Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Microbiology

Prevalence of Isoniazid Resistance in *Mycobacterium tuberculosis* in Western Rajasthan

Suneel Bhooshan[1*], Dr. P. K. Khatri[2]

[1]Senior Demonstrator, Department of Microbiology, Dr. S N Medical College, Jodhpur Rajasthan
[2]Senior Professor, Department of Microbiology, Dr. S N Medical College, Jodhpur Rajasthan

DOI: 10.36347/sjams.2022.v10i07.013 | **Received:** 08.06.2022 | **Accepted:** 19.07.2022 | **Published:** 30.07.2022

***Corresponding author:** Suneel Bhooshan
Senior Demonstrator, Department of Microbiology, Dr. S N Medical College, Jodhpur Rajasthan

Abstract **Original Research Article**

Drug resistance in MTB is alarming because of the limited choice of drugs available for treatment. First line drugs are safe, effective and cheaper than second line drugs. Any resistance in rifampicin or isoniazid will increase the chances of sensitive MTB strain to shift toward MDR or Pre-XDR strains. The male population (70.80%) was more frequently involved than the females (29.2%). The rural population was exposed at higher proportion accounted for 69.8% of cases. Comorbidity tobacco and alcohol consumption was associated in 31.2% and 25.8% of tuberculosis cases. In our study mono resistance in isoniazid was 14.20% which was higher than mono rifampicin resistance of 9.20%. Our findings suggest high resistance to isoniazid alone is alarming as these strains may shift towards MDR, Pre-XDR or XDR strains therefore, furthermore studies should be conducted at a large scale at the community level to estimate the prevalence and prevent this shift of resistant strains.

Keywords: *Mycobacterium tuberculosis*, Rifampicin, Isoniazid.

INTRODUCTION

Tuberculosis (TB) is air-born, communicable disease caused by *Mycobacterium tuberculosis*. It is on the top of the list of the leading causes of death by single infectious agents. It is worldwide prevalent with the capability of infecting any part of the body. It is curable, and preventable with appropriate and timely treatment. WHO has formulated guidelines in coordination with the government and private sectors to monitor the infected patients from the level of diagnosis to treatment and outcome? There are very efficient and less cytotoxic drugs available for the treatment of tuberculosis. However, the spread of drug-resistant tuberculosis has challenged the treatment outcome. Drug resistance has been reported worldwide irrespective of the economic status of the country. Well, implementation and adherence to strategies have shown very promising results in Europe by reducing 5% cases per year [1].

First-line treatment drugs include rifampicin, isoniazid, pyrazinamide and ethambutol which are very effective in the treatment and prevention of disease. The most prevalent drug resistance in *Mycobacterium tuberculosis* is seen in rifampicin and isoniazid. Patients with MDR/RR-TB roughly account for 4.1% of all new and 19% of retreatment TB cases globally, although wide regional and country differences occur. About 8% of TB cases worldwide are estimated to have Hr-TB, ranging from 5 to 11% in the WHO regions [2]. Isoniazid is a very essential component of the first-line treatment regimen and to date; no alternate drug is available to replace it from the first-line treatment regimen. It is bactericidal, less toxic and cost-effective. Resistance to isoniazid increases the likelihood of treatment failure and may convert to the strain multi-drug resistant tuberculosis [3, 4]. Resistance to INH is mainly driven by mutations in the katG gene, encoding a catalase-peroxidase that is essential for activation of INH, and mutations in the inhA promoter region, which result in upregulation of the drug target InhA, a protein reductase that plays an important role in mycolic acid synthesis. Although more than 300 different katG mutations have been identified, mutations at codon 315 of the gene are most prevalent, with, on average, 64% of isoniazid-resistant clinical isolates worldwide carrying a katG 315 mutation [5-7]. In a recent systematic review, the comparison of treatment outcomes between Hr-TB cases and patients with drug-susceptible TB receiving the WHO standard regimen for new patients, suggested that patients with confirmed isoniazid resistance had worse outcomes – i.e., higher treatment failure (11% vs 1%); relapse (10% vs 5%); as well as higher rates of acquired drug resistance (8% vs 0.3%) [3]. Easy access to INH-resistance testing is a challenge. Phenotypic (liquid DST) and molecular tests

(line probe assay) are recommended to check the resistance among first-line drugs. To perform liquid DST special technical skills are required and it is also time-consuming. Implementation of universal DST has brought some hope. The WHO-endorsed test Xpert MTB/XDR cartridge (Cepheid, Sunnyvale, CA, USA) detects rifampicin resistance to date, however, it is expected to be released and will include resistance testing for INH, fluoroquinolones, and second-line injectables [8]. Line probe assay (LPA), based on multiplex polymerase chain reactions, is used worldwide as a part of tuberculosis control programmes for simultaneous identification of *M. tuberculosis* complex (MTBC) and detection of drug resistance to rifampicin (RIF) and isoniazid (INH) among these strains.

We, therefore, set out to analyse the prevalence of mono-isoniazid drug resistance in presumptive cases.

MATERIAL AND METHODS

A cross-sectional study was carried out in TB C & DST laboratory, Kamala Nehru Chest Hospital, Jodhpur, Rajasthan. Specimens were collected from nine districts (Barmer, Bikaner, Jodhpur, Pali, Jalore, Hanumanghar, Sirohi, Sri Ganganagar and Jaisalmer). 500 (Pulmonary and extra-pulmonary) cases of presumptive tuberculosis were enrolled in the study. RNTCP diagnostic algorithm was followed with standard precaution. Identification techniques used in the study were tuberculosis microscopy, CBNAAT and liquid culture (MGIT 960). For drug resistance detection CBNAAT and first-line Line probe assay were performed. Data were analysed using SPSS 24 software.

Duplicate specimens were excluded from the study. To remove selection bias from the study, close contact with the tuberculosis-positive patient was not enrolled. Patients with a history of previous tuberculosis treatment, Non- tuberculous Mycobacteria (NTM), or incomplete demographic data were also excluded from this study.

Sample Collection and Identification

Two sputum specimens (spot and morning) were collected in 50 mL wide-mouthed sterile falcon tube from all participants. Ziehl-Neelsen (ZN) staining was performed on each specimen to detect the presence of *Mycobacterium tuberculosis* using a microscope. In the case of the smear-negative specimen, a subsequent liquid culture was performed by the N-acetyl-L cysteinesodium hydroxide method. Culture-positive specimens were subjected to immune- chromatographic assay for detection of the mpt64 antigen. MPT64 positive specimens were considered to be *Mycobacterium tuberculosis* complex and were further subjected to First-line LPA directly from the processed specimen.

Drug Resistance Detection

GeneXpert procedure: Drug resistance for rifampicin was detected by CBNAAT Briefly; the reagent was added at a 2:1 ratio to clinical specimens. The closed specimen container was manually agitated twice during the incubation period of 15 min at room temperature. The reagent sample mixture was transferred to the Xpert test cartridge. The cartridge was inserted into the GeneXpert device and the results generated automatically were read after 90 min.

LPA procedure: All smear-positive and smear-negative culture-positive isolates were further tested for drug resistance detection by LPA (Genotype MTBDRplus V 2.0 assay) as per the manufacturer's instructions. Ethical approval for the study was obtained from the Ethics Committee of Dr S N Medical College, Jodhpur.

RESULTS

500 presumptive tuberculosis patients were enrolled in the study. Out of which 354 (70.80%) patients were males and 146 (29.20%) females with a mean age of 38.66 (median 37.50 ±15.97). Male to the female sex ratio was recorded at 2.46:1. Young working-age group patients were the most infected in the study (11- to 60 years making up to 89.6%). While the lowest number of participants were observed in the age group of 81 to 90 years and one month to 10 years of age with 1 (0.20%) and 5 (5%) respectively. The preponderance of tuberculosis was seen in males in all age groups except 11 to 20 years of age.

Table: Distribution of patients according to their gender and age group (years)

Age group*	Male N (%)	Female N (%)	Total N (%)	Chi-Square test proportion
One month-10	5	0	5 (1%)	
11-20	27	35	62 (12.4%)	
21-30	76	48	124 (24.8)	
31-40	72	23	95 (19%)	
41-50	81	13	94 (18.8%)	73.615
51-60	54	19	73 (14.6%)	P < 0.0001
61-70	30	6	36 (7.20%)	
71-80	8	2	10 (2%)	
81-90	1	0	1 (0.2%)	
Total	**354 (70.80%)**	**146 (29.20%)**	**500**	

*Age group represented in years.

According to geographic area higher number of tuberculosis-infected patients was from rural areas which accounted for 349 (69.80%) while urban patients were 151 (30.20%).

Table: Distribution of the tuberculosis patients according to types of residence and their gender

	Male	Female	Total
	N (%)	N (%)	N (%)
Rural	257 (51.4%)	92 (18.4%)	349 (69.8%)
Urban	97 (19.4%)	54 (10.8%)	151 (30.2%)
Total	**354 (70.8%)**	**146 (29.2%)**	**500**

The most common comorbidity associated with tuberculosis patients was tobacco consumption followed by alcohol usage in our study population as shown in below table 2.

Table 2: Comorbidity associated with tuberculosis patients.

Association	User types	
	Alcohol N (%)	Tobacco N (%)
No	371 (74.2%)	344 (68.8%)
Yes	129 (25.8%)	156 (31.2%)
Both	**42 (8.4%)**	

Resistance in rifampicin was assessed by CBNAAT and first-line probe assay. Isoniazid's high and low-level resistance was assessed by targeting the katG and inhA gene respectively in the first line probe assay. Resistance in rifampicin was observed in a total of 44 (8.8%) strains by CBNAAT while FL-LPA showed 46 (9.20%) isolates rifampicin resistance. 2 rifampicin sensitive strains by CBNAAT showed rifampicin resistance in FL-LPA. For isoniazid total of 71 (14.20%) strains showed resistance. High-level isoniazid resistance was recorded in 61 (12.20%) isolates and low-level isoniazid-resistant was seen in 10 (2.00%) isolates. In terms of resistance inferred for rifampicin was observed in a total of 6 (1.20%) isolates out of 46 (9.20%). For isoniazid, high level inferred resistance was seen in 2 (0.40%) out of 61 (12.20%) isolates and for isoniazid low level only 1 (0.20%) isolate showed inferred resistance out of 10 strains. The majority of specimens 405 (81.00%) were sensitive to both drugs. The second specimen was not available in 8 cases of tuberculosis. 4 strains did not show a TUB band in FL-LPA.

The highest Mono resistance in rifampicin was seen in the Bikaner district with 7.69% followed by Barmer at 6.25%, Hanumangarh at 4.93%, and Jodhpur at 3.24% and Pali at 1.80%. Mono-Rifampicin resistance was not seen in Jaisalmer, Jalore, Shri Ganganagar and Sirohi. Isoniazid mono resistance was seen highest in Jaisalmer district in 21.73% of strains followed by Barmer 15.62%, Bikaner 11.53%, Hanumangarh 7.40%, Pali 7.27%, Jodhpur 6.94%, Sirohi 5.55% and Jalore 4.43%.

Districts	Mono R Resistance	Mono I Resistance	NO TUB	Second Sample Not Available
Barmer	2	5	1	0
Bikaner	2	3	0	0
Hanumangarh	4	6	0	0
Jaisalmer	0	5	0	0
Jalore	0	1	0	0
Jodhpur	7	15	2	4
Pali	1	4	1	0
Shri Ganganagar	0	0	0	0
Sirohi	0	2	0	0
Total	**16**	**41**	**4**	**4**

DISCUSSION

In our study males were more commonly infected in comparison to females. These findings were similar to the cross-sectional study conducted by (Rahma H A *et al*, 2017) where 67.5% of patients were male and 32.5% were females. Our data is also in coordination with the India TB report 2021 where infected male population percentages were 61.7% and females were 38.3% [9]. The mean ages of the patients were 38.66 (median 37.50 ±15.97) which is very close

to findings reported by Asho Ali, *et al,* from Pakistan (mean age 33.75 years) [10].

In contrast in the Philippines (Abdulrahman A A *et al,* 2002) in their study reported median age of the patients was 50 years. (Aricha S A *et al,* 2019), reported that 64.7% of males and 35.3% of females with a mean age of 36.8 (SD=13.3) were infected in their study. The most commonly infected age group in our study was 21 years to 30 years (24.8% cases) which is also reported in the India TB report 2021 (15 to 30 years, 38% cases). The overall working class is more commonly infected because of more exposure or contact with tuberculosis patients. We observed a significant difference in demographic (urban vs rural) prevalence of tuberculosis. The rural population was more commonly infected than the urban population 349 (69.8%) and 151 (30.2%). These findings were similar to a meta-analysis study conducted by Sathiyamoorthy *et al,* [11].

Our study population showed high usage of tobacco and alcohol. 32.1% of cases had a history of tobacco usage while 25.8% had a history of alcohol consumption. These comorbidities were not statically significant. These findings are similar to a study conducted by Beena Elizabeth Thomas (28% of cases answered yes to smoking) [12]. Zhang H *et al,* 2017 from China underscored the supposition that smoking is an independent risk factor for infection [13]. Our population had a high percentage of alcohol usage compared to other studies. It has been estimated that approximately 10% of all tuberculosis cases are attributable to alcohol use [14]. In respective to mono resistance in rifampicin or isoniazid, the present study data (rifampicin resistance 9.20% and isoniazid resistance 14.20%) is following the data presented by (Prabha D *et al,* 2014), 10.6% were rifampicin mono-resistant and 8.3% were INH mono-resistant. In contrast, P. Kumar *et al* 2015 reported a higher percentage of rifampicin resistance (18%) and a low percentage of Isoniazid (9.3%) resistance. D. M. D. Agdamag also reported a higher case of Rifampicin and isoniazid resistance was 51%, and 31% respectively [15].

The highest drug resistance in Bikaner district was observed (7.69%) reason behind this might the education profile of population or unawareness towards transmission of tuberculosis. Anna Dean *et al,* analysed data from 156 countries to find the prevalence of mono isoniazid resistance prevalence in new and old cases. They reported on average 7.4% of new cases possess isoniazid mono resistance which is very less than our findings [16]. This variation in the prevalence of mono isoniazid resistance may be due to the higher number of cases in India.

CONCLUSION

Earlier, resistance in isoniazid was not given as much attention as rifampicin in clinical practices. However, it is more common than rifampicin resistance in new tuberculosis cases. Recent studies suggest an association between isoniazid resistances to treatment outcome was negative. Additional resistance in mono isoniazid resistance strains will reduce the treatment efficacy and lead to generating a multidrug-resistant strain of tuberculosis. This may further lead to the spread of MDR strains in the community and complicate the treatment in an aspect of the financial, physical and mental status of patients. Therefore, it is very important to diagnose mono isoniazid resistance at the time of diagnosis of tuberculosis. This will help in preventing the spread of resistance and strains from becoming MDR.

REFERENCES

1. Tuberculosis surveillance and monitoring in Europe. (2017). European Centre for Disease Prevention and Control. https://www.ecdc.europa.eu/en/publications-data/tuberculosis-surveillance-and-monitoring-europe-2017
2. WHO. (2020). Global Tuberculosis Report. https://www.who.int/teams/global-tuberculosis-programme/tb-reports
3. Gegia, M., Winters, N., Benedetti, A., van Soolingen, D., & Menzies, D. (2017). Treatment of isoniazid-resistant tuberculosis with first-line drugs: a systematic review and meta-analysis. The Lancet Infectious Diseases, 17(2), 223-234.
4. Stagg, H. R., Lipman, M. C., McHugh, T. D., & Jenkins, H. E. (2017). Isoniazid-resistant tuberculosis: a cause for concern?. The International Journal of Tuberculosis and Lung Disease, 21(2), 129-139.
5. Ramaswamy, S. V., Reich, R., Dou, S. J., Jasperse, L., Pan, X., Wanger, A., ... & Graviss, E. A. (2003). Single nucleotide polymorphisms in genes associated with isoniazid resistance in *Mycobacterium tuberculosis*. Antimicrobial agents and chemotherapy, 47(4), 1241-1250.
6. Resistance to Isoniazid and Ethionamide in *Mycobacterium tuberculosis*. (2022). Genes, Mutations, and Causalities-PMC. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6636829/
7. Seifert, M., Catanzaro, D., Catanzaro, A., & Rodwell, T. C. (2015). Genetic mutations associated with isoniazid resistance in *Mycobacterium tuberculosis*: a systematic review. PloS one, 10(3), e0119628.
8. Detection of Isoniazid. (2022). Fluoroquinolone-, Amikacin-, and Kanamycin-Resistant Tuberculosis in an Automated, Multiplexed 10-Color Assay Suitable for Point-of-Care Use. https://pubmed.ncbi.nlm.nih.gov/27807153/

9. TB Annual Report. (2021). High Resolution. https://tbcindia.gov.in/WriteReadData/l892s/4504484964TB%20Annual%20Report%202021%20210321%20High%20Resolution.pdf
10. Ali, A., Hasan, R., Jabeen, K., Jabeen, N., Qadeer, E., & Hasan, Z. (2011). Characterization of mutations conferring extensive drug resistance to *Mycobacterium tuberculosis* isolates in Pakistan. Antimicrobial agents and chemotherapy, 55(12), 5654-5659.
11. Sathiyamoorthy, R., Kalaivani, M., Aggarwal, P., & Gupta, S. K. (2020). Prevalence of pulmonary tuberculosis in India: A systematic review and meta-analysis. Lung India: Official Organ of Indian Chest Society, 37(1), 45.
12. Thomas, B. E., Thiruvengadam, K., Kadam, D., Ovung, S., Sivakumar, S., Bala Yogendra Shivakumar, S. V., ... & CTRIUMPH-RePORT India Study. (2019). Smoking, alcohol use disorder and tuberculosis treatment outcomes: A dual co-morbidity burden that cannot be ignored. PLoS One, 14(7), e0220507.
13. Zhang, H., Xin, H., Li, X., Li, H., Li, M., Lu, W., ... & Gao, L. (2017). A dose-response relationship of smoking with tuberculosis infection: A cross-sectional study among 21008 rural residents in China. PloS one, 12(4), e0175183.
14. The association between alcohol use, alcohol use disorders and tuberculosis (TB) (2022). A systematic review. https://pubmed.ncbi.nlm.nih.gov/19961618/
15. Agdamag, D. M. D., Kageyama, S., Solante, R., Espantaleon, A. S., Sangco, J. C. E., & Suzuki, Y. (2003). Characterization of clinical isolates of *Mycobacterium tuberculosis* resistant to drugs and detection of RpoB mutation in multidrug-resistant tuberculosis in the Philippines. The International Journal of Tuberculosis and Lung Disease, 7(11), 1104-1108.
16. Sulis, G., & Pai, M. (2020). Isoniazid-resistant tuberculosis: A problem we can no longer ignore. Plos Medicine, 17(1), e1003023.