PATTERN OF DRUG RESISTANCE AMONG PATIENTS WITH TUBERCULOUS PLEURAL EFFUSION IN TB CULTURE & DRUG SUSCEPTIBILITY LABORATORY, JAMNAGAR

Comment [c1]: modify or re-write the topic

ABSTRACT

Background: Tuberculous pleural effusion (TPE) is among the most common forms of extra-pulmonary tuberculosis. India is such country which is classified as the highest multi drug resistance burden overall. There is limited information available regarding the drug resistance patterns in TPE, especially from high burden countries. This can be due to difficulty in obtaining specimens and limited facilities for drug susceptibility testing.
Aims and Objectives: This study was aimed to determine the prevalence of tuberculous pleural effusion and detection of drug resistant pattern associated with it.
Materials and Methods: Retrospective study for 469 Pleural effusion samples from suspected patients from January 2020 to December 2021 at the TB Culture & Drug Susceptibility Laboratory attached to a tertiary care center, Jamnagar was done. Samples processed for CBNAAT followed by liquid culture. Positive culture isolates were checked for drug resistance in First Line-Line Probe Assay (for Rifampicin and Isoniazid) and Second Line- Line Probe assay (for Flouroquinolones and second line injectable drugs Amikacin, Capreomycin, Kanamycin).
Results: Culture positivity rate was seen 7.03%. Among these, Rifampicin resistance was 9.09 % and isoniazid resistance was 12.1%. Resistance to Fluoroquinolones was 33.33% and no resistance seen to second line injectable drugs. No MDR-TB or XDR-TB detected in present study.
Conclusion: In our present study we have found that there is an increase trend of resistance to Anti-TB drugs which needs atmost attention and necessary steps have to be taken in early diagnosis and in administration of drug therapy among the patients in whom pleural TB is being suspected. For this Novel molecular techniques can help in early diagnosis and treatment to prevent disease progression and amplification of resistance.

Comment [c2]: Retrospective study was conducted on....

Keywords:
Drug resistant, Fluoroquinolones, Line-probe Assay, TPE (Tuberculous pleural effusion)

1. INTRODUCTION

Worldwide, an estimated 9.9 million people fell ill with TB in 2020, equivalent to 127 cases per 1,00,000 population[(1)]. TB can affect anyone, regardless of any age or sex. The highest burden of TB is in adult men, who accounted for 56% of all TB cases in 2020; by comparison, adult women accounted for 33% and children for 11%[(1).]

India accounts for 27 % of the 10 million TB cases globally which the highest in the world and thrice the share of TB cases in China, the country with second most TB cases (9%) [1]. As per the Global TB Report 2021, the estimated incidence of all types of TB in India for the year 2020 was 188 per 100,000 population. In India, the whole number of incident TB patients (new and relapse) notified during 2021 were 19,33,381 as opposition to that of 16,28,161 in 2020[2].

WHO uses five categories to classify cases of drug-resistant TB: isoniazid (INH)-resistant TB, (Rifampicin) RR-TB and MDR-TB plus pre-extensively drug-resistant TB (pre-XDR-TB) and XDR-TB. Pre-XDR-TB is resistance to rifampicin and any fluoroquinolone (a class of second-line anti-TB drug). XDR-TB is resistance to rifampicin, plus any fluoroquinolone, plus at least one of the drugs Bedaquiline and linezolid[1].

The estimated number of MDR and XDR-TB cases to possess been placed on treatment as per the global TB report 2021 was 4 per 100,000 and 1 per 100,000 population, respectively [2]. Drug resistance detection requires bacteriological confirmation of TB and testing for drug resistance using rapid molecular tests, culture methods or sequencing technologies.

Of the 1.5 million cases reported to the Revised National Tuberculosis Control Program (RNTCP), 10-15 per cent are extra pulmonary tuberculosis (EPTB), mostly TB lymphadenitis and pleural effusion [3]. EPTB generally constitutes 15-20 per cent of all cases of tuberculosis among immune-competent individuals and up to 50 percent in HIV-infected patients [3]. TB can involve any site and is classified as pulmonary and extra pulmonary TB (EP□TB). EP□TB is often a diagnostic and therapeutic dilemma. Similarly, EP DR□TB consists of a vast clinical spectrum and an intimidating challenge with scarce literature [4]. Depending on the site of disease, specimens is also difficult to get and also the lesions are usually pauci bacillary; hence bacteriological confirmation is the exception instead of the rule[3]. Tuberculous pleural effusion might be a manifestation of extra-pulmonary tuberculosis (TB) caused by delayed-type hypersensitivity to *Mycobacterium tuberculosis* antigens most commonly entering the pleural space after rupture of sub-pleural caseous foci within the lung [5]. Although it should resolve spontaneously (i.e., without treatment), the treatment of TPE is extremely important, as approximately two thirds of untreated cases later develop pulmonary TB [5].In countries with a high prevalence of TB, TPE is one of the foremost common causes of pleural effusion. Pleural TB is rare in children 2–12 years old and is usually found in adolescents 12–16 years old and adults .The typical clinical features are fever (in about 86% of cases), chest pain, cough, and dyspnea, and it is sometimes associated with loss of appetite, malaise, and weight loss[6]. The gold standard (100% specificity) for its diagnosis is that the isolation of *Mycobacterium tuberculosis* from pleural fluid (up to 35% sensitivity) or pleural biopsies (up to 76%sensitivity) [5]. However, omitting pleural biopsy ends up in significantly reduced isolation rates, thus increasing the amount of patients treated without drug susceptibility testing (DST) [5]. Given the worldwide increase in *Mycobacterium tuberculosis* drug resistance, this management strategy may cause treatment failure and contribute to the event of multidrug-resistant TB (MDR-TB) strains [5].

There's limited information within the literature regarding prevalence of drug resistance in EPTB especially from high burden settings like India [3]. The reasons for this include the problem in obtaining diagnostic specimens and therefore the limited number of laboratories within the country having the facility to perform culture and drug susceptibility testing (DST) for *Mycobacterium tuberculosis* from extra-pulmonary specimens. Clinical symptoms are foremost prognostic indicators in the case of EPTB and physicians suspect drug resistance only after failure or non-response to first line therapy [3].

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2. MATERIALS AND METHODS

In this retrospective study, a total of 469 suspected Tuberculous pleural effusion samples of all age group enrolled from January 2020 to December 2021 at the TB Culture & Drug Susceptibility Laboratory attached to a tertiary care center, Jamnagar were included. Samples were collected

from the patients in each peripheral designated microscopy centers (DMC) and transported in cold-chain to this laboratory. To accomplish the aims & objectives suspected samples were processed for isolation of *Mycobacterium tuberculosis* and drug resistance pattern. Samples were processed by N-acetyl-L-cysteine-Sodium hydroxide (NALC-NaOH) method of decontamination. All 469 samples proceeded for culture, among 469 samples CBNAAT was done for 290 samples. Culture positive isolates were proceeded for detection of resistance pattern to Rifampicin and Isoniazid to first line-line probe assay (FL-LPA). All samples except SS (Rifampicin and Isoniazid both sensitive) pattern in FL-LPA, were further proceeded for second line-line probe assay (SL-LPA) for detection of resistance pattern to fluoroquinolone and second line injectable drugs Kanamycin, Amikacin, Capreomycin. The test was performed as per manufacturer's guidelines.

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3. RESULTS

- A total of 469 pleural effusion samples were received between January 2020 to December 2021.
- Out of 469 samples 33 samples were culture positive for *Mycobacterium tuberculosis.* These consisted 7.03% of the total cases **(figure 1).** Adults (18 years and above) accounted to 31 (93.9%) and children consisted of 2 (6.06%) of total culture positive patients. The adult patients consisted of 25 (75.7%) men and 6 (18.1%) women. The children were 2 (6.06%) girls and 00 boys **(figure 2)**.

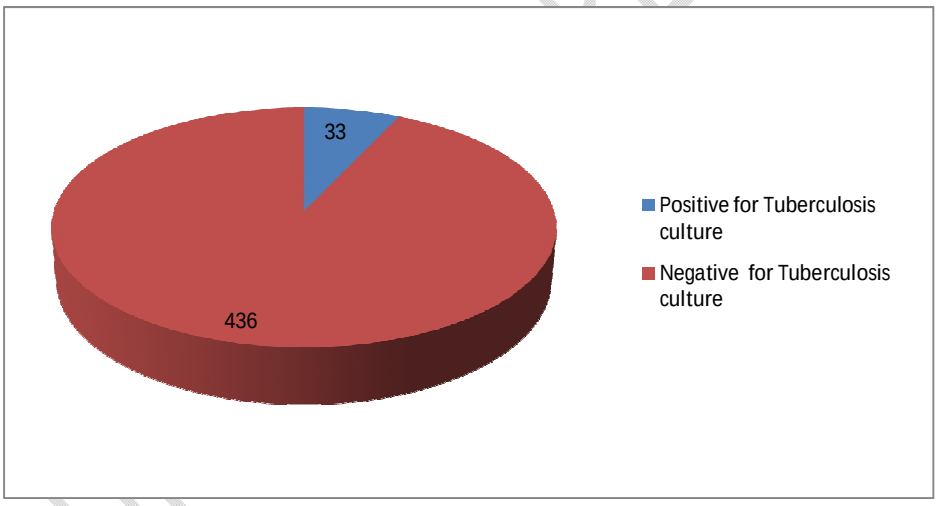

Figure 1: Positivity rate in Tuberculous pleural effusion suspected patients pleural effusion samples (n=439)

Comment [c5]: Culture positivity

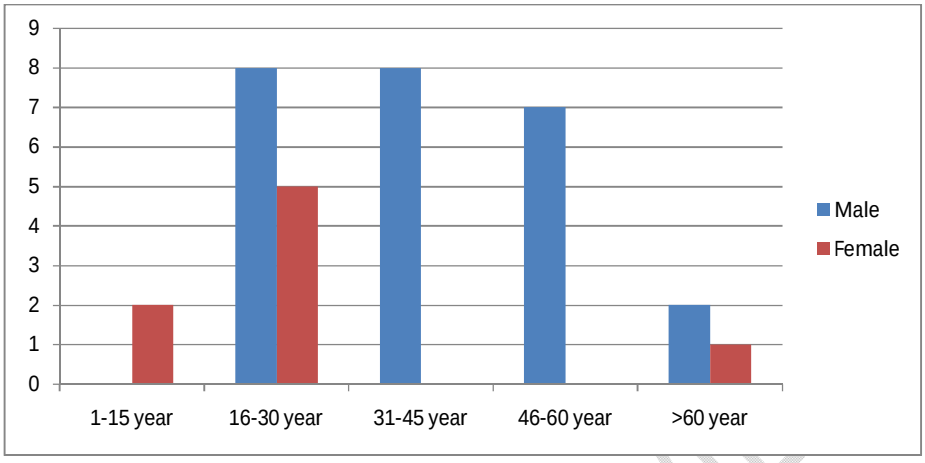

Figure 2 : Demographic distribution of culture positive pleural effusion patients (The figures represent number)

- All culture positive cases were subjected to first line-Line Probe Assay. In FL-LPA 26 (78.7%) patients had SS (Rifampicin and Isoniazid both sensitive) pattern, 03 (9.09%) patients had RS (Rifampicin resistant and Isoniazid sensitive) pattern, 04 (12.1%) patients had SR pattern (Rifampicin sensitive and Isoniazid resistant) and 00 patients had RR (Rifampicin and Isoniazid both resistant) pattern **(Table 1)**.

Table 1: Results of 1st Line Probe Assay in culture positive patients

Total samples tested	H & R Sensitive	H mono Resistance	R mono Resistance	MDR TB (H & R Resistance)
33	26 (78.7%)	4 (12.1%)	03 (9.09%)	00

H: Isoniazid, R: Rifampicin, MDR: Multi drug resistance

- All samples except SS pattern were subjected to second line-Line Probe Assay. In SL-LPA 2 (6.06%) patients were resistant to Fluroquinolones but sensitive to other injectable second‐line drugs and 00 patients were resistant to both Fluoroquinolones and injectable second‐line drugs **(Table 2)**.

Table 2: Results of 2nd Line Probe Assay

Total samples tested	FQ & SLI Sensitive	FQ Resistance	SLI Resistance	XDR TB (FQ & SLI Resistance)
7	5 (15.1%)	2 (6.06%)	0	0

FQ : Flouoroquinolones, SLID: Second line injectable drugs, XDR: Extensively drug resistance

- Among the 469 received samples 290 samples were also proceeded for CBNAAT. In CBNAAT testing out of 290 samples, 44 (15.1%) were found positive for tuberculosis infection. Among 44 positive samples 4 (9.09%) were resistant to first line Anti-TB drug Rifampicin and 40 (90.9%) were found sensitive to Rifampicin **(Table 3)**.

Table 3: Results of CBNAAT

CBNAAT	No. of tests conducted	MTB detected by CBNAAT	Rifampicin Resistant	Culture positive among MTB Detected
	290	44 (15.1%)	4 (9.09%)	20 (6.89%)

CBNAAT: Cartridge based nucleic acid amplification test

DISCUSSION:

This study aims at detecting the prevalence of tuberculous pleural effusion and drug resistance pattern associated with it. With a population of more than 1 billion, in India this has significant implications, due to the high potential proportion of resistance among individuals suspected of getting TB and MDR-TB.

In India, EPTB accounts for 10-15 per cent of all TB, mostly TB lymphadenitis and pleural effusion[3]. Patients with extra-pulmonary manifestations need specialized investigations and thus the diagnosis is based on clinical, radiographic or histopathological findings, rather than bacteriologic evidence. Therefore, there are only a few information available on drug susceptibility patterns of EPTB as these patients are usually not included in DR surveys, which focus mainly on pulmonary TB[3].

Drug resistance is the main cause of treatment failure; which can not only leads to premature death among TB patients but also leads to amplification of resistance within the community [17]. The isolation of *Mycobacterium tuberculosis* isn't easy in TPE. It is therefore not surprising that it has become common practice to diagnose TPE by combination of epidemiological and clinical features with pleural fluid ADA levels, and to administer a standard regimen in all patients[5]. This was the most probable reason for the gradual decline in numbers of culture-positive pleural TB cases in our study. This strategy, although attractive for its convenience, is inherently associated with a suboptimal rate of *Mycobacterium tuberculosis* isolation, as the isolation of mycobacterial strains is based exclusively on pleural fluid culture if biopsy is not obtained. The question then

arises as to how prudent it is to omit pleural biopsies, which maximize the yield of pleural M. tuberculosis isolation, in a setting of increasing drug resistance [5].

In present study data shows that prevalence of Tuberculous pleural effusion is 7.03%. Various studies have reported culture positivity rates varying from 29.7%[10] , 20.7%[14] , 21.4%[15]. Present study shows the prevalence of resistance to INH is 12.1% and 9.09% to RMP,V. Anastasakos mentioned 6% and 0% and S. Tahseen mentioned 9.4% and 4.7% . No MDR-TB and XDR-TB were found in present study while V. Anastasakos mentioned 3% and 1% and S. Tahseen mentioned MDR-TB is 3.1 (**Table 4**).

Comment [c6]: give possible reasons for low prevalence (compared to your result) compared to other findings.

Comment [c7]: give possible reasons for high prevalence

Comment [c8]: You mentioned studies done by V. Anastasakos and S. Tahseen only. You need to compare your results with more studies done worldwide.

Table 4 : Comparison of resistance pattern in different studies

Resistance pattern	V. Anastasakos (2017) [5]	S. Tahseen (2019)[10]	Present study
Rifampicin Resistance	0 %	4.7 %	9.09 %
Isoniazid Resistance	6 %	9.4 %	12.1 %
FQ Resistance	-	7.8 %	33.3 %
SLID Resistance	-	0 %	0 %
MDR TB	3 %	3.1 %	0 %
XDR TB	1 %	-	0 %

FQ: Flouroquinolones, SLID : Second line injectable drugs, MDR: Multi drug resistant, XDR: Extensively drug resistance

Despite the advances in diagnosis and management of pulmonary TB (PTB), EPTB remains largely neglected, with significant delays in diagnosis and an absence of evidence-based treatment strategies. Three main factors contribute to the paucity of enormous studies: the pauci-bacillary nature of EPTB; technical difficulties because of the location of TB (which causes inaccuracies in obtaining biopsy specimens); and lack of standard operating procedures for optimal sample collection, processing and testing methods. Empirical treatment often ends up in inevitable disease progression, disabling sequelae, avoidable drug toxicities and poor clinical outcomes in EPTB[13].

In clinical practice, an inadequately administered treatment allows drug resistant mutants to become the dormant strain. Recommended treatment regimens for MDR-TB may therefore require revaluation for better treatment outcomes and to curtail development of further drug-resistance. Such alarming data result is mandatory for creating awareness and better therapeutic regimens.

Trainings should be conducted on NTEP guidelines for TB case detection and treatment as well as on more judicious use of antibiotics for treatment of common infections, with a special emphasis on restricting the use of over the counter drugs. Medical associations, including those represents infectious disease specialists, pulmonologists as well as pharmacists need to play a more active role in communicating this message to providers and to the public.

CONCLUSION:

In present study we have found that there is an increase trend of resistance to Anti-TB drugs which needs atmost attention and necessary steps have to be taken in early diagnosis and in the administration of drug therapy among the patients in whom pleural TB is being suspected. Irrational use of antibiotics by the physicians, non-compliance or self-medication by patients may be some of the explanations for the high rates of drug resistance. Nowadays rapid DST methods are available and it is important that all TB laboratories are equipped to process TPE specimens which will enable these patients to access MDR-TB treatment, if required. For this Novel molecular techniques can help in early diagnosis and treatment to prevent disease progression and amplification of resistance.

ETHICAL APPROVAL:

Study got approval from Ethical Institution Committee of Shree M. P. Shah Government Medical college & Guru Gobind singh Hospital, Jamnagar

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