Biomedical Letters ISSN 2410-955X DOI: https://doi.org/10.47262/BL/9.1.20230103

Research article
2023 | Volume 9 | issue 1 | Pages 1-6

ARTICLEINFO

**Received**
January 03, 2023
**Revised**
February 07, 2023
**Accepted**
March 10, 2023

***Corresponding Author**
Khawar Ali Shahzad
**E-mail**
khawar7bar@yahoo.com

**Keywords**
Tuberculosis
Granulomatous
Rifampicin
Multidrug resistance
Smokers

**How to Cite**
Siddiq S, Sarwer MI, Shahzad KA. Causes, prevalence, and identification of Multi-Drug Resistant (MDR) Tuberculosis in patients with different age groups. Biomedical Letters 2023; 9(1):1-6.

Scan QR code to see this publication on your mobile device.

Open Access

# Causes, prevalence, and identification of multi-drug resistant (MDR) tuberculosis in patients with different age groups

Sabeera Siddiq[1#], Muhammad Imran Sarwer[2#], Khawar Ali Shahzad[1*]

[1]Department of Zoology, The Islamia University of Bahawalpur, 63100, Bahawalpur, Pakistan
[2]Department of Pathology, Quaid-e-Azam Medical College, Bahawalpur, Pakistan

## Abstract

Tuberculosis (TB) is a granulomatous infectious illness caused by gram-positive, acid-fast bacilli belonging to the *Mycobacterium* genus. *Mycobacterium tuberculosis* (MTB) causes tuberculosis in humans, which primarily affects the lungs and causes pulmonary tuberculosis. Extra pulmonary tuberculosis can damage the colon, meninges, bones, joints, lymph nodes, skin, and other body parts. The present study was conducted to find the causes, prevalence, and identification of multi-drug resistant tuberculosis in patients with different age groups. One hundred sputum samples were collected from patients with different age groups. Identification and confirmation of multidrug resistance tuberculosis were performed by smear microscopy, real-time PCR assay, and bacterium culture, and Out of 100 samples 86 were positive for tuberculosis and 14 were negative. Out of these 86 samples, 52 were males and 34 were females. The age group with the highest percentage of tuberculosis was 40-60 years. There were 47 smokers and 39 non-smokers. Out of 100 samples, only 19 had good living standards, 40 with average hygienic conditions, and 41 with poor hygiene. Through real-time assay it was concluded that 48 samples were Isoniazid resistant and 53 were Rifampicin resistant. According to our findings, poor quality of life, poor housing, overcrowding, population explosion, under nutrition, smoking, alcohol misuse, lack of education, large families, and lack of information about the cause and transmission of tuberculosis are some of the social causes. These elements are interconnected and play a role in tuberculosis occurrence and transmission.

## Introduction

An irresistible and likely hazardous disease that potentially harms the lungs but it can also affect the brain, kidneys, or spine in rare cases is TB [1]. A species of pathogenic bacteria named *Mycobacterium tuberculosis* (MTB*)* causes this disease, belonging to the family Mycobacteriacea [2]. MTB is infectious bacteria which implies that can spread from one person to the other, when a lung TB patient hack, sneeze, wheeze, laugh, talk or spit the microorganism might impel through the air and could be breathed by another individual. We can say that microorganisms could be available in air droplets (called droplet nuclei) and an individual can breathe in them [3].

TB is of two types Pulmonary (PTB) and extrapulmonary (EPTB). In PTB only lung tissues are get tainted but in EPTB one the bacteria might travel from the lungs into the circulatory system and may contaminate different locales including cerebrospinal fluid of the Central Nervous System, lymph nodes, kidneys, spine, pleural tissues that surround the lungs or bones and joints, heart tissues pericardium [4]. Also, it could be active or latent TB. The latent TB is non-transmissible because in it bacteria is inactive and shows no symptoms but if it remains unchecked or untreated it may become active TB [5]. But active TB is transmissible, and a person can, unfortunately, infects 10 or 15 more people their symptoms may include a bad dry cough that may last from two to three weeks, hemoptysis (coughing up blood or sputum), weight loss, chest pain, back pain, night sweats, fever, breathlessness, poor growth in children, etc.

People with lower incomes and less education that live in densely crowded areas are more likely to have TB. Poverty, which results from poor nutrition, impairs immunological function. On other hand, poor living conditions brought on by inadequate ventilation, crowded living spaces, and poor hygiene practices are likely to increase the transmission of TB [6]. The prevalence of TB is directly related to increasing age and is always higher in men than in women. The spread of smear-positive TB was 80% more in men than women [7]. Pakistan has the sixth-highest incidence of TB in the world [8]. Furthermore, Multidrug-resistant tuberculosis (MDR-TB) is highly resistant to both first-line drugs, *i.e*., INH and RIF. The mutations in one of two major sites i.e., the *katG* or *inhA* genes cause INH resistance and due to point mutations in the *rpoB* gene, RIF resistance is most likely to occur [9].

It may be a complicated procedure to diagnose TB. To check the presence of bacteria the samples of phlegm can even regularly be taken. To determine the best remedy for a person these gaugings are critical in favor [10]. The first and foremost procedure to diagnose TB is through smear microscopy [11]. Bacterium culture is the gold standard and widely accepted method although it takes 8 weeks [12]. Keeping in mind the above-given discussion, the present study was designed to analyze the prevalence of MDR-TB in patients from Bahawalpur City and its periphery, molecular characterization of MDR-TB by real-time PCR, and epidemiological distribution of MDR- TB.

## Materials and Methods

### *Chemicals*

The chemicals used were purchased from local suppliers and some are supplied by the organization which built the laboratory. These include Buffer solution, 0.1% Auramine phenol, 0.5% Decolorizer, 0.3% Methylene blue, fluorescent stain, Ziehl-Nelson stain, Lowenstein- Jensen (LJ) medium, Magnesium sulfate, NaOH 4%, phosphate buffered saline (PBS), Distilled water, Monopotassium Phosphate, Egg suspension, Potato flour, L-Asparagine, Magnesium citrate, Malachite green, Glycerol.

### *Media preparation*

LJ agar medium was used as selective media for the growth of MTB which was prepared by following the manufacturer's instructions [13]. The culture media was prepared by dissolving 18.65 grams of LJ medium base in 300 ml of distilled water. 6ml of reagent-grade glycerol was added. The solution was autoclaved for 30 minutes at 121ºC before being cooled. 500ml of a homogenized whole egg was mixed in it. The medium was then dispensed in 6-8 ml increments into 20 x 150 mm screw-capped tubes. These culture tubes were condensed for 50 minutes at 85ºC. When no bacterial contaminants were detected, the prepared culture media was incubated at 37ºC for 48 hours and stored in the refrigerator. To prevent evaporation during storage, all tubes were firmly capped.

### *Sample collection*

One hundred sputum samples of the suspected patients for TB were collected from the outdoor department of Bahawal Victoria Hospital, Bahawalpur, Pakistan for

a duration of three months. The patients were given a plastic cup and asked to go outside of the department to the sputum booth for sputum sample collection. Patients must take a huge inhale and hold it for a few seconds before slowly breathing out and coughing vigorously till some sputum comes up into their mouth. Into the plastic cup, spit the sputum. Continue doing so until the sputum reaches the 5ml line on the cup which is approximately one or a half teaspoon of sputum. At least ten samples per day were sent to the laboratory for further testing. Specific details of patients, *i.e*., their names and patient codes were labeled on the cups.

***Smear microscopy***

Smear was prepared from the sputum to confirm the presence of the bacteria and to be acquainted with its morphology. The smear preparation required certain conditions to be fulfilled such as collecting samples, storing the samples at a particular temperature i.e., 2-8ºC, and keeping them out of direct sunlight. Ziehl-Neelson method was used for acid-fast bacilli (AFB) staining.

***Semi-quantitative nested real-time PCR assay***

Each of the samples was examined using GeneXpert MTB/RIF assay RIF test. All samples were inoculated at the optimal room temperature for almost 15 minutes after being treated with sample reagent buffer (NaOH and isopropanol). The sample and buffer ratio was 1:2 respectively. After that, 2 mL of each sample was mixed in a vortex and filled in GeneXpert MTB/RIF cartridge. Finally, the GeneXpert machine was loaded with the cartridges. Using GeneXpert's software version 4.3, the findings were created and recorded after 2 hours.

***PCR assay for anti-TB drug-resistance detection***

The most consistent results were received utilizing the GeneXpert MTB/RIF assay. It is an automated molecular test that comes with all of the reaction fluids and primers needed for cell lysis, DNA extraction, amplification, and detection in a single plastic cartridge (**Table 1**). When preconditioned sputum was injected into the MTB/RIF cartridge and the GeneXpert machine showed findings for TB and first-line drug resistance within 2 hours, it automatically started PCR. Even this, in 2011, the WHO recommended its active usage for MDR-TB control. The findings of the real-time PCR assay show whether MTB was found in the sample. In some situations, the result may be "invalid," needing the test to be repeated. If MTB is discovered, the data will also provide if RIF resistance was discovered, was not discovered, or was uncertain.

***Bacterial culture***

As smear microscopy and PCR also detect dead bacteria with live ones without distinguishing between them, it is necessary to perform a culture method for detecting only live bacteria. For the production of bacterial culture, 4% NaOH was added to the sample and a vortex mixer was used to prepare the homogenized mixture. The resulting mixture was kept aside for at least 20 minutes before using the vortex mixer for 5 minutes again to mix them well. In the next step, 50ml of Phosphate Buffered Saline (PBS) was added to the sample to maintain its pH level. It was then placed in a refrigerated centrifuge at 3500 rpm, 4ºC for 10 minutes so that heat produced during spinning does not kill bacteria and to separate supernatant and sediment in the resulting mixture. The supernatant was discarded in 10% bleach and sediment was kept because it had the bacteria. Sediment was re-suspended with 2-3ml PBS with the help of a dropper. It was once again put in a vortex mixture and later inoculation was done i.e. 2-3 drops of sediment were put into LJ Media (double inoculation was done i.e., two slants/bottles of each sample were used as a precautionary measure). The slants were placed in an incubator at 37ºC for 8 weeks. The lids of the slants were kept loose for 2-3 days for aerobic purposes. All the growths should be monitored regularly for five to seven days of inoculation and then once a week for up to eight weeks. Typical wilted, unpigmented and uneven growths can be seen on LJ medium. Malachite green, one of the selective agents in LJ media works to limit the growth of most other pollutants and gives the medium its grassy color.

***Phenotypic drug susceptibility testing (DST)***

Drug susceptibility in MTB can be assessed by looking for metabolic growth or suppression in a medium containing an anti-tuberculosis drug [14]. To investigate antimicrobial susceptibility, the agar method of proportion on BACTEC was utilized. It was computed by contrasting growth counts on drug-containing agar to growth counts on drug-free agar. The organism must be deemed drug resistant if it

**Table 1**: Components used in real-time PCR assay.

| Amount | Component | Final Concentration |
|---|---|---|
| 5 µL | 10x PCR Buffer (L27989) | 1x |
| 1 µL | Deoxynucleotide Mix | 200 µM |
| 1µL | Forward primer (*5-GCCTTGCCAGCCCGCTCAGTCCGTGCACCCCACCcaytayggnmg-3*) (typically 15-30 bases in length) | 20pmol |
| 1µL | Reverse primer (*5- GCCTCCCTCGCGCCATCAGCCCACGGCCTGCckytgcatrtt-3*) (typically 15-30 bases in length | 20pmol |
| 0.5 µL | *Taq* DNA Polymerase | 2.5 units/µL |
| 1µL | Template DNA | ~$10^5$Molecules |
| 3 µL | 25 mM $MgCl_2$ | 1.5 mM |
| 37.5 µL | Water | |
| 50 µL | Total reaction volume | |

grows at a rate of less than 1% on the medium having drug contrast to the media having no drug [15].

## Results

### *Culture on LJ medium*

Although one or two samples were found to be negative in smear microscopy by using the culture method on a solid LJ medium 86 out of 100 samples were confirmed to be positive for TB. MTB forms bronze-colored, gritty colonies after eight weeks, which are also known as tough, buff, and rough colonies.

### *Comparison of positivity rate among pulmonary and extrapulmonary samples*

An immunochromatographic (ICT) test was utilized for the detection of plasma and serum samples. Out of 100 samples, 86 were confirmed to be positive (**Table 2**).

**Table 2:** Observation of +ve and –ve samples.

| Sr.# | Sample type | Samples received | +ve | %age |
|---|---|---|---|---|
| 1 | Plasma | 2 | 2 | 100% |
| 2 | Serum | 2 | 2 | 100% |
| 3 | Urine | 6 | 4 | 66.7% |
| 4 | CSF | 0 | 0 | 0 |
| 5 | Ascitic Fluid | 0 | 0 | 0 |
| 6 | Pleural fluid | 8 | 3 | 37.5% |
| 7 | Pericardial fluid | 0 | 0 | 0 |
| 8 | Pus | 0 | 0 | 0 |
| 9 | Bone marrow | 0 | 0 | 0 |
| 10 | Sputum | 72 | 65 | 90% |
| 11 | BAL | 10 | 10 | 100% |
| 12 | Total | 100 | 86 | 82.4% |

### *Male/Female ratio*

There were 100 total samples collected out of which 38 were females and 62 were males, out of these 100 samples 86 stand positive for MTB and 14 were negative. Of these 14 negative samples, 10 were males and 4 were females so 34 remaining females stood positive for MTB and 52 remaining males were positive for MTB (**Table 3**). So, males show higher positivity rates in Plasma, BAL, and serum while females show higher rates of being positive in urine sputum, pleural fluids, and urine.

**Table 3:** Male/Female ratio in +ve samples.

| Sr. # | Sample type | +ve samples | Males | Females |
|---|---|---|---|---|
| 1 | Plasma | 2 | 2/2 | 0 |
| 2 | Serum | 2 | 2/2 | 0 |
| 3 | Urine | 4 | 1/1 | 3/3 |
| 4 | Pleural fluid | 3 | 0 | 3/3 |
| 5 | Sputum | 65 | 39/39 | 26/26 |
| 6 | BAL | 10 | 8/8 | 2/2 |
| 7 | Total | 86 | 52 | 34 |

### *Positivity rates in different age groups*

The effect of age on sample positivity was also investigated, and it was discovered that the most positive samples were found in the 40–60-year age group, while the second most positive samples were located in the 20–40-year age group (**Table 4**).

### *Effect of Smoking on MTB positivity*

Out of the hundred total collected samples, 54 were smokers and 46 were non-smokers, while as, out of 86 samples that were confirmed positive for MTB 47 were smokers and 39 were non-smokers (**Table 5**).

### *Home hygienic conditions*

Good, average, and poor are the scales of measurement referring to the home hygienic conditions of TB patients (**Table 6**).

### *Antibiotic sensitivity*

Around 48 isolates were found resistant to INH

**Table 4:** Positivity rates in different age groups.

| Sr. # | Types of +ve samples | 10-20 years | 20-40 years | 40-60 years | Above 60 years |
|---|---|---|---|---|---|
| 1 | Plasma=2 | 0 | 0 | 2 | 0 |
| 2 | Serum=2 | 1 | 0 | 1 | 0 |
| 3 | Urine=4 | 2 | 1 | 1 | 0 |
| 4 | Pleural fluid=3 | 0 | 0 | 3 | 0 |
| 5 | Sputum=65 | 2 | 31 | 25 | 7 |
| 6 | BAL=10 | 2 | 2 | 5 | 1 |
| 7 | Total=86 | 7 | 34 | 37 | 8 |

antibiotic and 53 isolates were resistant to RIF (**Table 7**).

## Discussion

TB is a consequential and tenacious public health problem, particularly in developing countries, with a high morbidity and mortality rate. Because the need for an immediate, fast, and sensitive diagnosis is growing, we looked into a diagnostic method that could help advance the diagnosis of MTB [16]. According to our findings, the clinical utility of the MTB PCR test for the rapid diagnosis of both extrapulmonary and pulmonary TB showed results with the high sensitivity in males than in females, as there was a significant difference in TB positivity in males (60.4%) and females (41.8%). When it came to positivity rates in various specimens, males had greater positivity rates in plasma, serum, and BAL samples, whereas females had higher positivity rates in sputum, urine, and pleural fluids samples. However, Marais, Gupta [17] Reveals that except for the private trust hospital, the overall M:F ratio is 1:0.4, which is very closely reflected in the various centers for which data was obtained. Males outnumbered females in the public health facilities (M:F: 1:0.8 and 1:0.6). Lin, Chen [18] observes that females were more likely than males to have concurrent EPTB in this study, and this gender disparity was most noticeable in patients aged 45 years old compared to those aged 45 years old. And men of every age have more prevalence of PTB than women. According to Rhines [19] TB epidemiology is marked by huge disparities in prevalence between men and women around the world, with male cases outnumbering female cases by a factor of 2:1 in some areas.

The effect of age on the positivity of samples was also investigated. The majority of positive samples (43%) were identified in the age category of 41 to 60 years. Crampin, Glynn [20] showed that the age effect has been studied in a population, but the results vary, being younger was found to be a potential risk factor for getting EPTB in one study that compared EPTB cases to PTB cases. Moreover, our findings reveal that the real-time PCR assay is a much more sensitive and specific test for accurate TB diagnosis than conventional diagnostic procedures (microscopic and smear culture). Other studies conducted in different populations revealed inconsistent results. Agrawal, Bajaj [21] real-time PCR Assay has a higher sensitivity than AFB smear microscopy in respiratory samples. According to Shi, Dong [22] the results of this study demonstrate that real-time PCR assay outperforms mycobacterium culture in detecting MTB from salivary sputum, given the important role of PCR and mycobacterium culture in detecting TB.

**Table 5:** Effect of Smoking on MDR positivity ratio.

| | Smokers | Non-smokers |
|---|---|---|
| Total samples (n=100) | 54 | 46 |
| MTB positive (n=86) | 47 | 39 |

**Table 6:** Observation of home hygienic conditions.

| Good | Average | Poor |
|---|---|---|
| 19 | 40 | 41 |
| | Total=100 | |

## Conclusion

We concluded that poor living and working conditions are linked to a higher risk of TB transmission, as well as factors that weaken the host's defense against TB infection and diseases, such as HIV infection, malnutrition, smoking, diabetes, alcohol abuse, and indoor air pollution, appear to be important risk factors at the population level. Identification of TB is performed by using molecular techniques such as smear microscopy, real-time PCR Assay, and bacterium culture (gold standard) method. Results explain that both PTB and EPTB cases were higher in men (60.4%) as compared to women (41.8%). When it came to positivity rates in various specimens, males had greater positivity rates in plasma, serum, and BAL samples, whereas females had higher positivity rates in sputum, urine, and pleural fluids samples. Despite significant investments and efforts, annual TB incidence reductions are too slow to reach the end of

**Table 7:** Drug susceptibility testing.

| Drug susceptibility test | Types of drug resistance | No. of Isolates | |
|---|---|---|---|
| | | Susceptible | Resistant |
| DST test | INH | 0 | 48 |
| | | Total=48 | |
| | RIF | 0 | 53 |
| | | Total=53 | |

TB targets. Interventions aimed at known high-risk populations have proven to be successful and efficient.

## Conflict of interest

The authors declare no conflict of interest.

# References


[1] Ziskind B, Halioua B. Tuberculosis in ancient Egypt. Revue des maladies respiratoires. 2007;24:1277-83.

[2] Koch R. Die Ätiologie der Tuberkulose (1882). Robert Koch: Springer; 2018. p. 113-31.

[3] Behr MA, Waters WR. Is tuberculosis a lymphatic disease with a pulmonary portal? The Lancet infectious diseases. 2014;14:250-5.

[4] McMillen CW. Discovering Tuberculosis: Yale University Press; 2015.

[5] Horsburgh Jr CR, Goldberg S, Bethel J, Chen S, Colson PW, Hirsch-Moverman Y, et al. Latent TB infection treatment acceptance and completion in the United States and Canada. Chest. 2010;137:401-9.

[6] Ayles H, Schaap A, Nota A, Sismanidis C, Tembwe R, De Haas P, et al. Prevalence of tuberculosis, HIV and respiratory symptoms in two Zambian communities: implications for tuberculosis control in the era of HIV. PloS one. 2009;4:e5602.

[7] Qadeer E, Fatima R, Yaqoob A, Tahseen S, Ul Haq M, Ghafoor A, et al. Population based national tuberculosis prevalence survey among adults (> 15 years) in Pakistan, 2010–2011. PloS one. 2016;11:e0148293.

[8] Metzger P, Baloch N, Kazi G, Bile K. Tuberculosis control in Pakistan: reviewing a decade of success and challenges. EMHJ-Eastern Mediterranean Health Journal, 16 (Supp), 47-53, 2010. 2010.

[9] Organization WH. The global MDR-TB & XDR-TB response plan 2007-2008. Geneva: World Health Organization; 2007.

[10] Peter JG, van Zyl-Smit RN, Denkinger CM, Pai M. Diagnosis of TB: state of the art. European Respiratory Monograph. 2012;58:124-43.

[11] Ansari S, Amanullah MF, Ahmad K, Rauniyar RK. Pott's spine: diagnostic imaging modalities and technology advancements. North American journal of medical sciences. 2013;5:404.

[12] Kendig Jr EL, Kirkpatrick BV, Carter WH, Hill FA, Caldwell K, Entwistle M. Underreading of the tuberculin skin test reaction. Chest. 1998;113:1175-7.

[13] Piersimoni C, Scarparo C, Callegaro A, Tosi CP, Nista D, Bornigia S, et al. Comparison of MB/Bact alert 3D system with radiometric BACTEC system and Lowenstein-Jensen medium for recovery and identification of mycobacteria from clinical specimens: a multicenter study. Journal of clinical microbiology. 2001;39:651-7.

[14] Díaz-Infantes MS, Ruiz-Serrano MJ, Martínez-Sánchez L, Ortega A, Bouza E. Evaluation of the MB/BacT mycobacterium detection system for susceptibility testing of Mycobacterium tuberculosis. Journal of clinical microbiology. 2000;38:1988-9.

[15] Hall L, Jude KP, Clark SL, Wengenack NL. Antimicrobial susceptibility testing of Mycobacterium tuberculosis complex for first and second line drugs by broth dilution in a microtiter plate format. JoVE (Journal of Visualized Experiments). 2011:e3094.

[16] Khan M, Islam M, Ferdous J, Alam M. An Overview on Epidemiology of Tuberculosis. Mymensingh medical journal: MMJ. 2019;28:259-66.

[17] Marais BJ, Gupta A, Starke JR, El Sony A. Tuberculosis in women and children. The Lancet. 2010;375:2057-9.

[18] Lin C-Y, Chen T-C, Lu P-L, Lai C-C, Yang Y-H, Lin W-R, et al. Effects of gender and age on development of concurrent extrapulmonary tuberculosis in patients with pulmonary tuberculosis: a population based study. PloS one. 2013;8:e63936.

[19] Rhines AS. The role of sex differences in the prevalence and transmission of tuberculosis. Tuberculosis. 2013;93:104-7.

[20] Crampin A, Glynn J, Floyd S, Malema S, Mwinuka V, Ngwira B, et al. Tuberculosis and gender: exploring the patterns in a case control study in Malawi. The International Journal of Tuberculosis and Lung Disease. 2004;8:194-203.

[21] Agrawal M, Bajaj A, Bhatia V, Dutt S. Comparative study of GeneXpert with ZN stain and culture in samples of suspected pulmonary tuberculosis. Journal of clinical and diagnostic research: JCDR. 2016;10:DC09.

[22] Shi J, Dong W, Ma Y, Liang Q, Shang Y, Wang F, et al. GeneXpert MTB/RIF outperforms mycobacterial culture in detecting mycobacterium tuberculosis from salivary sputum. BioMed research international. 2018;2018.