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Isolation of Multi Drug Resistant Mycobacteria from Extra-pulmonary samples in a Tertiary Care Hospital: A Retrospective Analysis

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ABSTRACT

Background: Drug resistant tuberculosis has frequently been encountered in India. The prevalence of extra-pulmonary multi drug resistant tuberculosis in this country is found to be 3% in new cases and 12-17% in treated cases, which needs to be evaluated. Settings and Design: A retrospective analysis of processed extra-pulmonary tubercular samples in Intermediate Reference Laboratory of Department of Microbiology, Goa Medical College, was carried out over a period of one year, from November 2013 to October 2014. **Methods:** Lowenstein Jensen Media was used for culture processing of 202 samples from clinically suspected cases of extra-pulmonary tuberculosis patients. Drug susceptibility testing was done on the isolated strains using the economic variant of proportion method. **Results:** Out of 202 samples, 30 (14.85%) were observed to be culture positive for Mycobacterium tuberculosis of which six (21.43%) were found to be resistant to both Isoniazid and Rifampicin. **Conclusion:** With the advent of immunocompromised states, one has to be aware of multi drug resistant extra-pulmonary tuberculosis as a double edged sword. The early detection in such cases is a need of the hour to initiate prompt treatment.

Keywords: Mycobacterium tuberculosis, Multidrug resistant Tuberculosis, Extra-pulmonary tuberculosis, Lowenstein Jenson Medium, Intermediate Reference Laboratory, Extensively drug resistant Tuberculosis.

INTRODUCTION

Tuberculosis remains a major health problem in the developed and developing countries including India.[1,2] According to World Health Organisation (WHO) statistics for India, the incidence of new cases of tuberculosis was observed to be 2.79 million/year.[3-5]

Multi-drug resistant (MDR) tuberculosis is a form of tuberculosis caused by Mycobacterium tuberculosis that does not respond to Isoniazid (INH) and Rifampicin.[3,4] In 2016, an estimated 4,90,000 and 1,10,000 people worldwide developed MDR-TB and Rifampicin-resistant (RR) TB respectively. The countries with the largest numbers of MDR/RR-TB cases (47% of the global total) were China, India and the Russian Federation.[4] About 6.2% of MDR-TB cases had extensively drug resistant tuberculosis (XDR-TB) in 2016.[6]

Diagnosis of extra-pulmonary tuberculosis (EPTB) usually poses a dilemma for most pulmonary physicians due to paucity of clinical manifestations.[7] Out of 1.5 million cases reported to Revised National Control Program, 10-15 % is extra-pulmonary mostly including lymphadenitis and pleural effusion.[7]

The data regarding the pattern of drug resistance in EPTB cases is quite limited in India. The main factor can be attributed to the difficulty in obtaining diagnostically significant samples and inadequate number of laboratories handling such samples.[7,8]

Extra-pulmonary tuberculosis has become more important as chances of its development in immunocompromised patients i.e. AIDS patients,

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are higher than in their immunocompetent counterparts.[9]

Advocacy to Control TB Internationally (ACTION) says that India's TB control program is failing to take sufficient action to diagnose such cases. Diagnosis of EPTB is not covered by RNTCP; and these cases are forwarded to the DOTS regimen for treatment. Tertiary care centres appear to be an excellent place for medical education and operational research in this regard.[10,11]

Our present study focuses on isolation rate of Mycobacterium tuberculosis bacilli from extra-pulmonary samples and determining the drug resistance pattern in an intermediate reference laboratory from a tertiary care hospital.

MATERIALS AND METHODS

Retrospective data of 202 samples from cases of extra-pulmonary tuberculosis was collected in the Intermediate Reference Laboratory (IRL) of Department of Microbiology, Goa Medical College during the period from November 2013 to October 2014. The samples included purulent discharge collected from cervical lymph node aspirations, cold abscess, breast abscess, postauricular abscess, chronic discharging sinus, pleural abscess, Pott's spine and osteomyelitis.

Isolation and Identification

Specimens were subjected to fluorescent staining by Auramine O dye.[12] The smear was observed for the typical morphology of tubercle bacilli, described as long, slender and beaded in appearance.

Processing of culture

The purulent samples were processed by the conventional 2% N acetyl L cysteine, 4% NaOH method (Modified Petroff's Method).[13,14] After decontamination, the sediment was inoculated on two slopes of Lowenstein Jenson (LJ) medium in a biosafety cabinet, under strict aseptic precautions. The LJ media was incubated at 37ºC for a period of eight weeks. Once the growth appeared, colonies were again tested by Ziehl Neelsen (ZN) staining for AFB. Mycobacterium tuberculosis species were confirmed by standard conventional protocol based on slow growth rate, absence of pigmentation, morphology of the colonies, Niacin test positivity, absence of growth on LJ medium with para nitro benzoic acid (PNB) and catalase test at 68ºC.

Drug susceptibility testing (DST) of Mycobacterium tuberculosis

The drug susceptibility testing method was standardized as per World Health Organization (WHO) & International Union against TB and lung disease (IUATLD) guidelines. The testing was carried out in Intermediate Reference Laboratory within one to two weeks of appearance of growth.[13] The method employed for drug susceptibility testing, was economic variant of proportion method. The drugs tested for sensitivity were Isoniazid, Rifampicin, Ethambutol and Streptomycin, their critical concentrations being 0.2µg/ml, 40µg/ml, 2µg/ml and 4µg/ml respectively. All inoculated media including control (drug free) media were properly labelled and incubated at 37ºC. The slopes were read at fourth week and sixth week for the presence of colonies. Mycobacterium tuberculosis strain H37Rv (reference strain) was used as a quality control for every batch of freshly prepared medium.[15-16]

RESULTS

Total of 30 (14.85%) out of 202 processed samples showed positive culture for Mycobacterium tuberculosis, of which 13 (43.43%) samples were positive by smear while 17 (56.66%) samples were positive by culture alone. Two out of 176 (1.16%) culture negative samples were positive by smear alone.

Distribution of culture positive samples among various departments revealed that 18 (60%) were from Surgical wards, followed by Pulmonary Medicine and Otorhinolaryngology (four; 13.33% each) [Figure 1]. Amongst surgical wards, the highest isolation rate of Mycobacterium tuberculosis was from purulent aspirate of cervical lymph nodes i.e., eight (44.44%) out of 18 cases [Figure 2].

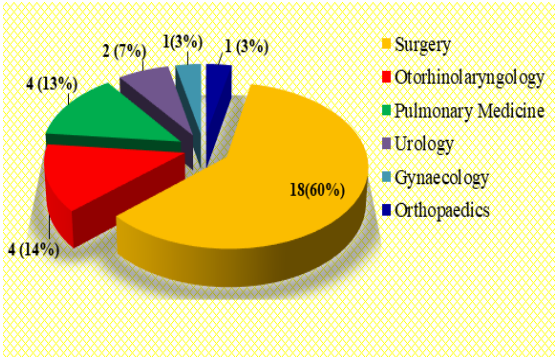

Figure 1: Distribution Of Culture Positive Samples Among Various Departments

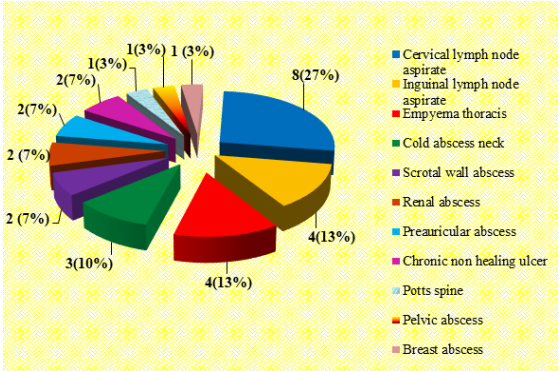

Figure 2: Site Distribution Of Culture Positive Samples

Drug susceptibility testing (DST) was set up for the 30 culture positive samples of which two showed repeated contamination hence had to be discarded. Resistance was observed to all four anti tubercular drugs namely Streptomycin, Isoniazid (INH), Rifampicin and Ethambutol among four strains (14.28%). One (3.57%) strain was resistant to Streptomycin, INH and Rifampicin; one (3.57%) strain was resistant to INH and Rifampicin and one (3.57%) was mono-resistant to Rifampicin [Figure 3]. The total MDR-TB cases isolated in the study were six (21.42%). Remaining 21 strains were sensitive to all the four anti tubercular drugs.

Out of the six MDR – TB cases, four (66.67%) were old treated cases of tuberculosis whereas two (33.33%) were newly diagnosed. The highest isolation of MDR TB strain was from cervical lymph node aspirate sample followed by empyema thoracis.

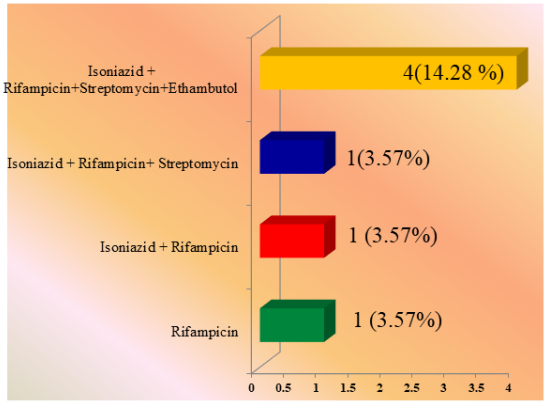

Figure 3: Drug Resistant Pattern Of Anti Tubercular Drugs Among Culture Positive Isolates

DISCUSSION

Our study observed a high rate of isolation of multi-drug resistant strains from extra-pulmonary samples at a tertiary care hospital in Goa. The overall isolation rate of Mycobacterium tuberculosis strain from 202 clinically suspected extra-pulmonary samples was 14.85% (30/202). A similar study conducted by Wankhade et al, showed isolation rate of 13.33% (16/120) from purulent discharge samples collected from extra-pulmonary sites.[15] Other studies conducted by Mahadev et al,[14] and Bhat et al,[17] in India reported an isolation rate of 23.5% and 21.3% respectively which were consistent with our study. Similar results have been reported by Husain et al (2004), carried out in Bangladesh.[18] Guraung et al have reported an isolation rate of 10.5% from 513 extra-pulmonary TB samples in Nepal.[19]

We obtained a maximum number of culture positive isolates from cervical lymph node aspirates i.e. eight out of 30 (26.67%). These findings are concordant with studies conducted by Bhat et al (2003) and Maurya et al, (2012), each showing positivity rate of 35.6 %.[17,20]

In the present study, 17 out of 30 culture positive cases were positive by culture alone and were smear negative. The incongruency between the smear positivity rate and the culture isolation rate can be attributed to the sample being paucibacillary due to the presence of very few numbers of acid fast bacilli, especially in an extrapulmonary tubercular sample. Further, it stands to reason that isolation by culture is more sensitive than smear detection. Hence a definitive diagnosis solely depends upon the gold standard method of isolation of Mycobacterium tuberculosis by solid culture on Lowenstein Jensen medium.[21-23]

Multi-drug resistant tuberculosis is a major public health problem in India and in the world.[21,22] Earlier studies have reported a high rate of MDR TB in EPTB cases, 12.5% from Nepal and 10 % from Delhi, India.[19,23] In the present study the isolation rate of MDR TB cases was seen to be 21.43 % in lymph node and purulent discharge samples alone. The reason for this high rate of MDR TB may be due to selection of highly suspected drug resistant cases for culture and DST by the clinician in our tertiary care hospital.

The high frequency of chronic cases and treatment failure may explain the high resistant rate in previously treated cases. In our study, four patients out of the confirmed six MDR cases had previous history of treatment with antitubercular drugs. This observation is further validated in studies conducted by WHO in the year 2013 (20%),[1] Swasiland national survey (33.8%),[21] WHO European region (48.7%)[2] and 2 studies in China (25.6% and 33.7% respectively).[3,4]

Possible cause of resistance in extra-pulmonary cases can be attributed to inadequate treatment, poor drug supply, poor quality of drugs and non-adherence of patients to the prescribed drug regimens and indiscriminate use of anti-tubercular drugs in private sector.[22-24]

The major limitation of this present study is the small sample size of extra pulmonary samples and therefore does not represent the entire EPTB cases in our hospital. However despite the possible limitations, the current study strongly suggests a high rate of MDR cases in extra pulmonary suspects.

CONCLUSION

EPTB is an important clinical problem in India. Most physicians treat the patients with extra-pulmonary TB based on the clinical symptoms.

Multidrug resistant tuberculosis has emerged as a significant global health concern. There are alarming reports of increasing drug resistance globally which potentially threaten to disrupt the gains achieved in tuberculosis in last decade. MDR

TB is essentially a manmade disease due to inadequate treatment. Many cases can be treated with the right combination and rational use of available anti-tubercular drugs.

Revised National Tuberculosis Control Programme (RNTCP) recommends the same treatment category for extra-pulmonary TB cases as for diagnostic sputum positive pulmonary cases. A comprehensive research plan is required to estimate the disease burden country wide. Health care providers must remain cognisant of emerging issues such as increase in multi-drug resistance to anti tubercular drugs. Conventional culture and drug susceptibility testing by solid culture still remains the gold standard of testing and should be employed wherever discordance arises. A need of the hour is a continuous monitoring of drug resistant trends in order to assess the efficacy of current DOTS programme and epidemiological surveillance for proper planning and execution.

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