OF ANTI-TUBERCULOSIS DRUG RESISTANCE FROM TERTIARY CARE HOSPITAL UDAIPUR (RAJASTHAN)

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ABSTRACT

Objective: Resistance against Mycobacterium tuberculosis (MTB) is important in the sense that it has an implication in the control of tuberculosis. The terms used to describe resistance to antituberculosis drugs are resistance among new cases (or primary resistance) and resistance among previously treated patients. The resistance among previously treated patients may be due to faulty treatment like prescription of inadequate treatment regimens, interrupted availability or poor quality of drugs, or incomplete treatment adherence while subsequent transmission of these resistant organisms to others will lead to development of disease which is resistant from the beginning called primary resistance. Pakistan is ranked eighth in terms of global estimated burden of tuberculosis cases. Multi-Drug Resistant (MDR) tuberculosis among new cases and MDR among previously treated patients is 3.2% and 35% respectively.

Material and methods: -

AFB smear examination and grading: - AFB smear examination was carried out by direct microscopy using the Ziehl Neelsen (ZN) method. Sputum smear result was examined and interpreted according to the AFB grading.

AFB culture and drug susceptibility test: - Culture examinations were done on all diagnostic specimens of AFB smear positivity. Sputum specimens from each patient were processed with sodium hydroxide (NaOH) method-Modified Petroff 's procedure and cultured on Lowenstein-Jensen (LJ) slopes.10 All inoculated LJ drug and control media were incubated at 37°C. All cultures were examined 48-72 hours after inoculation to detect gross contaminants. Thereafter, cultures were examined weekly, up to eight weeks on a specified day of the week.

Typical colonies of M. tuberculosis were rough, crumbly, waxy, non-pigmented (buff coloured) and slow-growers, i.e., only appeared two to three weeks after inoculation. The colony was confirmed by ZN staining. Detection time for MOTT was 25 days. M. tuberculosis positive strains were culture negative when they grew on p-nitro benzoate (PNB) containing medium. Only a few colonies of non-tuberculosis Mycobacteria (NTM – often pigmented, with smooth morphology or PNB positive) were grown as visible colonies on PNB containing medium.

Anti-TB drug susceptibility testing: - anti-susceptibility testing perform on pre-formed LJ media with antitubercular drugs

Tuberculosis First Line Kit (Total 7 slants) Containing five antitubercular agent (Isoniazid, Streptomycin, Ethambutol, Rifampicin and Pyrazinamide) 2 controls without any antimicrobial agent.

Results: out of 119 samples antitubercular testing against first line antitubercular drugs such as Pyrazinamide were shows 12 (10.08%) sample were resistance which accounts maximum resistance among first line antitubercular another first line antitubercular drugs shows resistance as follows Streptomycin (9.24%), Ethambutol (8.40%), Isoniazid (7.56%), Rifampicin (6.72%), drugs out of 119 samples in which 107 samples were susceptible to the Pyrazinamide drug in in-vitro antitubercular susceptibility testing. Antitubercular resistance against second line antitubercular drugs were shows as follows out of 119 samples antitubercular testing Ethionamide were shows 9 (8.18%) sample were resistance which accounts maximum resistance among second line antitubercular another second line antitubercular drugs shows resistance as follows Clarithromycin (6.72%), Ciprofloxacin (5.88%), D- Cycloserine (5.88%), Amikacin (5.04%), Kanamycin (4.20%), P-aminosalicylic acid (4.20%) and Rifabutin (3.36%) drugs out of 119 samples in which 107 samples were susceptible to the Pyrazinamide drug in in-vitro antitubercular susceptibility testing. MDR-TB emerged in patients who were resistant to Rifampicin and Isoniazide was 6 in number during this study.

KEYWORDS : MDRTB, antitubercular, Rifampicin, Isoniazid

INTRODUCTION

Tuberculosis scenario: -TB is the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS. In 2016, there were an estimated 1.3 million TB deaths among HIV-negative people (down from 1.7 million in 2000) and an additional 374 000 deaths among HIV-positive people (1). In India, each year, approx. 220,000 deaths are reported due to Tuberculosis. Between 2006 and 2014, the disease cost Indian economy USD 340 billion. India is the highest TB burden country with World Health Organization (WHO) statistics for 2011 giving an estimated incidence figure of 2.2 million cases of TB for India out of a global incidence of 9.6 million cases. This public health problem is the world's largest tuberculosis epidemic. Rajasthan is leading among states having highest deaths due to Chronic Obstructive Pulmonary Disease in the country in 2016 (3). The figures of the study, which was prepared by the Indian Council of Medical Research (ICMR), Public Health Foundation of India and the Institute for Health Metrics and Evaluation through funding by Bill & Melinda Gates Foundation; Indian Council of Medical Research, Department of Health Research, Ministry of Health and Family Welfare, Government of India, was released in New Delhi. In Rajasthan, malaria, tuberculosis and diseases of the respiratory tract cause the highest number of deaths (4). People who are HIV-positive and infected with TB are 20 to 40 times more likely to develop active TB than people not infected with HIV living in the same country (5)Pulmonary nocardiosis mimics pulmonary tuberculosis in both clinical manifestation, being chronic in nature and radiological characteristics makes it difficult to differentiate from M. Tuberculosis and may as well be often wrongly treated with anti TB drugs patients might also be confused with other chronic lung infection such as invasive fungal infection (14).

Resistance against Mycobacterium tuberculosis (MTB) is important in the sense that it has an implication in the control of tuberculosis. The terms used to describe resistance to anti-tuberculosis drugs are resistance among new cases (or primary resistance) and resistance among previously treated patients (27). The resistance among previously treated patients may be due to faulty treatment like prescription of

inadequate treatment regimens, interrupted availability or poor quality of drugs, or incomplete treatment adherence (28) while subsequent transmission of these resistant organisms to others will lead to development of disease which is resistant from the beginning called primary resistance (29) Pakistan is ranked eighth in terms of global estimated burden of tuberculosis cases. Multi-Drug Resistant (MDR) tuberculosis among new cases and MDR among previously treated patients is 3.2% and 35% respectively (30). There are small studies on resistance pattern from Pakistan. The objective of this study was to retrospectively determine the resistance pattern against both first and second line drugs in MDR-TB patients during a decade at a specialized tuberculosis treatment center.

MATERIAL AND METHOD

a) **Study Design:** -This was an analytical cross-sectional laboratory based prospective study of 1year and 6 month duration.

b) **Inclusion criteria:** -Patients with sign and symptoms of suspected cases of pulmonary TB were investigated for confirmation of pulmonary tuberculosis at the PMCH, Udaipur.

c) **Exclusion criteria:** - Patients with other broncho pulmonary diseases were excluded from study.

d) **Definition of TB Contact:-** A close contact is defined as living in the same household or frequent contact with a source case (e.g., caregiver) with sputum smear-positive TB.

Preparation of inoculum:

a. Take a loopful aseptically from the M. tuberculosis growth, primarily isolated on L. J. medium slant.
b. Suspend the sample in 1.0ml of sterile distilled water in a screw capped bottle. Homogenize the mixture on a vortex mixture up to 10 minutes.
c. Keep standingfor 10 minutes before opening the bottle.
d. Adjust opacity of suspension to match McFarland 0.5 standard with saline givingapproximately 1.5x 10^8cfu/ml. Dilute this suspension to 1:10000.

Principle and interpretation

Based on invitro correlation between the clinical response to antimicrobial agent and the result of invitro susceptibility testing kit (SL023) helps in diagnosing the sensitivity pattern of M. tuberculosis (HIMEDIA Labs) affected patient and accordingly provide treatment, drug therapy for the patients.

Microbiological investigations:- Sputum samples were examined for Mycobacteria and Nocardia by direct microscopy of smears stained by Gram's Method and Ziehl Neelsen Staining Method by using 20% sulphuric acid and modified Ziehl Neelsen Staining Method using 1% H_2SO_4 as decolouriser and lacto-phenol cotton blue preparation were also studied for mycelial morphology of Nocardia.

a) **Culture of Mycobacteria:-** All the sputum samples were decontaminated by N-acetyl L-cysteine sodium hydroxide method (as per RNTCP guideline) method for culture of M. tuberculosis. In brief, 3-5 ml of sputum was homogenized for 15 min in a shaker using an equal volume of 4% NaOH. After centrifugation at 3,000 rpm for 15 min, the deposit was neutralized with 20 ml of sterile distilled water. The samples were again centrifuged. From the sediment, LJ medium was inoculated and smear was made. The culture slants were incubated at 37°C.

All slopes were observed for occurrence of growth daily for first week and then at weekly intervals for 8 weeks. The isolates were identified by following tests: rate of growth, optimum temperature of growth, colony morphology, pigmentation, niacin test, and nitrate reduction tests, which confirmed that all isolates are M. tuberculosis (8).

Absence of growth at the end of 8th weeks was regarded as negative culture. Contamination, if any, was recorded separately. The number of culture failures for a certain decontamination method, included the number of specimens with negative culture as well as number of contaminated cultures (9).

RESULTS AND DISCUSSION

Out of 119 samples of mycobacterium tuberculosis were tested for antitubercular susceptibility in-vitro testing in which 11 (9.24%) samples were shows resistance against streptomycin and 108 were susceptible to it. Pyrazinamide were shows 12 sample were resistance out of 119 samples in which 107 samples were susceptible to the Pyrazinamide drug in in-vitro antitubercular susceptibility testing. Out of 119 samples of mycobacterium tuberculosis were tested for antitubercular susceptibility in-vitro testing with Isoniazid in which 9 (7.56 %) samples were shows resistance against Isoniazid and 110 were susceptible to it. Out of 119 samples of mycobacterium tuberculosis were tested for antitubercular susceptibility in-vitro testing with Ethambutol in which 10 (8.40 %) samples were shows resistance against Ethambutol and 109 samples were susceptible to it. Out of 119 samples of mycobacterium tuberculosis were tested for antitubercular susceptibility in-vitro testing with Rifampicin in which 8 (6.72 %) samples were shows resistance against Rifampicin and 111 samples were susceptible to it.

Second line antitubercular drug susceptibility testing:- second line drugs most commonly prescribed when the first drug doesn't work perfectly.

Second line antitubercular drugs susceptibility testing

Out of 119 samples of mycobacterium tuberculosis were tested for antitubercular susceptibility in-vitro testing with Amikacin in which 6 (5.04 %) samples were shows resistance against Amikacin and 113 samples were susceptible to it. Out of 119 samples of mycobacterium tuberculosis were tested for antitubercular susceptibility in-vitro testing with Kanamycin in which 5 (4.20 %) samples were shows resistance against Kanamycin and 114 samples were susceptible to it. Out of 119 samples of mycobacterium tuberculosis were tested for antitubercular susceptibility in-vitro testing with Rifabutin in which 4 (3.36 %) samples were shows resistance against Rifabutin and 114 samples were susceptible to it. Out of 119 samples of mycobacterium tuberculosis were tested for antitubercular susceptibility in-vitro testing with D-Cycloserine in which 7 (5.88 %) samples were shows resistance against D- Cycloserine and 114 samples were susceptible to it. Out of 119 samples of mycobacterium tuberculosis were tested for antitubercular susceptibility in-vitro testing with Ciprofloxacin in which 7 (5.88 %) samples were shows resistance against Ciprofloxacin and 112 samples were susceptible to it. Out of 119 samples of mycobacterium tuberculosis were tested for antitubercular susceptibility in-vitro testing with P-aminosalicylic acid in which 5 (4.20 %) samples were shows resistance against P-aminosalicylic acid and 114 samples were susceptible to it. Out of 119 samples of mycobacterium tuberculosis were tested for antitubercular susceptibility in-vitro testing with Clarithromycin in which 8 (6.72 %) samples were shows resistance against Clarithromycin and 111 samples were susceptible to it. Out of 119 samples of mycobacterium tuberculosis were tested for antitubercular susceptibility in-vitro testing with Clarithromycin in which 9 (8.18 %) samples were shows resistance against Clarithromycin and 110 samples were susceptible to it.

MDR-TB emerged in patients who were resistant to Rifampicin and Isoniazide was 6 in number and 113 samples were negative for MDR-TB

CONCLUSION

High resistance rates in MDR-TB to remaining first line and

second line drugs were found. Continuous monitoring and regular surveillance of drug resistance pattern especially of MDR isolates and treatment in specialized centres is a crucial need for future TB control in India. In conclusion, although the findings of a low burden of drug resistance to first-line TB drugs and low MDR as compared to other parts of the world is encouraging. Nevertheless, further studies are required including different geographic locations of the country that should be receiving support from the national TB control program. It would appear that monitoring of drug resistance should be enhanced by periodic surveys to assess trends of antibiotic-resistant patterns, and to prevent transmission of MDR-TB and progression of active disease.

The current study reveals that the overall resistance to first line anti-TB drugs is high. The highest mono drug resistance is detected against H. While the proportion of MDRTB is relatively low, signifying conditions favoring the spread of MDR TB is on rising. HIV co-infected patients were more likely to develop resistance to any one of the drugs tested compared with HIV negative patients. For early case detection and treatment, expanding diagnostic capacity for Mycobacterial culture and DST is a vital step to limit further spread of drug resistant TB strains in the study area.

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