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Research article

Detection of pulmonary tuberculosis and rifampicin resistant tuberculosis from sputum samples in a tertiary care hospital, Delhi & NCR, India

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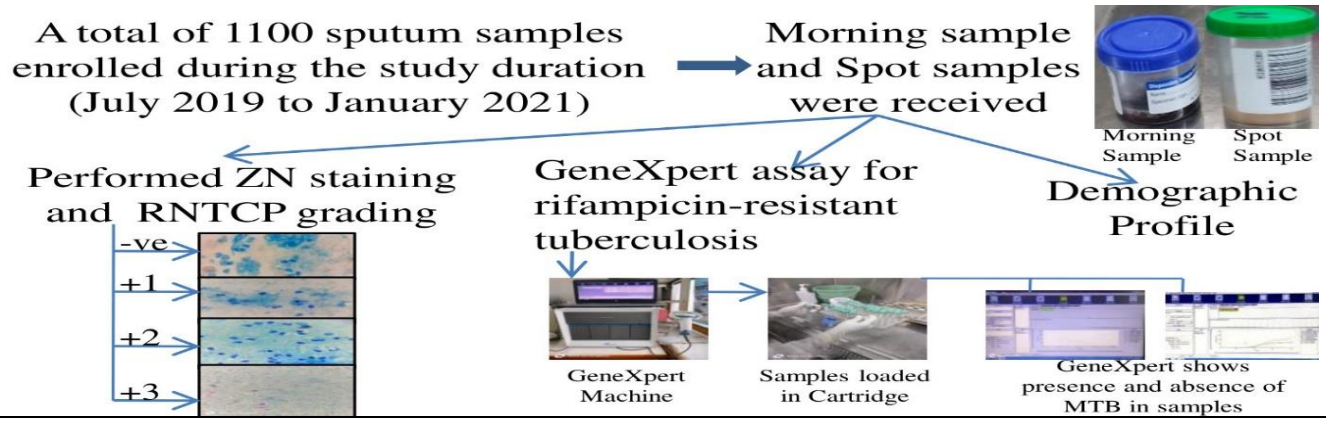
ABSTRACT

This study examines pulmonary TB diagnosis and Rifampicin resistance in sputum samples from a Delhi tertiary hospital. Findings indicate a 3.08% TB prevalence, significant gender differences, and morning specimens' diagnostic superiority.



Keywords: Latent TB, *Mycobacterium*, Rifampicin, Multi-drug Resistant, Pulmonary Tuberculosis.

INTRODUCTION

Mycobacterium tuberculosis (TB) ranks second only to the Human Immunodeficiency Virus (HIV) in terms of infectious disease-related deaths, and it also plays a significant role in the development of antibiotic resistance worldwide [1]. The *Mycobacterium tuberculosis* complex is responsible for transmitting it, making it one of the oldest diseases known to affect humans and a major cause of death on a global scale [2]. *Mycobacterium tuberculosis* is a thin, aerobic bacterium that measures 0.5 x 3 m, is rod-shaped, and is non-spore-forming in nature. Acid-fast microorganisms are known as mycobacteria. Acid fastness is primarily caused by the organisms' high mycolic acid concentration, cross-linked long-chain fatty acids and cell-wall lipids [3]. Coughing, sneezing, speaking, or singing when suffering from TB disease of the lungs or throat causes the discharge of TB germs into the air. These viruses could persist in the air for a number of hours, depending on the surrounding environment [4]. Those who breathe in air contaminated with these TB bacteria may get a latent TB infection. Depending on the part of the body where the TB bacteria are developing, different people will have different TB illness symptoms. Pulmonary TB is a condition where the TB bacteria typically proliferate [5]. Sign & symptoms of TB lung disease can include a severe cough that lasts for three weeks or longer, chest pain, and bleeding or mucus in the cough. Other signs of TB illness include weakness or exhaustion, weight loss, loss of appetite, chills, fever, and night-time perspiration [5]. People

with latent TB infections do not feel unwell, show no symptoms, and cannot infect others with the disease.

The burden of mortality caused by tuberculosis is still present worldwide. The burden of mortality caused by tuberculosis is still present worldwide. According to the India TB report 2023, there was a slight decrease in TB notifications in 2020 & 2021; however, NTEP reclaimed and achieved beyond these numbers. India's TB surveillance efforts, 2022 is a landmark year with a record-breaking 24.2 lakh cases reported, up 13% from 2021 [6]. The tuberculosis disease still spreads as a result of the numerous limitations in the diagnostic techniques that are currently accessible. Although it can potentially affect other organs, tuberculosis frequently affects the lungs. Concern should be expressed over the rising incidence of extra pulmonary tuberculosis associated with the HIV pandemic [1]. The inability to collect sputum from severely ill patients and youngsters also slows down a tuberculosis diagnosis. Due to these difficulties, various sample types with quick turnaround times should be investigated. Therefore, it is crucial to examine sputum under a microscope to look for acid-fast bacteria (AFB). Additional complications can be avoided with early detection [4]. The current gold standard for identifying M. tuberculosis is mycobacterial culture, although it is time-consuming and requires extra safety precautions in labs. Although convenient, serological techniques lack sensitivity and specificity [4]. Although the PCR approach is quick, it is too expensive to be used frequently in developing nations, where tuberculosis cases are most common. Due to its efficiency, affordability, and strong positive predictive value for tuberculosis, conventional smear microscopy with the Ziehl-Neelsen (ZN) stain is a useful and quick approach for identifying acid-fast bacilli (AFB), especially in low-income countries [4]. According to the WHO or the Revised National Tuberculosis Control Programme (RNTCP), pulmonary tuberculosis is defined as having at least one sputum smear or culture positive for AFB or tubercle bacilli [1]. A Mycobacterium tuberculosis infection is defined as MDR-TB if it is resistant to both isoniazid (H) and rifampicin (R), with or without additional drug resistance. Rifampicin-resistant tuberculosis (RR-TB) is characterised by rifampicin resistance that has been found by genotypic or phenotypic approaches, with or without RR-TB from other first-line anti-TB medications [7]. The purpose of this study is to diagnose pulmonary tuberculosis and detect Rifampicin-resistant TB from sputum samples in a tertiary care hospital in the Delhi and NCR regions of India.

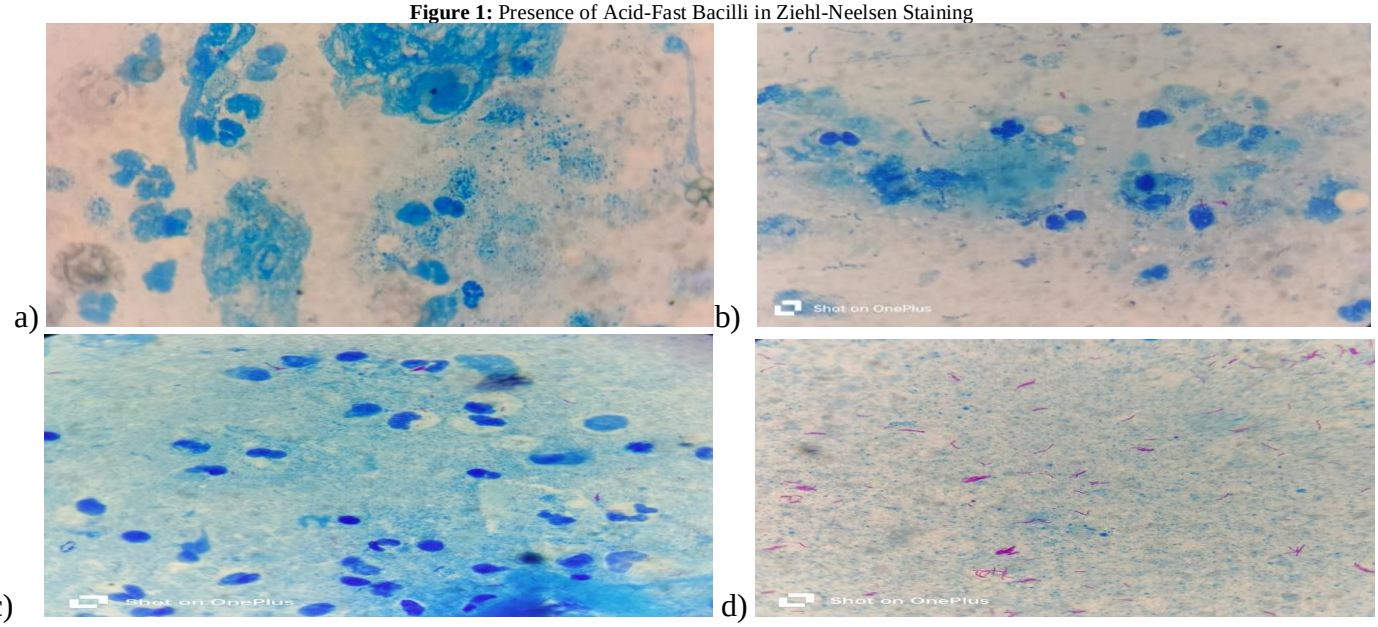
MATERIALS AND METHODS

This study was conducted in a tertiary care facility from July 2019 to January 2021 in clinically suspected MTB inpatients and outpatients. A total of 1100 sputum samples were taken from individuals suspected of having pulmonary TB. Spot and early morning sputum samples were taken in a new and sterile, wide-mouth, leak-proof specimen container after the patients received the proper instructions. A fresh specimen of sputum approx. 5 mL or less is considered a good specimen. The samples were brought to the lab as soon as they could be after being collected. All required safety precautions were taken while processing the samples in a BSC-2. Smears were made, and they were stained using ZN staining [8]. The smears were graded in accordance with RNTCP recommendations [9]. The Gene-Xpert assay was used to detect rifampicin-resistant tuberculosis (RR-TB).

DATA ANALYSIS: The data was entered into a Microsoft Excel spreadsheet, and the social sciences statistical programme SPSS (Version 21) was used to conduct the statistical analysis. The results were presented using mean, SD, counts, t-tests, p-values, and percentages. P-value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Figure 1: Presence of Acid-Fast Bacilli in Ziehl-Neelsen Staining



a) Negative AFB staining; b) +1 AFB; c) +2 AFB; d) +3 AFB

Table 1: Sociodemographic and clinical characteristics of pulmonary tuberculosis patients

Descriptive Analysis							
					95% Confidence Interval		
	Age Group	N	Mean	SE	Lower	Upper	SD
	≤25	254	537	20.9	496	578	332
	26-50	477	547	13.9	520	574	304
	51-75	343	555	17.6	521	590	325
	≥76	26	684	57.2	572	796	292
Sex M/F							
	Female	421	563	15.6	532	593	319
	Male	679	543	12.2	519	567	317
Key Population (1= Contact of TB/DRTB case; 2=; 4= Miner; 9= Others)							
	1	177	925	12.49	900.2	949	166.1
	2	7	250	135.61	-15.6	516	358.8
	4	41	961	10.2	940.8	981	65.3
	9	875	458	9.24	439.9	476	273.4
History of >1 month ATT (Yes/No)							
	No	1026	566	9.86	547	585	316
	yes	74	337	30.77	277	397	265
Visual Appearance (A)							
	Mucopurulent	503	537	14.3	509	565	322
	Saliva	597	562	12.9	537	587	314
Visual Appearance (B)							
	Mucopurulent	609	551	12.9	526	576	318
	Saliva	491	550	14.3	522	578	317
Spot Samples							
	1+	14	283	97.92	91.3	475	366
	2+	17	335	86.59	164.9	504	357
	3+	4	861	92.48	679.2	1042	185
	NEGATIVE	1065	556	9.62	537.4	575	314
Morning Samples							
	1+	12	299	104.65	93.9	504	363
	2+	15	435	96.03	246.5	623	372
	3+	4	861	92.48	679.2	1042	185
	NEGATIVE	1069	554	9.64	534.9	573	315

N= number of samples; SE= Standard error; SD= Standard Deviation; ATT= Anti-tuberculosis therapy

Table 2: ***Mycobacterial findings of tuberculosis samples among gender and sample type***

Results	Spot sample (n=1100)		Morning samples (n=1100)	
	Female	Male	Female	Male
1+	0.71% (3)	1.62 % (11)	0.71 % (03)	1.33 % (09)
2+	1.19 % (5)	1.77 % (12)	1.19 % (05)	1.47 % (10)
3+	0.00 % (0)	0.59 % (04)	0.00 % (00)	0.59 % (04)
Negative	98.10 % (413)	96.02 % (652)	98.10 % (413)	96.61 % (656)
Total	**421**	**679**	**421**	**679**

Table 3: Percentage of pulmonary tuberculosis during Jan 2022 to July 2022 basis of sex and in different age groups.

AGE	Total cases	Male cases	Female cases
≤25	09 (25.71%)	04 (14.81%)	05 (62.5%)
26-50	13 (37.14%)	11 (40.74%)	02 (25%)
51-75	11 (31.42%)	10 (37.03%)	01 (12.5%)
≥76	02 (5.73%)	02 (7.40%)	00 (00)
Total	**35**	**27 (77.15%)**	**08 (22.85%)**

Table 4: Distribution of Rifampicin activity between male and females

Rifampicin DST result	Gender		Mean	SD	t-test	p-value
	Females	Males				
Sensitive(n=1066)	38.75% (413)	61.25% (653)	557	314	3.799	< .001
Resistant (n=34)	20.58% (7)	79.42% (27)	339	351		

DISCUSSION

In India, TB has long been a crucial public health concern. Despite all the improvements in management and treatment, TB continues to be a public health issue in India, with adverse economic and social effects. The emphasis of current tuberculosis control recommendations is on early case discovery to facilitate patient

treatment and hence restrict the spread of the bacteria. The quick and precise identification of those who are affected is the mainstay of its control [10]. AFB is recognised as evidence of an infective stage when it is found. The pulmonary tuberculosis diagnosis depends heavily on the laboratory. Sputum microscopy is by far the quickest, least expensive, and most accurate way to diagnose pulmonary tuberculosis in developing nations [10].

Out of 1100 samples used in this investigation, 35 (3.08%) were positive for ZN smear. By using ZN staining, similar results for pulmonary tuberculosis were obtained by et al. In a different investigation, Ulukanligil *et al*. [11], Suria *et al*. [12], and Surani *et al*. [13] discovered that, in accordance with the results of acid-fast bacilli (AFB) smear staining, 9.89%, 12.4%, and 14.8% of the samples, respectively, were positive. In a study by Brijesh Thakur *et al.*, higher Ziehl-Neelsen smear positives for mycobacteria were identified in 26.67% (24/90) of the samples [14]. The type (spot and early morning sample) and quality of the sputum sample, as well as the presence of tubercle bacilli in the sputum sample, determine the rate of smear positivity. Due to the overnight concentration of the bacilli's release, we came to the conclusion that early morning sputum were more effective than spot sputum samples in this investigation. Surani *et al*. [13, 15] also reached the same conclusion.

Similar to the research conducted by Sharma S, Nagmoti JM, *et a*l., which revealed that the age group most affected was 21–40 years (45.5%) [16], the majority of positive samples in this investigation came from those in the 26–50 age range (37.14%). Another study, by Masali *et al.* [17], discovered a similar finding in their research. They discovered that the age group of 26–45 years was frequently affected by pulmonary TB, which was compatible with studies by Jadhav *et al*. [18], which showed the age group of 21–40 years, and Lawrence *et al*. [19], which showed the age group of 18–30 years as the mostly affected age group. This data confirms the widespread prevalence of tuberculosis in the economy.

According to our analysis, there were significantly more male patients (77.15% [27/35]) than female patients (21.85% [8/35]). Other studies conducted by Masali *et al.* [17] reported majority of prevalence were from male patients (67.2%) compared to female patients (32.8%), Neelu Sree *et al*. [20] reported a high prevalence of 52.3% in males and 47.7% in females, and Makesh Kumar *et al.* [21] revealed a ratio of 59.8% in men and 40.2% in women. The study provides compelling evidence that males are more likely than females to get pulmonary TB. According to the TB Annual Report [20], males are more likely than females to develop tuberculosis, and incidence is substantially higher in older age groups.

Our analysis revealed a 3.09% (34/1100) prevalence pattern of rifampicin resistance. Similar findings were reported by Prasad R., Singh A., *et al.* in their study, which found that all patients were HIV-seronegative and exhibited drug resistance to at least isoniazid and rifampicin (a mean number of 3.02 medications) [22]. 15% of lung cases, according to a second study by Steingart KR et al. were rifampicin-resistant.

Our study underscores the limitations of the conventional culture testing method, which not only requires several days to yield results but also cannot detect rifampicin resistance concurrently. While ZN staining remains a widely used approach for pulmonary tuberculosis detection, its efficacy is hindered by demanding personnel requirements and time-intensive processes, necessitating the adoption of alternative techniques for prompt case identification. GeneXpert emerges as a promising diagnostic tool for suspected pulmonary tuberculosis cases, offering rapid results irrespective of AFB smear status. Its expedited turnaround time makes it particularly valuable in clinical settings where timely diagnosis is crucial. Furthermore, modern molecular techniques like PCR demonstrate superior sensitivity and precision compared to traditional microscopic methods, potentially revolutionizing tuberculosis diagnosis. However, the widespread implementation of molecular approaches faces challenges, notably due to their high cost and lengthy processing times, which may render them inaccessible for resource-limited settings. This underscores the need for continued research to optimize cost-effective strategies for tuberculosis diagnosis, ensuring equitable access to advanced diagnostic technologies worldwide.

CONCLUSION

In conclusion, our study highlights the superiority of morning sputum specimens over spot specimens due to the concentrated release of bacilli overnight. Despite the prolonged processing time for AFB smears hindering timely TB diagnosis, molecular techniques like GeneXpert offer rapid and accurate results. Nonetheless, the cost and limited applicability of these advanced methods pose challenges for broader implementation in patient care.

Conflict of interests Nil

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