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Original Research Article

The diagnostic concordance between micro real-time PCR and Lowenstein Jensen (LJ) media assays for pulmonary tuberculosis detection with associated clinical characteristics

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ABSTRACT

Background: Tuberculosis (TB) is an infectious disease that can affect various parts of the body, with lung infections being the most common cause. In this study, drug susceptibility testing (DST) using LJ media was compared to Truenat testing to detect rifampicin resistance in sputum smear-positive cases with related clinical characteristics.

Materials and Methods: The Vedantaa Institute of Medical Sciences conducted a laboratory-based study from July 2023 to December 2023, enrolling 102 clinical isolates. Sputum smears with acid-fast bacilli were cultured in LJ medium, isolated, and grown with rifampicin for resistance observation and a correlation with Truenat.

Results: The study found that individuals aged 21–30 had the highest prevalence of TB. The highest *Mycobacterium tuberculosis* detection ratio was shown in the Below Poverty Line (BPL) at 84.2%, and Rifampicin (RIF) resistance was detected at 75%. A TB positive ratio of 68.4% were found to be both alcoholic and smoker population and 42.1% were found to have asthma and liver disease, no Rifampicin (RIF) resistance was detected in both the population. In HIV patients 50% RIF resistance was detected. MTB coinfections were observed in 68.4% of pneumonia patients. The LJ culture test had 95% sensitivity and 100% specificity for MTB detection, while the Truenat test had 100% sensitivity and 100% specificity for both MTB testing and MTB/RIF resistance detection.

Conclusion: Demographic, clinical, and social variables, including alcoholics and smokers, asthma and liver disease, hypertension, diabetes, and co-infection with pneumonia, were the main factors for pulmonary tuberculosis patients. Micro-real-time PCR has higher clinical sensitivity for MTB detection, while conventional tests predict rifampicin resistance.

1. Introduction

Tuberculosis is an infectious illness that can affect practically any region of the body but is most commonly associated with lung infections. People with pulmonary tuberculosis frequently spread the disease through coughing or sneezing, which releases bacteria into the air. According to a WHO report released on October 14, 2020, 1.4 million people died from tuberculosis in 2019. In 2019, an estimated ten million people worldwide were diagnosed with TB. TB is one of the top 10 causes of mortality globally, and it is the major infectious agent-related cause of death. Globally, the incidence of tuberculosis is declining at a pace of about 2% per year, with a 9 percent drop between 2015 and 2019. This was less than half-way to the goal of a 20% decrease in tuberculosis between 2015 and 2020 set by the TB plan. The

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United Nations Sustainable Development Goals include the health goal of ending the tuberculosis pandemic by 2030.[1,2]

MTB is a common opportunistic bacterial infection that causes severe morbidity and death in both healthy and immunocompromised people. Those living in the same country who are HIV-positive and infected with TB are 20–40 times more likely to acquire active TB than people who are not infected with HIV.[3,4] MDR-TB, or multidrug-resistant tuberculosis, is still a public health concern and a security danger. In 2019, a total of 0.20163 million patients with multidrug- or rifampicin-resistant tuberculosis were diagnosed and notified worldwide, up 10% from 0.1869 million in 2018. The mechanism of TB resistance is due to a point mutation in the *Mycobacterium tuberculosis* genome that occurs once per 10^8 cell divisions. MTB resistance has evolved as a direct result of evolutionary purging following prolonged drug exposure, typically through missense mutations in drug targets or activating enzymes.[5,6] Drug resistance genes such as inhA, katG, ahpC or isoniazid, rpoB for rifampicin, and pncA for pyrazinamide are responsible for drug resistance in TB. Antitubercular medication resistance might be primary or acquired as a result of ineffective treatment. For active and drug-susceptible TB patients, a 6-month short-course regimen containing a mixture of four anti-TB medications such as rifampicin, isoniazid, pyrazinamide, and ethambutol has been used as a conventional treatment. The most common cause of MDR in nature is rifampicin resistance, which leads to treatment failures and deaths. Rifampicin resistance in MTB is caused by changes in the residues of the drug's molecular target, *Mycobacterium tuberculosis* RNA polymerase (MTB-RNAP). More than 95% of rifampicin-resistant strains had mutations in a short region of MTB-RNAP known as the "rifampicin resistance determining region.[7–9]

The most common mutations in the rifampicin resistance-determining region in *Eschericia coli* are S456, H451, and D441, which correspond to S531, H526, and D516. According to studies, 70% of rifampicin-resistant clinical isolates exhibit point mutations in two residues (S456 and H451),[5] with H451 being most commonly swapped for Asp (D), Tyr (Y), and Arg (R).[10–12] Rifampicin resistance is linked to three mutations at position 451 (H451D, H451Y, and H451R).[13] To identify drug resistance, sputum specimen processing, culture, and drug susceptibility testing (DST) are required.[14] Traditional DST, on the other hand, is time-consuming, and there are significant issues with test consistency and drug stability in various cultural media.[15,16] The late detection of medication resistance could be a crucial factor in MDR-TB transmission. As a result, quick diagnostic assays are essential for efficient drug-resistant tuberculosis control. The World Health Organization (WHO) has approved genotype MTBDRplus LPA (line probe assay) for fast detection of drug-resistant tuberculosis.[17] Resistant tuberculosis is difficult and costly to treat. It includes the use of expensive antibiotics for longer periods of time, which are less effective and more likely to cause side effects than traditional TB chemotherapy.[18,19] A timely diagnosis of treatment resistance in the infectious agent is critical for curing the patient and eliminating the risk of infection spreading throughout a population.[20,21] In the current study, drug susceptibility testing (DST) using LJ media was compared to Truenat testing for the purpose of detecting rifampicin resistance in sputum smear-positive cases with related clinical characteristics.

2. Materials and Methods

2.1. Study setting

A prospective study was conducted in the Department of Microbiology at the Vedantaa Institute of Medical Sciences, Vedantaa Hospital, and Research Center for the duration of July 2023 to December 2023. The sample size was 102 sputum samples. Inclusion criteria: Sputum samples were collected from all patients suspected of having pulmonary tuberculosis due to the *Mycobacterium tuberculosis* complex. Samples were collected from those patients who fulfilled the following: World Health Organization (WHO) criteria were included: cough for 2 weeks or more; chronic fever for more than 2 weeks; night sweats; weight loss (unintentional). Exclusion criteria: extra-pulmonary tuberculosis cases were excluded.

2.2. Data collection

Our research institution was recognized as DMC and Truenat testing under the RNTCP government program in Palghar, Maharashtra. Considering the number of patients with this type of pulmonary tuberculosis, we referred to our hospital from the surrounding tribal population catered to by us, there was a need to undertake such a study.

Patients consent was taken. From the study proforma, basic information such as age, gender, address, socio-economic status, habits, and co-morbidities was collected.

2.3. Technique

Lowenstein Jensen medium was used to culture sputum smears that showed acid fast bacilli on Ziehl Neelsen stain. Smear positive sputum samples were isolated in drug free Lowenstein Jensen (LJ) medium and grown on LJ medium with rifampicin (20 μl/ml) for observation of resistance in the form of visible growth. For correlation, Truenat data were also retrieved from patient records.

2.3.1. Culture

The smear positive sputum samples were processed using the NALC technique for digestion, decontamination, and

concentration before being cultured on LJ media. The cultured media were incubated for up to 4 weeks and examined weekly for growth. Growth was observed in the form of rough, tough and buff-coloured colonies which were subjected to acid fast staining to confirm acid fast bacilli. Then the isolates were subjected to re-culture on LJ medium containing Rifampicin drug for antimicrobial sensitivity testing using the standard strain H37Rv as the negative control. The isolates which were showing growth of ≥1% in rifampicin containing media when compared to drug free media were considered as rifampicin resistant. Finally, the rifampicin resistance was compared with Truenat report.

2.3.2. Truenat (micro real-time PCR)

The Trueprep Auto MTB sample pretreatment pack was used to homogenize and concentrate samples, releasing bacilli and discarding inhibitory substances. The sample was loaded into the Trueprep AUTO Universal Cartridge-Based Sample Prep kit for extraction and purification of bacterial DNA. This lightweight, portable device operates at room temperature, requires minimal hands-on time, and is quick, reliable, and efficient. Waste was contained within the cartridge dump area. The Truenat MTB chip was used in a micro PCR test. The fluorophore releases exponentially upon positive amplification; this phenomenon was detected by the integrated sensor and shown on the analyzer screen. The cycle threshold (Ct) was the number of amplification cycles required for the fluorescent signal to cross the threshold. At the end of the test run, a "detected" or "not detected" result is displayed, and the internal positive control (IPC) validates the test run from sample to result. Samples positive for MTB were tested using the Truenat MTB/RIF micro PCR chip and TruelabTM Real-Time Micro PCR Analyzer.

2.4. Statistically analysis

Data were analyzed online using the MedCalc Statistical Software (https://www.medcalc.org/calc/diagnostic_test.php). In the collected data, the values of total positive (TP), false positive (FP), total negative (TN), and false negative (FN) were determined. Furthermore, the provided data was analyzed to determine the sensitivity and sensitivity values. Additionally, estimated 95% confidence interval (CI) values for the pooled sensitivity and specificity of LJ culture media and Truenat were obtained.

2.5. Ethics Statement

The study was approved by the Ethics Committee of Vedantaa Institute of Medical Sciences, Palghar (approval number: EC/05/2023).

3. Results

During the study period, a total of 102 clinical isolates of M. tuberculosis were enrolled based on inclusion and exclusion criteria in the Microbiology department in the tertiary care hospitals. In our study they were analysed for prospective, cohort study.

3.1. Sample cohort statistics

The Local population was divided into different groups as follows groups, 0-20 years, 21-30 years, 31-40 year, 41-50 years, 11-60 years, 61-70 years and 71-80 years. The maximum number of TB patients were in the age group 21-30 year, followed by group 41-50 years and group Out of the total 102 registered cases, 19 (18.63%) were found to be positive and 83 (81.37%) were negative. The TB-RIF resistance-detected cases were 4 (21.05%) as shown in Figure 2.

3.2. Demographic, clinical and social variables characteristics

Among the total number of patients, 76 (74.5%) belonged to the below-average poverty line (BPL), followed by 22 (21.6%) above the average poverty line (APL), and 4 (3.9%) belonged to the middle class. The highest MTB detection ratio was shown in BPL (16 (84.2%) and RIF resistance detected at 75%, respectively (Figure 3a). Our study revealed that MTB detected 13 (68.4%) and RIF resistance detected 50% of patients who were both alcoholics and smokers (Figure 3b). Co-morbidities were the main factors for pulmonary tuberculosis patients. A high TB positive ratio of 8 (42.1%) showed asthma and liver disease, but RIF resistance detection was not found. Followed by hypertension and diabetes (26.3%).HIV patients showed 2/4 (50%) RIF resistance detected (Figure 3c). The MTB coinfections were noted in the pneumonia patients 13/19 (68.4%), and RIF resistance detection 2/4 (50%) followed oral candidiasis 4/19 (21.1%)(Figure 3-d). The patients were grouped into different categories based on their treatment regimens. 12/19 (63.2%) of the patients with MTB detected were new patients in the intensive phase, followed by 10.5% of the new patients in the continuation phase. 50% of the patients with RIF resistance detected were new patients in the intensive phase (Figure 3e).

3.3. Sensitivity and specificity of MTB and RIF resistance detection by LJ culture and the Truenat method

Considering the Truenat data as a reference, the number of true positive, true negative, false positive, and false negative samples was enumerated as shown in Table 1. Using online statistics analysis software, the assay sensitivity for the LJ culture test was calculated to be 95% (95% CI, 75.13% to

Figure 1: Truenat MTB Test Result Screen;**a:** Not detected; **b:** Detected; **c:** RIF resistance not detected; **d:** RIF resistance detected.

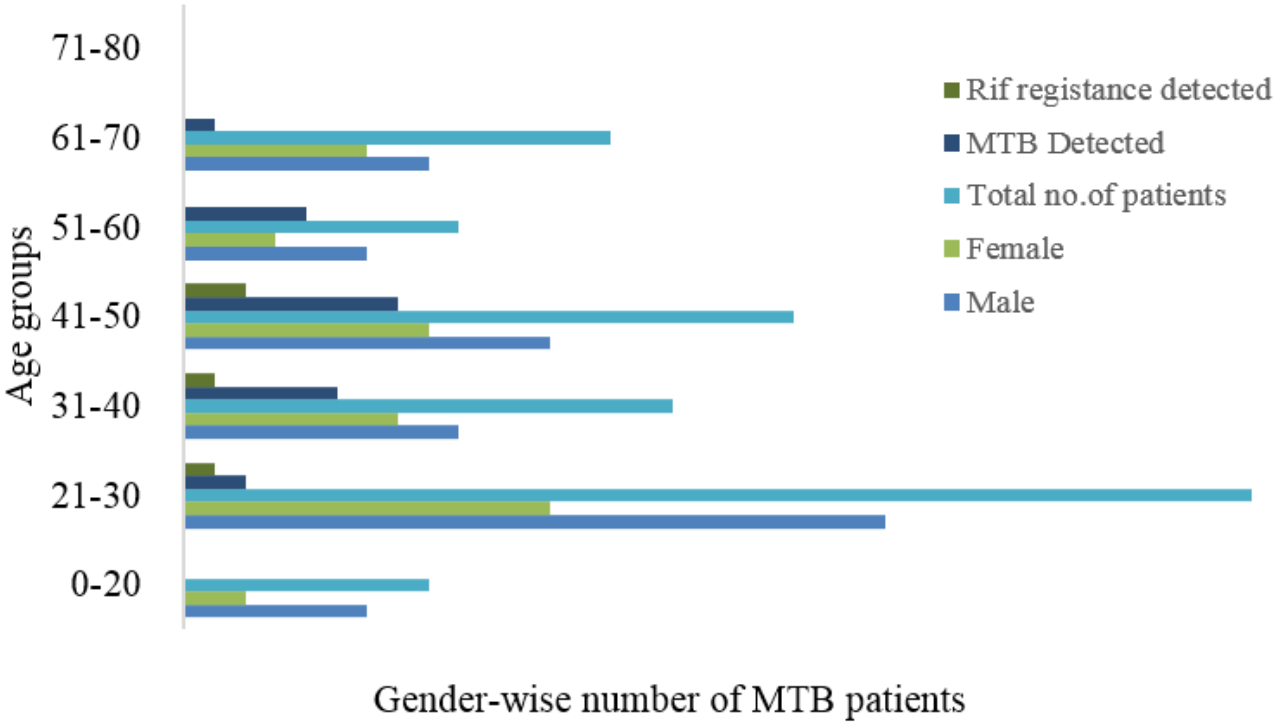

Figure 2: Age and gender wise distribution of TB patients (n = 102)

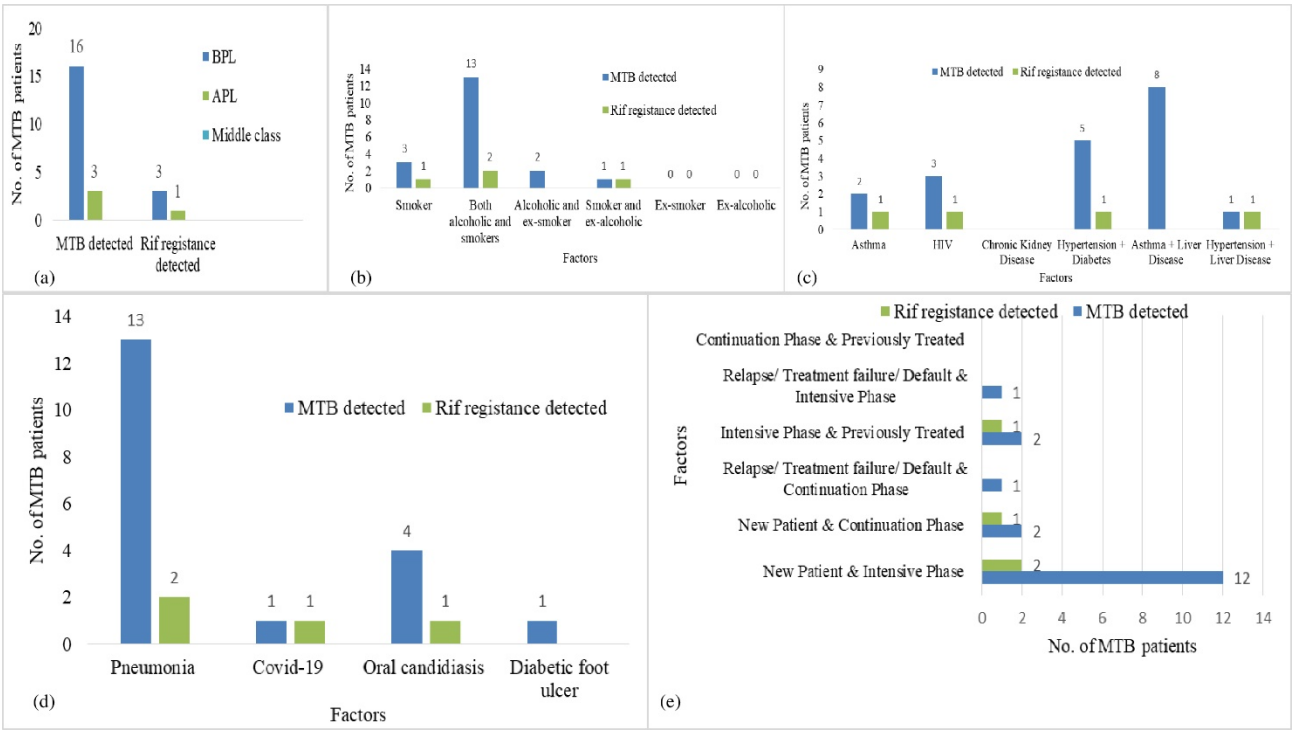

Figure 3: Demographic and clinical characteristics of positive pulmonary tuberculosis andRIF resistance patients (n=102); **a**: Socio-economic status; **b:** Addictive habits; **c:** Co-morbidities; **d:** Co-infection; **e:** Treatment regimen for TB

Table 1: Sensitivity and specificity of the molecular method and the phenotypic method for MTB and RIF resistance detection. (n=102)

	Methods	TP	FN	TN	FP	Clinical sensitivity (95 % CI)	Clinical specificity (95 % CI)
MTB detection	LJ media culture	18	1	83	0	95%(75.13% to 99.87%)	100%(95.65% to 100.00%)
	Truenat	19	0	83	0	100%(82.35% to 100.00%)	100%(95.65% to 100.00%)
MTB/RIF detection	LJ media culture	4	0	15	0	100%(39.76% to 100.00%)	100%(78.20% to 100.00%)
	Truenat	4	0	15	0		

**; TP=True positive; FN=False negative; TN=True negative; FP=False positive

99.87%) with a specificity value of 100% (95% CI, 95.65% to 100.00%) for MTB detection. The assay sensitivity for the LJ culture test was calculated to be 100 (39.76% to 100.00%) with a specificity value of 100% (78.20% to 100.00%) for MTB/RIF detection.

4. Discussion

During our study, 102 clinical isolates of MTB were collected over a period of six months (July 2023 to December 2023). Smear-positive samples were subjected to culture on LJ medium and DST by the proportion method on rifampicin-containing LJ medium. The results were compared with our study.

The age group of 21–30 years old had the highest prevalence of tuberculosis in the current study, whereas the age groups of 0–20 and 60+ years old had the lowest infection rates. Similarly, according to the Xpert MTB/RIF technique, the age group of 21 to 30 years old in Jumla had the highest rate of tuberculosis infection.[22] In Patan Hospital, the oldest patients (32.54%) had the highest rate of tuberculosis, although the youngest patients (5.79%) had the lowest prevalence.[23] In Ethiopia, a higher prevalence of tuberculosis was in the 15–24 age group, followed by 25–34 years.[24] But in Nigeria, the highest prevalence (17%) was in the age group 30-43 years,[25] in Malaysia 21–40 years (37%), and in Pakistan < 20 years (48.08%).[26]

In Satara district, the frequency of pulmonary tuberculosis in children appears to be steadily rising, with men having a nearly twofold higher prevalence than females.[27] The result was similar in the our study. A similar result had also been shown at Lumbini Provincial Hospital.[28] TB was more common in men than in women throughout most of the nation, including Nepal.[29] According to a different survey, men were more common than women in certain districts of Nepal, including Ramechhap, Gorkha, Jumla, and Chitwan.[22,23,29] However,

in Pakistan, women (57.63%) had a higher infection rate than men (42.37%).[26] It was possible because, Males increased exposure to the environment, frequent travel, social contact, and professions associated with higher tuberculosis risk, such as mining, contribute to increased transmission.[30–33]

In our study, we found that 76 patients (74.5%) were below the poverty line, 22 were above the APL, and 3.9% were middle-class, with the highest MTB detection ratio and RIF resistance in BPL. similar to other studies conducted in India, 36 percent of TB patients in Karnataka, India were unemployed, 71 percent belonged to BPL.[34] All patients were studied for their addictive habits; 68.4% were both alcoholics and smokers. In a similar study, drinking and smoking were linked in men, with 38% of control men reporting alcohol and smoking, while less drinking and smoking habits were reported in women (14%; 1,112/8,021).[34,35] Compared to non-smokers and non-alcohol drinkers, smokers and alcohol users had higher odds of PTB [OR 3.2; 95% CI 516.4–1986.4; p = 0.003; OR 3.2; 95% CI 480.8–2254.8; p = 0.009], respectively. Individuals who smoked and consumed alcohol had odds of PTB that were equal to those who did not smoke and drink (OR 4.1; 95% CI 477.6–2581.6; p = 0.001).[36]

In our study, a high TB positive ratio (42.1%) identified asthma, liver disease, hypertension, and diabetes, but no RIF resistance detection was found, followed by HIV patients with 50% RIF resistance. Similarly comorbid conditions, including diabetes mellitus in 64 cases (21.33%), chronic kidney disease (CKD) in 22 instances (7.33%), and co-infection with the human immunodeficiency virus (HIV) in 36 cases (12.00%), were noted. We reported that MTB coinfections were observed in 68.4% of pneumonia patients, with RIF resistance detection in 50% of cases. Similarly, due to the significant global incidence of both diseases, co-infection between *Pneumoccus* and *Mycobacterium tuberculosis* was unavoidable.[37,38] Patients were categorized based on treatment regimens, with 63.2% new MTB patients in the intensive phase, 10.5% in the continuation phase, and 50% in the intensive phase with RIF resistance. Reported by Sotgiu,[39] the 6-month treatment was nearly 100 percent successful; however, following a two-year follow-up period, the relapse rate can range from 0% to 7%. In our study, the LJ culture test has a 95% sensitivity and 100% specificity for MTB detection and 78.20% to 100.00% for MTB/RIF detection. According to Gashaw et al.[40] using LJ media, 384 samples of Mycobacterium smear-positive samples were cultivated; of these, 29.2% (112/384) showed culture-positive results. The MTBDR plus assay and traditional MGIT test demonstrated fair agreement in detecting *Mycobacterium tuberculosis*, with sensitivity, specificity, and positive/negative predictive values of 94.2, 30.2, 68.4, and 76.5%, respectively. The samples that tested positive for LJ culture produced 95 appropriate results for the MTBDR plus assay, and 16/95 (16.8%) showed drug resistance.

5. Conclusion

We found that the demographic, clinical, and social variables included alcoholics and smokers. Co-morbidities were the main factors for pulmonary tuberculosis patients, such as asthma and liver disease, hypertension, and diabetes. The MTB coinfections were noted in the pneumonia patients. The micro-real-time PCR had higher clinical sensitivity than conventional culture methods for MTB detection. The conventional drug susceptibility test had the equal sensitivity and specificity as Truenat in predicting resistance to rifampicin.

6. Conflict of Interest

The authors declare that there is no conflict of interest

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References

1. Global T.B Report; 2019. Available from: https://iris.who.int/bitstream/handle/10665/329368/9789241565714-eng.pdf?sequence=19.
2. Khutade K, Shah H, Yadav D, Patel H, Patil S. Smear Positive Pulmonary Tuberculosis Patients-An Epidemiological Perspective. *Int J Health Sci Res*. 2023;13(9):7–11.
3. World Health Organization. Monograph on integrated monitoring of TB/HIV- a case study in Malawi. Geneva: WHO; 2009. Available from: https://iris.who.int/bitstream/handle/10665/44134/9789241598279_eng.pdf?sequence=1.
4. Olivier C, Luies L. WHO Goals and Beyond: Managing HIV/TB Co-infection in South Africa. *SN Compr Clin Med*. 2023;5(1):251. doi:10.1007/s42399-023-01568-z.
5. Musser JM. Antimicrobial agent resistance in mycobacteria: molecular genetic insights. *Clin Microbiol Rev*. 1995;8(4):496–514.
6. Mancuso G, Midiri A, De Gaetano S, Ponzo E, and CB. Tackling drug-resistant tuberculosis: new challenges from the old pathogen Mycobacterium tuberculosis. *Microorganisms*. 2023;10(9):2277. doi:10.3390/microorganisms11092277.
7. Morlock GP, Plikaytis BB, Crawford JT. Characterization of spontaneous, in vitro-selected, rifampin-resistant mutants of Mycobacterium tuberculosis strain H37Rv. *Antimicrob Agents Chemother*. 2000;44(12):3298–301.
8. Zaw MT, Emran NA, Lin Z. Mutations inside rifampicin-resistance determining region of rpoB gene associated with rifampicin-resistance in Mycobacterium tuberculosis. *J Infect Public Health*. 2018;11(5):605–10.
9. Monama MZ, Olotu F, Bishop Ö. Investigation of Multi-Subunit Mycobacterium tuberculosis DNA-Directed RNA Polymerase and Its Rifampicin Resistant Mutants. *Int J Mol Sci*. 2023;24(4):3313. doi:10.3390/ijms24043313.
10. Etim NG, Mirabeau Y, Olorode A, Nwodo U. Risk Factors of Tuberculosis and Strategies for Prevention and Control. *Int J Innovative Healthcare Res*. 2024;12(1):1–13.

11. Telenti A, Imboden P, Marchesi F, Matter L, Schopfer K, Bodmer T, et al. Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis. *Lancet*. 1993;341(8846):647–50.
12. Caws M, Duy PM, Tho DQ, Lan NT, Hoa DV, Farrar J, et al. Mutations prevalent among rifampin- and isoniazid-resistant Mycobacterium tuberculosis isolates from a hospital in Vietnam. *J Clin Microbiol*. 2006;44(7):2333–7.
13. Zhang Q, An X, Liu H, Wang S, Xiao T, Liu H, et al. Uncovering the resistance mechanism of Mycobacterium tuberculosis to rifampicin due to RNA polymerase H451D/Y/R mutations from computational perspective. *Frontiers in Chemistry*. 2019;7:819. doi:10.3389/fchem.2019.00819.
14. Varma JK, Wiriyakitjar D, Nateniyom S, Anuwatnonthakate A, Monkongdee P, Sumnapan S, et al. Evaluating the potential impact of the new Global Plan to Stop TB: Thailand. *Bull World Health Organ*. 2004;85(8):586–92.
15. Victor TC, Warren R, Butt JL, Jordaan AM, Felix JV, Venter A, et al. Genome and MIC stability in Mycobacterium tuberculosis and indications for continuation of use of isoniazid in multidrug-resistant tuberculosis. *J Med Microbiol*. 1997;46(10):847–57.
16. Chaiyachat P, Kaewseekhao B, Chaiprasert A, Kamolwat P, Nonghanphithak D, Phetcharaburanin J, et al. Metabolomic analysis of Mycobacterium tuberculosis reveals metabolic profiles for identification of drug-resistant tuberculosis. *Sci Rep*. 2023;13(1):8655. doi:10.1038/s41598-023-35882-2.
17. WHO. Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB). Policy statement; 2008.
18. Douglas JG, Mcleod MJ. Pharmacokinetic factors in the modern drug treatment of tuberculosis. *Clin Pharmacokine*. 1999;37(2):127–46.
19. Ohiengbomwan OT, Oguzie J, Eromon P, Kayode AT, Afolabi TS, Komolafe IO, et al. Gene mutation patterns of Mycobacterium tuberculosis complex and associated factors among suspected multidrug-resistant tuberculosis patients in Osun State, South-West, Nigeria. *Sci Afr*. 2023;22:1968. doi:10.1016/j.sciaf.2023.e01968.
20. Naidoo K, Perumal R, Ngema SL, Shunmugam L, Somboro AM. Rapid Diagnosis of Drug-Resistant Tuberculosis-Opportunities and Challenges. *Pathogens*. 2023;13(1):27. doi:10.3390/pathogens13010027.
21. Jureen P, Engstrand L, Eriksson S, Alderborn A, Krabbe M, Hoffner SE, et al. Rapid detection of rifampin resistance in Mycobacterium tuberculosis by Pyrosequencing technology. *J Clin Microbiol*. 2006;44(6):1925–9.
22. Magar BP, Thapa E, Rana KR, Joshi NR, Maharjan B, Thapa N, et al. Prevalence of Tuberculosis by GeneXpert Method in Karnali Academy of Health Sciences Teaching Hospital. *Biomed Sci*. 2020;6(3):56–60.
23. Sah MK, Maharjan K, Aryal P, Jha AK, Jha G, Shrestha SR, et al. Pulmonary tuberculosis at Patan Hospital, Nepal: One year audit. *J Clin Tuberc Other Mycobact Dis*. 2021;22:100207. doi:10.1016/j.jctube.2020.100207.
24. Ali H, Zeynudin A, Mekonnen A, Abera S, Ali S. Smear Posetive Pulmonary Tuberculosis (PTB) Prevalence Amongst Patients at Agaro Teaching Health Center, South West Ethiopia. *Ethiop J Health Sci*. 2012;22(1):71–6.
25. Imam TS, Oyeyi TI. A retrospective study of Pulmonary Tuberculosis (PTB) prevalence amongst patients attending infrectious diseases hospital. *Bayero J Pure Appl Sci*. 2008;1(1):10–5.
26. Khan JA, Irfan M, Zaki A, Beg M, Hussain SF, Rizvi N, et al. Knowledge, attitude and misconceptions regarding tuberculosis in Pakistani patients. *J Pak Med Assoc*. 2006;56(5):211–4.
27. Mohite RV, Mohite VR, Ganganahalli PG, Lale SV. Trend of sputum smear positivity among presumptive tuberculosis cases visiting respiratory disease centre of Western Maharashtra, India. *Asian J Med Sci*. 2015;6(4):30–4.
28. Paudel S, Maharjan M. Prevalence of tuberculosis among PTB suspected patients visiting Lumbini Zonal Hospital. *Asian J Sci Technol*. 2018;9(2):7648–71.
29. Smith I. Gender and tuberculosis in Nepal. *J Nepal Med Assoc*. 2024;24(117):49–58.
30. Tamrakar DK. Prevalence of pulmonary tuberculosis in-relation to economical and socio-behavioural aspects in Ramechhap district of Nepal. Kathmandu, Nepal; 2002.
31. Amgain K. Prevalence of pulmonary tuberculosis in Jutpani VDC Chitwan, Nepal; 2013. Available from: elibrary.tucl.edu.np.
32. Narasimhan P, Wood J, Macintyre CR, Mathai D. Risk factors for tuberculosis. *Pulm Med*. 2013;p. 828939. doi:10.1155/2013/828939.
33. Oni T, Gideon HP, Bangani N, Tsekela R, Seldon R. Smoking, BCG and Employment and the Risk of Tuberculosis Infection in HIV-Infected Persons in South Africa. *PLoS One*. 2012;7(10):47072. doi:10.1371/journal.pone.0047072.
34. Poornima MP, Shruthi MN, Chingale AL, Veena V, Nagaraja SB, Madhukeshwar AK, et al. Cost of tuberculosis care in programmatic settings from Karnataka, India: is it catastrophic for the patients. *Tuberc Res Treat*. 2020;p. 3845694. doi:10.1155/2020/3845694.
35. Patra J, Jha P, Rehm J, Suraweera W. Tobacco smoking, alcohol drinking, diabetes, low body mass index and the risk of self-reported symptoms of active tuberculosis: individual participant data (IPD) meta-analyses of 72,684 individuals in 14 high tuberculosis burden countries. *PLoS One*. 2014;9(5):e96433. doi:10.1371/journal.pone.0096433.
36. Scholze AR, Alves JD, Berra TZ, Ramos AC, Pieri FM, Pillon SC, et al. Tuberculosis among people living on the street and using alcohol, tobacco, and illegal drugs: analysis of territories in extreme vulnerability and trends in Southern Brazil. *Int J Environ Res Public Health*. 2022;19(13):7721. doi:10.3390/ijerph19137721.
37. Patil S, Narwade S, Mirza M. Bronchial wash Gene Xpert MTB/RIF in lower lung field tuberculosis: Sensitive, superior, and rapid in comparison with conventional diagnostic techniques. *J Transl Int Med*. 2017;5(3):174–81.
38. Gweryina RI, Madubueze CE, Bajiya VP, Esla FE. Modeling and analysis of tuberculosis and pneumonia co-infection dynamics with cost-effective strategies. *Results Control Optimization*. 2023;10:100210. doi:/10.1016/j.rico.2023.100210.
39. Sotgiu G, Centis R, D'ambrosio L, Migliori GB. Tuberculosis Treatment and Drug Regimens. *Cold Spring Harb Perspect Med*. 2015;5(5):a017822. doi:10.1101/cshperspect.a017822.
40. Gashaw F, Erko B, Mekonnen Y, Yenew B, Amare M, Gumi B, et al. Phenotypic and genotypic drug sensitivity profiles of Mycobacterium tuberculosis infection and associated factors in northeastern Ethiopia. *BMC Infect Dis*. 2021;21(1):1–2.

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