Original Article

Molecular Detection of Mycobacterium tuberculosis in the Indigenous Population of Tharparkar, Sindh, Pakistan. Using GeneXpert Assay: Age and Gender Stratified Analysis

Allied Medical Research Journal
Vol:3 No.4: 64-71

Bhawani Shankar[1], Atif Ahmed Patoli[1], Bushra Bano Patoli[1], Iram Nabi Begum[2]

[1]*Institute of Microbiology University of Sindh, Jamshoro, Pakistan*
[2]*Servicio Andaluz de salud, Costa del Sol SAS, Malaga, Spain*

ABSTRACT

Background: Mycobacterium tuberculosis (MTB) the causative agent of Tuberculosis (TB), spreads through air from infected persons via coughing, sneezing etc. It affects various body organs such as kidneys, brain, spine but in majority of cases it affects lungs and causes pulmonary TB. The distribution of the Pulmonary tuberculosis burden across Pakistan is diverse, with incidence rates ranging from 110 cases per 100,000 to 462 cases per 100,000 inhabitants. Given that Tharparkar is a rural and underdeveloped area, the TB burden is underreported. The current paper evaluates the burden of TB cases in the indigenous population of Tharparkar region of Province Sindh with age and gender wise stratified analysis.

Methods: A total of 1,099 sputum samples from clinically and radiologically suspected TB patients were tested for MTB and rifampicin resistance using the GeneXpert/RIF assay. Data was analyzed using IBM SPSS Statistics version 20, applying chi-square tests and odds ratios (95% CI) to assess differences in MTB positivity across age groups and genders.

Results: Of the 1,099 samples, 198 (18%) were MTB positive. Notably, no case of MDR-TB was detected. MTB prevalence was slightly higher in males (n=133, 18.9%, median age: 40) than in females (n=65, 16.4%, median age: 35). The highest positivity (n=11, 25.6%) was observed in the 71–80 age group. Gender-specific trends showed peak prevalence in males aged 71–80 (n=9, 33%) and in females aged 11–20 (n=10, 27%, mean age: 18.08 years). Differences in prevalence between genders exceeded 60% in the 41–50 and 71–80 age groups, though not statistically significant (p-values = 0.083 and 0.130).

Conclusion: Tharparkar data indicates a significantly lower prevalence, approximately 61.5% lesser, than in KPK and FATA, earlier onset in females and absence of MDR-TB, indicating region's unique environmental or sociocultural factors need further investigation.

Keywords: *Gene Xpert MTB/RIF, Mycobacterium tuberculosis, Multi-Drug-Resistant TB, Pulmonary TB, Sputum analysis.*

Received: August 7, 2025**; Revised:** September 5, 2025**; Accepted:** October 10, 2025

Corresponding Email: bushra.patoli@usindh.edu.pk

DOI: https://doi.org/10.59564/amrj/03.04/010

INTRODUCTION

Tuberculosis (TB) is considered among the oldest human diseases recognized by the mankind.[1] It is caused by Mycobacterium tuberculosis (MTB) that spread through air from infected persons via coughing, sneezing etc.[2] It is known to affect various body organs such as kidneys, brain, spine however in majority of cases it is associated with lungs and causes pulmonary TB. TB is generally treatable and curable; however, without appropriate treatment, it can be fatal. During the golden age of antibiotics (1940s–1960s) Streptomycin and p-amino-salicylic acid, isoniazid, ethambutol, rifampicin and pyrazinamide were considered to be the most significant antibiotics for treatment.[3] During the 1960s, tuberculosis was widely believed to be under control and on the path to eradication as a public health threat. However, the disease re-emerged in the 1980s, driven by the spread of acquired immune deficiency syndrome (AIDS) and the rise of drug-resistant TB strains.[4] MTB expressing resistance against isoniazid and rifampicin are branded as Multiple Drug Resistant (MDR), while additional Strains

that exhibit resistance to any fluoroquinolone and at least one of the injectable second-line drugs such as kanamycin, capreomycin, or amikacin are classified as Extensively Drug-Resistant (XDR) tuberculosis.[2]

According to the WHO 2024 annual report, tuberculosis remains the leading cause of death from a single infectious agent. In 2023, about 10.8 million people developed active TB worldwide. The disease caused nearly 1.25 million deaths among HIV-negative individuals and around 161,000 deaths among people with HIV. Most TB cases occurred in the 30 high-burden countries identified by WHO. Five countries alone accounted for 56% of the global total: India (26%), Indonesia (10%), China (6.8%), the Philippines (6.8%), and Pakistan (6.3%). Pakistan was ranked fifth (5th) for high-TB burden countries accounting 7.8% global TB burden. The WHO report on TB 2024, indicated that in 2023, 75% of MDR-TB cases were contributed by ten countries, Pakistan being 5th largest contributor.[2]

Chest radiography is used as a screening tool and, when combined with symptom assessment, helps identify individuals who should undergo further diagnostic testing. Apart from direct visualization of tubercle bacilli in the specimen and culture methods, the TB is diagnosed through amplification and detection of MTB specific genetic material. Assays such as Xpert MTB/RIF and Line Probe Assays (LPA), offer rapid and accurate results.[5] GeneXpert (MTB/RIF) assay is an independent cartridge-based test to identify MTB along with detection of RIF resistance in a single tube by using specific primers. GeneXpert targets MTB specific *rpoB* gene and mutations within 81-bp Rifampicin Resistance Determining Region (RRDR) of *rpoB* gene for Rifampicin resistance respectively.[6] Rifampicin resistance is associated with INH resistance, therefore, Rifampicin resistance (RR) is used as surrogate marker of MDR-TB.[7] Due to its specificity and less detection time the Xpert/RIF assay is preferred method for molecular detection.[5] WHO recommends the use of GeneXpert as initial diagnostic test in all individuals. The GeneXpert assay was introduced in Pakistan in 2011 to enable rapid detection of rifampicin resistance in patients suspected of having drug-resistant tuberculosis.

Although many studies are conducted in Pakistan to state the prevalence of pulmonary TB. In some cities such as; Karachi, Rawalpindi, and Queta, TB along with its resistance pattern against rifampicin and isoniazid is reported[8-11], while for others such as; Hyderabad, Islamabad, Lahore, and Okara the data based on direct microscopic visualization of acid-fast bacilli in sputum smear, PCR amplification of TB specific gene and Interferon Gamma Release Assay is reported.[12–15] Tharparkar is an underdeveloped region of southeastern Sindh. The district Tharparkar is characterized by its arid, desert topography and comprises seven tehsils (sub-districts): Chachro, Dahli, Diplo, Islamkot, Kaloi, Nangar Parkar and Mithi. The local economy primarily relies on small-scale businesses, vegetation, livestock and tourism. The region is significantly impacted by climate change. Common challenges faced by the population include poverty, hunger, low socioeconomic status, limited access to healthcare services, and inadequate medical infrastructure.[16] Additionally, widespread poverty, illiteracy, life style and a lack of awareness about contagious infections contribute to increased vulnerability towards acquiring life threatening diseases such as Tuberculosis.

Since the data pertaining to Tharparkar are under-reported, therefore current study evaluates the burden of TB cases in the indigenous population of Tharparkar region using Gene Xpert/RIF assay with reference to age-gender categorized analysis.

METHODOLOGY

Study design, duration and setting

This was a cross-sectional study conducted from July 2020 to January 2023. Patients from various sub-districts of Tharparkar including Chachro, Dahli, Diplo, Islamkot, Kaloi, Nangar Parkar and Mithi were selected. A purposive sampling method was employed and individuals who met specific inclusion criteria such as those showing clinical symptoms of TB and having abnormal X-ray findings were included. Individuals exhibiting

clinical symptoms indicative of TB such as cough lasting a couple of weeks, weight loss without any particular reason, night sweats, low-grade fever, and hemoptysis were first evaluated through physical examination and chest radiography (X-ray). Radiographic findings consistent with pulmonary TB (e.g., cavitary lesions, infiltrates, or consolidation in the upper lobes) strengthened clinical suspicion. Based on the combination of clinical presentation and abnormal X-ray findings, suspected TB cases of all ages were referred for confirmatory testing using the GeneXpert MTB/RIF assay and included in the current study.

GeneXpert Assay

The GeneXpert MTB/RIF assay (Cepheid) was conducted following the manufacturer's instructions. In brief, the sample reagent was added to the decontaminated sputum specimen at a 3:1 ratio and mixed thoroughly for 15 minutes. The prepared mixture was then transferred into a test cartridge, which was subsequently loaded into the GeneXpert instrument for analysis. Sample preparation, nucleic acid amplification, and detection of MTB genomic DNA were fully automated in the GeneXpert system. The assay detects MTB and rifampicin (RIF) resistance caused by mutations in the *rpoB* gene of M. tuberculosis with the help of fluorescent probes which are known as molecular beacons. Rifampicin resistance is identified through hybridization of the amplified DNA with five overlapping probes targeting the 81-base pair rifampicin resistance-determining region (RRDR) of the *rpoB* gene.[17]

Ethical Consideration

The research study was approved by the Research and Graduate Studies, vide letter No. DRGS/1050/30-7-2020 University of Sindh. In adherence to ethical responsibilities, no personal or identifiable information of the patients is disclosed in this study.

Statistical Analysis

Data were processed to determine significant differences for MTB positive cases between various age groups of both genders. IBM SPSS version 20 was used for data processing. Percentage differences were calculated using standard formula using online statistical calculator i.e. https://www.calculatorsoup.com. The Odds Ratio (OR) with 95% Confidence Interval (CI) were calculated through online statistics calculator (www.select-statistics.co.uk).

Chi-square test employing 2x2 contingency table was used for the calculation of p-values via online statistics calculator (www.socscistatistics.com).

RESULTS

A total of 1099 sputum samples from suspected TB patients were taken and processed for the detection of MTB and its resistance against isoniazid and rifampicin using Gene Xpert Assay. Out of 1099 samples 198 (18 %) were positive for MTB while 901 (82 %) were negative for MTB. None of the MTB was detected as MDR-TB. Of all the samples 704 (64%) samples belonged to Male category while 395 (36%) samples belonged to female category with median age 45 and 40 years respectively. Among male category 133 (~19%) were positive for MTB and 571 (81%) were negative for MTB. Among female category 65 (16.4%) were positive for MTB and 330 (73.6%) were negative for MTB (Fig.1).

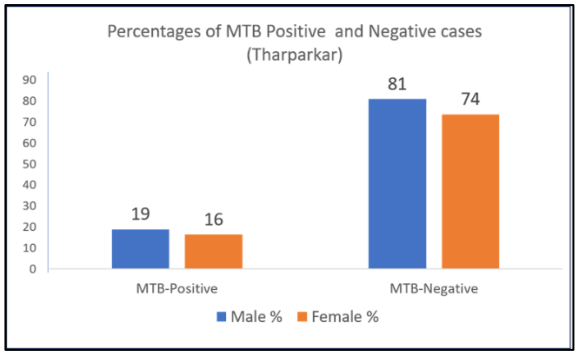

Fig.1 Diagram representing the comparative percentages of MTB positive and negative cases in both gender categories.

Age and Gender Based Analysis

To analyze the data for MTB detection with respect to age of the suspected TB patients, the data (n = 1,099) were categorized into age groups with 10-year intervals. The highest proportion of MTB-positive cases (25.6%) was observed in the 71–80-year age group, while the lowest (10%) was recorded in the 81–90-year group (Table 1).

Table 1. Absolute numbers and relative percentages of MTB cases in various age groups for both gender categories along with statistical data

Age (Year)	MTB	Total N [%]	Male N [%]	Mean Age	Female N [%]	Mean Age	% Dif	OR	CI [95%]	p-value
	+ ve	2 [13.3]	1 [11]	7.9	1 [20]	8.8	58.06	0.5	[0.02 , 10.25]	0.649
	- ve	13 [86.7]	8 [89]		4 [80]					
11 -- 20	+ ve	23 [21.9]	13 [18.8]	17.2	10 [27]	18.08	35.8	0.63	[0.02 , 10.25]	0.330
	- ve	82 [78.1]	56 [81.2]		27 [73]					
21 -- 30	+ ve	40 [18.5]	24 [19.4]	26.74	16 [17.5]	27.07	10.3	1.13	[0.56 , 2.27]	0.741
	- ve	176 [81.5]	100 [80.6]		75 [82.5]					
31 -- 40	+ ve	37 [18.1]	24 [19.5]	36.89	13 [16]	36.7	19.7	1.27	[0.6 , 2.66]	0.530
	- ve	167 [81/9]	99 [80.5]		68 [84]					
41 -- 50	+ ve	29 [14]	22 [17.3]	48.38	7 [8.7]	47.78	66.1	2.19	[0.89 , 5.38]	0.083
	- ve	178 [86]	105 [82.7]		73 [91.3]					
51 -- 60	+ ve	28 [15.1]	19 [14.6]	57.4	9 [16.6]	57.66	12.8	0.86	[0.36 , 2.03]	0.724
	- ve	157 [84.9]	111 [85.4]		45 [83.4]					
61 -- 70	+ ve	27 [23.9]	20 [23.8]	67.4	7 [23.3]	67.03	2.1	1.03	[0.38 , 2.75]	0.958
	- ve	86 [76.1]	64 [76.2]		23 [76.7]					
71 -- 80	+ ve	11 [25.6]	9 [33]	78.1	2 [12.5]	78.06	90.1	3.5	[0.65 , 18.85]	0.130
	- ve	32 [74.4]	18 [67]		14 [87.5]					
81 – 90	+ ve	1 [10]	1 [11]	84.8	0 [0]	85	[NA]	[NA]	[NA]	[NA]
	- ve	9 [90]	8 [89]		1 [100]					
91 -- 100	+ ve	0 [0]	0 [0]	94	0 [0]	0	[NA]	[NA]	[NA]	[NA]
	- ve	1 [100]	1 [0]		0 [0]					
Total	+ ve	198 [18]	133 [18.9]		65 [16.4]					
	- ve	901 [82]	570 [81.1]		330 [83.6]					

[NA] = Not Applicable, MTB = Mycobacterium tuberculosis

Among males, the highest MTB prevalence (33%) occurred in the 71–80-year age group, while among females, the highest prevalence (27%) was seen in the 11–20-year group (Figure 2). Although percentage differences exceeded 60% in the 41–50 and 71–80 age groups, statistical analysis (odds ratios and Chi-square test) showed no significant difference between males and females (Table-1).

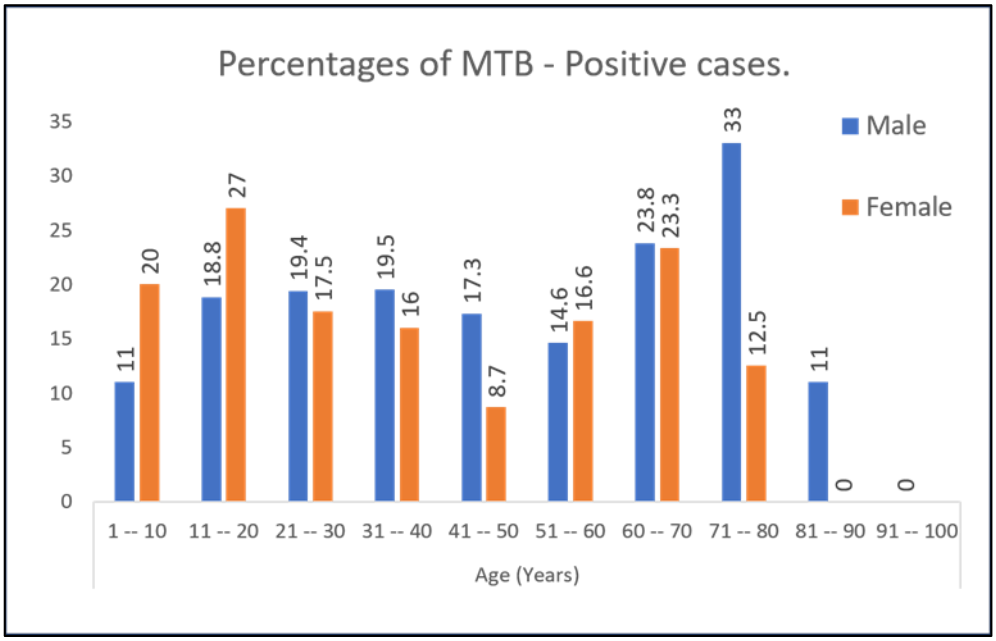

Fig.2 Comparative bar diagram representing the percentages of MTB cases in different age groups

DISCUSSION

The global increase in the number of people that fell ill with TB (incident cases) was 10.8 million in 2023. Of the five countries accounting for 56% of the worldwide total, Pakistan contributed 6.3%, while, China and the Philippines 6.8% each, Indonesia 10% and India 26%.[2] Currently, there is no specific data available on the prevalence of TB in Tharparkar district, Sindh. However, general statistics indicate that Sindh province has a higher TB incidence rate compared to other regions in Pakistan.

According to Pakistan TB Prevalence Survey 2010–11.[18], the Sindh region bears the highest tuberculosis burden in Pakistan, with an incidence rate of 292 cases per 100,000 inhabitants. This is followed by Khyber Pakhtunkhwa, Punjab, and Baluchistan, which have incidence rates of 269, 243, and 178 per 100,000 inhabitants, respectively.[19]

The distribution of the tuberculosis burden across Pakistan is diverse, with incidence rates varying from 110 cases per 100,000 inhabitants in Harnai, Balochistan, to 462 cases per 100,000 inhabitants in Khairpur, Sindh.[19] Given that Tharparkar is a rural and underdeveloped area, the TB burden is underreported.

The present study shows some significant and distinctive findings for this region. A total of 1099 sputum samples from suspected TB patients were taken and processed for the detection of MTB and its resistance against rifampicin using Gene Xpert Assay. Out of 1099 samples 198 (18%) with median age of 40 years were positive for MTB. In 2023 Jawad and others reported 34% of MTB prevalence for Bajou agency FATA (20), while a prevalence of 34% was also reported for KPK by Sajid Ali and other in 2020[21]. However, a combined prevalence of 42.9% for Pulmonary and Extra-pulmonary tuberculosis was documented by M. Zahid and others in 2020.[8] Additionally, prevalence ranging from 23% to 58% have been reported through studies based on microscopic visualization of Acid-Fast Bacilli (AFB) for Sindh.[22,23] Interestingly during the current study none of the MDR-TB was detected for Tharparkar region. Intriguingly, based on Drug-Sensitivity Testing, 43.2% of

MDR-TB was reported by Nazia Khursheed and others in 2022 for Karachi[10], a comparatively higher literacy urban city of Sindh province.

Moreover, about 6.9% of MDR-TB based on GeneXpert MTB/RIF assay was reported by Muhammad Ahmed Mughal and others in 2025 for Lahore.[24] To analyze the data for MTB detection with respect to age of the suspected TB patients, the data (n=1099, Median Age = 44.5 Years) were categorized into various age groups. The magnitude of each group was selected as 10 years randomly. The maximum MTB positive cases (25.6%) were seen in the age groups of 71-80 with mean age of 77.9 years. A prevalence of 40% in the age group of 25-34 years and 69.6% in the age group of 11-20 years have been documented for KPK and FATA respectively [20,21] while minimum MTB positive cases (10%) were seen in the age group of 81-90. Out of 1099 samples 64% samples belonged to Male category and 36% samples to female category. Among male category 18.9% were positive for MTB and 16.4% among female category. Previously, a relatively higher prevalence of 48% in males and 52% in females was reported for Hyderabad [12]. Among the male category the highest prevalence of MTB cases (33%) was seen in the age group of 71-80 years with mean age of 78.1 years, while highest prevalence of MTB cases (27%) in case of female category was witnessed in the age group of 11-20 years with mean age of 18.08 years The percentage differences between the two categories were assessed across all age groups. Substantial differences, exceeding 60%, were observed in the 41–50 and 71–80 age groups. However, subsequent statistical analysis comparing observed and expected frequencies revealed that these differences were not statistically significant, with *p-values* of 0.084 and 0.130, respectively.

Study Limitations, Strengths and Recommendations.

This study utilized a purposive sampling approach targeting clinically suspected TB cases, which may have excluded asymptomatic or subclinical infections. Additionally, the absence of detailed information on participants' socioeconomic status, comorbidities, and HIV status could have influenced the observed TB prevalence patterns.

The study provides much-needed epidemiological data from Tharparkar, a district where TB surveillance and published literature are extremely limited. Generating primary data from such an underrepresented region is a significant contribution.

Future studies should collect detailed information on socioeconomic status, comorbidities (such as diabetes), nutritional status, and HIV status, as these factors significantly influence TB susceptibility and transmission patterns.

CONCLUSION

The prevalence of MTB in Tharparkar district, as presented here, is approximately 61.5% lesser than previously reported rates for FATA and KPK, despite being part of a high-burden province. The absence of MDR-TB and the earlier onset of infection in females (notably in the 11–20 age group) highlight unique regional patterns. The earlier onset of MTB in females compared to males may be attributed to their relatively longer exposure to enclosed, poorly ventilated home environments.

Interestingly, the incidence of MDR-TB is not witnessed for Tharparkar, however for other cities of Sindh such as Karachi, 43.2% MDR-TB is reported. In KPK and FATA, MTB predominantly affects individuals under the age of 34, whereas in Tharparkar, a higher incidence is observed among those over 71 years old. However, in this region, 50% of the positive cases occur in males under 40 and females under 35 years of age. The lower incidence in this underdeveloped district compared to more developed cities such as Hyderabad and Karachi, indicates underreporting and limited access to diagnostic and healthcare services. This under-detection may facilitate ongoing community transmission. The observed gender and age-specific trends,

with earlier infection onset among females and higher prevalence among elderly males, highlight potential sociocultural and occupational risk factors that require targeted public health interventions.

Acknowledgments
None.

Author Contributions
Bhawani Shankar contributed to the study conception, design, and data collection. **Atif Ahmed Patoli** supervised the research process and provided critical input during analysis, editing, and final approval of the manuscript. **Bushra Bano Patoli** assisted in data interpretation and manuscript drafting. **Iram Nabi Begum** contributed to literature review and manuscript drafting. All authors read and approved the final version.

Ethical Approval
The study received approval from the Ethical Review Board of University of Sindh (DRGS/1050/30-7-2020).

Grant Support and Funding Disclosure
None.

Conflict of Interests
None.

REFERENCES

1. Mousavi-Sagharchi SMA, Ghorbani A, Meskini M, Siadat SD. Historical examination of tuberculosis; from ancient affliction to modern challenges. *J Infect Public Health.* 2025;18(3):102649. **DOI:** https://doi.org/10.1016/j.jiph.2024.102649
2. Tereza Kasaeva (WHO Report). *Global tuberculosis report 2024.* WHO Global Tuberculosis Programme; 2024. Available from: https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024
3. Kadhiravan T, Sharma SK. Medical management of genitourinary tuberculosis. *Indian J Urol.* 2008;24(4):362. **DOI:** https://doi.org/10.4103/0970-1591.42619
4. Hamada Y, Getahun H, Tadesse BT, Ford N. HIV-associated tuberculosis. *Int J STD AIDS.* 2021;32:780–90. **DOI:** https://doi.org/10.1177/0956462421992257
5. Sajjad Ahmad SAAU, AS. Evaluation of GeneXpert MTB/RIF Assay for Detection of Pulmonary Tuberculosis on Sputum Samples. *J Coll Physicians Surg Pak.* 2019;29(1):66–9. **DOI:** https://doi.org/10.29271/jcpsp.2019.01.66
6. Haas AL, Ma A, Pham J, Verma P, Malhotra U, Church EC, et al. Limitations of the MTB/RIF Assay: An Xpert Review of 4 Clinical Cases. *Open Forum Infect Dis.* 2025;12(4). **DOI:** https://doi.org/10.1093/ofid/ofaf132
7. Sailo CV, Lalremruata R, Sanga Z, Fela V, Kharkongor F, Chhakchhuak Z, et al. Distribution and frequency of common mutations in rpoB gene of *Mycobacterium tuberculosis* detected by Xpert MTB/RIF. *J Clin Tuberc Other Mycobact Dis.* 2022;29:100342. **DOI:** https://doi.org/10.1016/j.jctube.2022.100342
8. WHO Press. *Rapid Implementation of the Xpert MTB/RIF Diagnostic Test: Technical and Operational "How-to."* WHO Library Catalogue; 2011. Available from: http://www.who.int/about/licensing/copyright_form/en/index.html
9. National TB Control Program, The Islamic Republic of Pakistan. *National TB Control Program 2020–23.* 2020. Available from: https://ntp.gov.pk/wp-content/uploads/2020/04/TB%20NSP%20-2020-2023
10. Mengal MZ, Ali H, Asmat R, Naeem M, Abbas F, Samad A, et al. Detection of mutations in rpoB gene using GeneXpert MTB/RIF in clinical specimens from Quetta, Pakistan. *Pak J Zool.* 2020;53(1):1–7. **DOI:** https://doi.org/10.17582/journal.pjz/20190522060512
11. Hammad M, Sharjeel M, Fazal I, Aziz A, Shahid F, Iqbal M. Pattern of drug resistance among patients presenting with relapse of pulmonary tuberculosis. *Pak Armed Forces Med J.* 2024;74(2):362–6. **DOI:** https://doi.org/10.51253/pafmj.v74i2.7772
12. Khursheed N, Asif S, Bano S, Ali MM, Adnan F. Susceptibility pattern of *Mycobacterium tuberculosis* at Indus Hospital and Health Network, Karachi. *Pak J Med Sci.* 2022;38:399–404. **DOI:** https://doi.org/10.12669/pjms.38.ICON-2022.5778
13. Baig S. MDR TB – The lethal sound of silent mutations. *J Coll Physicians Surg Pak.* 2024;34:867–8. **DOI:** https://doi.org/10.29271/jcpsp.2024.08.867
14. Laghari M, Sulaiman SAS, Khan AH, Memon N. A prospective study of socio-demographic, clinical characteristics and treatment outcomes of children with tuberculosis in Sindh, Pakistan. *BMC Infect Dis.* 2019;19(1). **DOI:** https://doi.org/10.1186/s12879-019-3702-3
15. Sagheer N, SSNSSAAM. Examining tuberculosis cases using sputum testing across two tehsils of district Okara, Punjab, Pakistan (2019–2023). *Azerbaijan Pharm Pharmacother J.* 2024;4(23):31–40. **DOI:** https://doi.org/10.61336/appj/24-04-03
16. Sadaf R, Munir T, Farrukh S, Abbasi S. Prevalence of latent tuberculosis infection in healthcare workers in tertiary care hospitals of Pakistan. *Pak J Med Sci.* 2020;36(2):198–202. **DOI:** https://doi.org/10.12669/pjms.36.2.936
17. Jabeen C, Mushtaq MH, Khan JA, Umbreen G, Saeed MS, Sadiq S, et al. Burden and factors associated with occupational tuberculosis infection among high-risk workers in Lahore District, Pakistan. *J Infect Dev Ctries.* 2024;18(7):1041–9. **DOI:** https://doi.org/10.3855/jidc.17965
18. Li K, Li L. Correlation between Xpert MTB/RIF results and *rpoB* mutations within probe-targeted regions in *Mycobacterium tuberculosis* isolates from Sichuan Basin. *Infect Drug Resist.* 2025;18:5161–71. **DOI:** https://doi.org/10.2147/IDR.S541544
19. Memon A, Ali Laghari Z, Ali Samo A. Factors associated with prevalence of stunting and thinness among adolescents of Tharparkar, Sindh, Pakistan: A community-based study. *PLoS One.* 2025;20(4). **DOI:** https://doi.org/10.1371/journal.pone.0318055
20. Schwalb A, Samad Z, Yaqoob A, Fatima R, Houben RMGJ. Subnational tuberculosis burden estimation for Pakistan. *PLOS Glob Public Health.* 2024;4(9). **DOI:** https://doi.org/10.1371/journal.pgph.0003653
21. Jawad M, Bilal A, Khan S, Rizwan M, Arshad M. Prevalence and awareness survey of tuberculosis in suspected population of Bajaur Agency, Pakistan. *Pak J Health Sci.* 2023;4(6):56–61. **DOI:** https://doi.org/10.54393/pjhs.v4i06.793
22. Ali S, Khan MT, Khan AS, Mohammad N, Khan MM, Ahmad S, et al. Prevalence of multidrug-resistant *Mycobacterium tuberculosis* in Khyber Pakhtunkhwa, Pakistan. *Pol J Microbiol.* 2020;69(2):133–7. **DOI:** https://doi.org/10.33073/pjm-2020-005

23. Khan K, Unar AA, Unar K, Rajper FQ, Baig MT. Evaluation of drug resistance and treatment outcomes among TB patients with diabetes mellitus in Sindh, Pakistan. *J Pharm Res Int.* 2021;33(27B):39–46. **DOI:** https://doi.org/10.9734/jpri/2021/v33i27B31501
24. Ahmad Mughal M, Imran A, Khan HU, Farooq M, Ikram A, Arshad F, et al. Prevalence of multidrug-resistant tuberculosis and its association with previous treatment history in adults. *Cureus.* 2025;17(7):e88204. **DOI:** https://doi.org/10.7759/cureus.