

Isolation and Identification of Mycobacterium from Extrapulmonary Specimen at NTRL, NIDCH

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Abstract

Objective of the study was to see the frequency of isolation of Mycobacterium among different extrapulmonary specimens. The study was carried out at NTRL (National Tuberculosis reference laboratory), NIDCH Bangladesh during January 2008-June 2009. This study was carried out retrospectively by analyzing NTRL laboratory data. A total of 514 extra-pulmonary specimens from different treatment centre of Dhaka was analyzed. Clinical specimens, such as lymph node aspirate, pleural fluid, urine, stool, gastric lavage, pus, ascitic fluid, cerebrospinal fluids, etc was collected. Lowenstein-Jensen media was used for culture and antimicrobial susceptibility testing. Mycobacteria were isolated from 113 extra-pulmonary specimens. Male and female ratio was almost equal among positive cases. The commonest source of isolation was lymph nodes (frequency 55.8%) and lymph node aspirate (frequency 68.4%) pleural fluid (frequency 10.6%). Anti-microbial susceptibility of the isolates to the four first line anti-tuberculosis drugs, rifampicin, isoniazid, streptomycin and ethambutol was tested, susceptibility rate was 100%. The results suggest that, emphasis should be placed on laboratory diagnosis and treatment of extra-pulmonary tuberculosis.

Keyword: Pulmonary tuberculosis, extrapulmonary, Microbial Sensitivity Test, Laboratory Technics and Procedures, Bangladesh

Introduction

Tuberculosis (TB) continues to be a major health concern, especially in the developing world with more than 8 million new cases each year.¹ This recent increase in TB was attributed to HIV epidemic, homelessness, war, immigration, poor socioeconomic situation, and the weak function of health systems in detection and treatment of TB cases.² While pulmonary tuberculosis is the most common presentation, extrapulmonary tuberculosis (EPTB) is also an important clinical problem.³ The term EPTB has been used to describe isolated occurrence of tuberculosis at body sites other than the lung.⁷ Tuberculosis has the potential to infect any organ in the body due to dissemination via lymphohaematogenous route in the early period of the pulmonary infection and a significant number of tuberculosis cases occur in extra-pulmonary sites especially among the immunocompromised.^{3,4,5}

EPTB constitutes about 15 to 20 per cent of all cases of tuberculosis in immuno competent patients and accounts for more than 50 per cent of the cases in HIV-positive

individuals.³ The most common site of extrapulmonary tuberculosis is lymph node affecting mostly the cervical group of lymph nodes. Other forms of extrapulmonary tuberculosis are- tuberculous pleural effusion, tuberculous pericardial effusion, intestinal, genitourinary, meningeal, bone, skin, and breast tuberculosis.³ The diagnosis of extrapulmonary tuberculosis poses a special challenge as it is often missed or misdiagnosed due to its atypical presentations. The first step in its diagnosis is its awareness and a high index of suspicion by the physicians.^{5,6} Even then, it is difficult to isolate Mycobacterium tuberculosis due the small number of organisms present at these sites.³ Various international studies in the world have focused on the problem of extra-pulmonary tuberculosis, reporting a high frequency.^{3,4,7-10} Bangladesh is a high TB burden area with 300,000 new cases and 45000 deaths per year. However, to best of our knowledge, no bacteriological data regarding EPTB has been published in Bangladesh. The aim of the study was to see the frequency of isolation of Mycobacterium among different extra pulmonary specimens.

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Materials and Method

National Tuberculosis Reference Laboratory (NTRL), NIDCH is the premiere referral center in national level for the diagnosis of tuberculosis. This retrospective study was conducted by analyzing the data of the extra pulmonary specimens sent for detection of tuberculosis at NTRL during the period January to June 2009. Records showed that a total of 514 specimens were analyzed during the said period which included lymph node aspirate, pleural fluid, urine, stool, gastric lavage, pus, ascetic fluid, cerebrospinal fluid, synovial and other body fluids. After appropriate preparation the material were cultured in Lowenstein Jensen (L-J) medium and sensitivity was tested for four 'first line' anti tubercular drugs which included Rifampicin, Isoniazid, Ethambutol and Streptomycin.

Results:

Out of the 514 cases analyzed 299 were from male and 188 from female patients while no gender related data were available for 27 specimens. 62 Lymph nodes were sent for analysis and was the highest number among the tissue samples out of which 38 turned out to be positive for EPTB, while among the body fluids pleural fluid was the highest number of specimen with 132 samples were sent for analysis but only 14 turned out to be positive. The rest of the break down figures is shown in Tables I-IV. Out of the 514 samples positive yield was found in 113 samples (22%) of which 111 were Mycobacterium tuberculosis and 2 were atypical mycobacteria. The drug sensitivity report showed 100% sensitivity to all the four drugs in all 111 specimens.

Table-I
Sample Type

Type	No	Positive
Lymph node	62	38
FNA Lymph node	57	39
Pus	75	4
Other Tissue	24	1
GastricLavage Fluid	6	2
Ascitic fluid	3	0
Pleural fluid	132	14
CSF	3	0
Synovial fluid	4	0
Urine	34	3
Stool	2	0
Brochoalveolar Lavage	112	12
Total	514	113

Table-II
Result of samples

Result	Number	Percentage (%)
Positive	113	22.00
Negative	401	78.00
Total	514	

Table-III
Identity of the isolates

Type	No
Mycobacterium tuberculosis	111
Atypical Mycobacterium	02

Table-IV
Drug susceptibility (n=111)

Drugs	No Susceptibility	%
Streptomycin	111	100
Isoniazid	111	100
Rifampicin	111	100
Ethambutol	111	100

Discussion:

Clinical information is often inconclusive for making a diagnosis of EPT. Laboratory aid is commonly required to come to a conclusion. But question remained regarding the out come of these laboratory investigations. The gold standard is culture or direct visualization of mycobacteria. The major problem is the very low yield of mycobacteria in extrapulmonary samples resulting in a low sensitivity of Acid Fast Bacilli (AFB) smear and culture.

Direct visualization through Ziel Neelsen (ZN) stain is commonly missed in single or paucibacillary specimen due to the very few number of AFB. A definitive diagnosis therefore remains solely in the hand of a culture growth.¹¹

Direct visualization of AFB or cytological examination is not a full proof method. Nataraz et al in their study on 250 patients correlated between cytological and smear positivity with culture. They had found that culture yielded a significantly higher diagnostic confirmation.¹²

Out of the 514 samples analyzed for EPT 113 (21.98%) were positive. Mahdev et al and Butt et al in India found 23.5% and 21.3% isolates respectively in their samples which were similar to this study. Forsshohm et al showed an almost double figure of isolates in his study of 5675.¹³⁻¹⁵

Out of the 113 confirmed EPT cases 60 (53.1%) were male and 53 (46.9%) were female, showing an almost equal rate of involvement among the sexes. Similar results have been observed before by Hussain et al in their study in Rajshahi Medical College Hospital.¹⁶

The sample most commonly sent for analysis and diagnosis of EPT was pleural fluid. Out of the 132 samples sent only 14 was positive, indicating a very low yield of organism from this specimen. The reason might be that most of the cases of pleural effusion in suspected patients were either reactionary or an alternated diagnosis is behind it.

Lymphnode biopsy or aspirate showed the highest positive yield in culture, 38 out of 62 and 39 out of 57 samples respectively. Previous studies have shown variable results according to specimen. Butt et al found pus to have the highest culture positivity while Forssshohm et al and Rijalal et al found urine the most common specimen. Tubercular lymphadenopathy (TBL) has been found as the most common form of EPT in Bangladesh in previous studies. Husain et al in their study on 2582 patients found TBL as the most common form of EPT followed by pleural involvement cases. The result is consistent with the findings in this study.¹⁴⁻¹⁷

One of the most talked about issues now a day is the finding of Multi Drug Resistant (MDR) tuberculosis and its prevalence in our country. In this study sensitivity of all the four first line drugs were tested for and no resistance were found. Previous studies abroad have shown high resistance for this 1st line drugs. This might give us some encouragement for treatment in the future and a hope for better tuberculosis control in Bangladesh.

Conclusion

Finally it can be said that the most common form of EPT in Bangladesh is TBL while the most common samples sent for analysis are pleural fluids. The drugs currently used as first line of treatment are well in action giving a glimpse of a bright future in the field of TB control.

Conflict of interest: None

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