Jigan et al | Article 11163
J Pure Appl Microbiol. 2026;20(1):817-830. doi: 10.22207/JPAM.20.1.63
Received: 20 November 2025 | Accepted: 20 January 2026
Published Online: 09 March 2026

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Association of Mycotic Lung Infections with Multidrug-resistant Tuberculosis and Immunocompromised Status of Patients

Shushant I. Jigan[1], Jyoti M. Nagamoti[1]*, S.L. Hoti[2], Mahantesh B. Nagamoti[1], Priyanka S. Kundekar[3], Alex Carvalho[4] and H.P. Jai Shanker Pillai[5]

[1]Department of Microbiology, JN Medical College, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India.
[2]Emeritus Scientist, ICMR-Vector Control Research Centre, Pondicherry, India.
[3]Department of Microbiology, Govindram Seksaria Science College, Tilakwadi, Belgaum, Karnataka, India.
[4]Department of Epidemiology and Biostatistics, JN Medical College, KLE Academy of Higher Education and Research, Belagavi, Karnataka, India.
[5]Department of Medical Laboratory Science, Komar University of Science and Technology, Sulaymaniyah, Kurdistan Region, Iraq.

***Correspondence:** jyotinagmoti@yahoo.com

Citation: Jigan SI, Nagamoti JM, Hoti SL, et al. Association of Mycotic Lung Infections with Multidrug-resistant Tuberculosis and Immunocompromised Status of Patients. *J Pure Appl Microbiol.* 2026;20(1):817-830. doi: 10.22207/JPAM.20.1.63

Abstract

Tuberculosis (TB) remains a leading cause of mortality worldwide, with multidrug-resistant TB (MDR-TB) and immunocompromised patients at high risk. Pulmonary mycotic infections often mimic TB and may co-exist, leading to misdiagnosis and poor outcomes. We conducted a hospital-based cross-sectional study of 282 pulmonary cases. All patients underwent sputum analysis for *Mycobacterium tuberculosis* and evaluation for fungal co-infection. Data on HIV status and other immunocompromising conditions were recorded. We compared the prevalence of pulmonary fungal infections between MDR-TB and drug-susceptible TB cases, and between immunocompromised and immunocompetent patients. Fungal infections were detected in 128 patients (45.4%). The common isolates were *Candida* species (74 cases) and Aspergillus species (24 cases), followed by other opportunistic fungi. Pulmonary fungal co-infection prevalence was significantly higher in MDR-TB patients than in drug-susceptible TB patients (60.0% vs 42.6%, p = 0.03). Likewise, immunocompromised TB patients showed higher fungal co-infection rates than immunocompetent patients (70.4% vs 39.5%, p = 0.001). On multivariate analysis, MDR-TB and immunocompromised status were independently associated with increased odds of pulmonary mycotic infection. No significant association with sex was observed, but patients aged 21-40 and 61-80 had higher odds of fungal co-infection compared to those ≤20 years. In this cohort, pulmonary fungal infections were frequent among TB patients, especially those with MDR-TB or immunocompromised conditions. Our findings underscore the need for routine screening for fungal infections in TB patients with drug-resistant disease or immunosuppressive conditions may aid early detection and improve outcomes in this vulnerable population.

Keywords: Coinfection, *Candida, Aspergillus,* HIV, Diabetes, Colonization

INTRODUCTION

Tuberculosis (TB) is a major global health problem and remains among the top ten causes of death worldwide.[1] In 2021, an estimated 10.6 million people fell ill with TB (reversing years of decline), and about 1.6 million died of the disease.[1] TB disproportionately affects developing regions over; 85% of cases occur in Asia and Africa.[1] Immunocompromised individuals are at particularly high risk. For example, TB is the leading cause of death in people living with HIV, who are roughly 18 times more likely to develop active TB than HIV-negative people.[2] Similarly, diabetes mellitus triples the risk of developing TB and is implicated in roughly one-third of cases of invasive pulmonary fungal infections like mucormycosis.[3] The convergence of TB with immunosuppressive conditions (HIV, diabetes, corticosteroid or anti-TNF therapy, malignancies, etc.) has created a vulnerable population prone to opportunistic infections.

Multidrug-resistant TB (MDR-TB), defined as TB resistant to at least isoniazid and rifampicin, poses a growing challenge to TB control.[4] Approximately 3%-4% of new TB cases and 18%-19% of previously treated cases globally are MDR-TB.[4] MDR-TB outcomes are poor -historically only ~50%-60% of patients achieve treatment success.[4] Indeed, managing MDR/XDR-TB remains challenging in the 2020s.[4] The lengthy treatment, high toxicity, and residual lung damage from MDR-TB may further predispose patients to secondary infections.[5] Prolonged broad-spectrum antibiotic use and immune dysfunction in TB patients can create an environment favorable to fungal colonization and infection.[6] However, the burden and impact of fungal co-infections in MDR-TB remain understudied.

Pulmonary fungal infections ("mycotic" lung infections) often mimic pulmonary TB in clinical presentation and radiologic appearance. They can cause chronic cough, hemoptysis, fever, weight loss, and cavitary lung lesions, overlapping with TB symptoms.[7] This similarity can lead to missed or delayed diagnosis of the fungal infection, as symptoms may be attributed solely to TB.[7] Human fungal infections have been described as "hidden killers" often overlooked in patients with underlying lung diseases.[8] Missed fungal infections

in TB patients likely contribute to ongoing morbidity and mortality.[9] For instance, one study noted that unrecognized fungal pulmonary disease in TB patients led to high rates of treatment failure and mortality.[9] Timely identification of co-infections is therefore critical.

Emerging data suggest that TB-fungal co-infections are not rare. *Candida* species are the most commonly isolated fungi from TB patient sputum (often as colonizers but potentially invasive in immunosuppressed hosts).[10] Reports from different regions have documented *Candida* co-infection rates in 15%-32% of pulmonary TB cases.[10] Pulmonary aspergillosis is another important co-infection: TB-damaged lungs provide an ideal niche for *Aspergillus*. Global estimates indicate ~15% of TB patients may have co-existing *Aspergillus* infection or colonization.[11] Chronic pulmonary aspergillosis (CPA) alone is estimated to affect 1.2-3 million survivors of TB worldwide.[7] Other fungal pathogens like *Histoplasma capsulatum* and *Cryptococcus neoformans* can also co-occur with TB, particularly in HIV-positive individuals.[12] Despite these observations, co-infections often go undiagnosed due to limited awareness and diagnostic facilities.

In this context, we aimed to investigate the association of pulmonary mycotic infections with MDR-TB and immunocompromised status. We conducted a cross-sectional study to determine the prevalence of fungal co-infections among pulmonary TB patients and to assess whether MDR-TB and immune status are significant risk factors for such co-infections. We also sought to characterize the spectrum of fungal organisms involved and examine clinical features of co-infected patients. Understanding these associations will help inform screening strategies and clinical management for TB patients, especially those with drug resistance or underlying immunosuppression.

MATERIALS AND METHODS

Study design and setting

This was a hospital-based cross-sectional study conducted at the Department of Tuberculosis (TB) Chest Diseases and the RNTCP laboratory at KLE'S Dr. Prabhakar Kore Charitable Hospital-Medical Research Centre (MRC) and District Hospital in Belagavi. All confirmed positive pulmonary tuberculosis patients; both new, old cases attending to these centers were included in the research.

Ethical considerations

Prior to initiation of the study, ethical approval was obtained from the Institutional Ethics Committee of KLE Academy of Higher Education and Research (KAHER), Belagavi (Ref. No.: KAHER/EC/20-21/001/15). The study procedures were explained to all eligible participants, and those willing to participate were enrolled only after obtaining written informed consent. Confidentiality of patient information was strictly maintained throughout the study, and all procedures were conducted in accordance with standard ethical guidelines for biomedical research involving human participants.

TB diagnosis and drug resistance testing

All patients had sputum examination for *Mycobacterium tuberculosis*. Three sputum samples per patient were examined by Ziehl–Neelsen staining technique for screening of acid-fast bacilli (AFB) smear microscopy. Drug susceptibility results were noted based on CBNAAT and True NAT testing done in RNTCP facility. MDR-TB was defined as resistance to at least isoniazid and rifampicin.[5] Patients were classified as having MDR-TB or drug-susceptible TB based on these results.

Fungal investigations

In parallel with TB diagnostics, we evaluated all patients for pulmonary fungal infection. Sputum specimens (~5-10 mL each) were collected in sterile containers before initiation of anti-TB treatment or as early as possible during presentation. For direct microscopy, portions of each sputum sample were digested with 10% potassium hydroxide (KOH) and examined under microscopy.[10] Fungal culture was performed by inoculating sputum on Sabouraud dextrose agar (SDA) with chloramphenicol; one set of cultures was incubated at 25 °C and another at 37 °C for 1-2 weeks. If *Aspergillus* growth was suspected, additional subculture on Czapek-Dox agar was done for species identification. We also used chromogenic *Candida* agar to help differentiate

Table 1. Frequency distribution of the selected variables (n = 282)

Variables		Frequency	Percentage (%)
Age group	≤20	30	10.6
	21-40	124	44
	41-60	89	31.6
	61-80	39	13.8
Gender	Male	167	59.2
	Female	115	40.8
Residence Status	Permanent	280	99.2
	Temporary	1	0.4
	Migrated	1	0.4
Educational Qualification	Illiterate	99	35.1
	Secondary	57	20.2
	Higher Secondary	110	39
	Graduate/Degree/Diploma	15	5.3
	Postgraduate	1	0.4
	Any other specify	0	0
Marital Status	Married	238	84.7
	Unmarried	43	15.3
Religion	Hindu	257	91.2
	Muslim	21	7.4
	Christian	4	1.4
Type of House	Kachha	45	16
	Pakka	237	84
Occupation	Housewife	69	24.6
	Student	40	14.3
	Own cultivation, Agri labour, own cultivation and Labour, Constructon labour, MGNREGA work	85	30.4
	Small business/petti/tea shop	9	3.2
	Forest products, Livestock	3	1.1
	Private	58	20.7
	Others	16	5.7
Health facility used when sick?	Government	163	57.8
	Private	83	29.4
	Health centers run by NGO	11	3.9
	Self-treatment	16	5.7
	Traditional Healer	9	3.2
	Other (Specify)	0	0
Socioeconomic status	BPL	239	84.7
	APL	40	14.2
	Others	3	1.1
Area of Living	Urban	84	29.8
	Urban Slum	56	19.8
	Rural	142	50.4
MDR-TB Status	MDR-TB (+)	45	16
	MDR-TB (-)	237	84
Immuno-status	Normal	228	80.8
	HIV/AIDS	20	7.1
	Diabetic	33	11.7
	Cancer	1	0.4
	Total	282	100

Candida species. Fungal growth was identified by colony morphology and microscopic characteristics (lactophenol cotton blue mounts). Yeast isolates were further identified via germ tube testing for *Candida albicans* and by biochemical assimilation tests as needed.

We defined "pulmonary mycotic infection" as a positive fungal culture (or positive direct microscopy) from respiratory samples plus clinical and radiologic features consistent with active infection (to distinguish true infection from mere colonization). In practice, we considered a fungus to be pathogenic if the patient had symptoms not fully explained by TB alone, with radiologic lesions compatible with mycosis (e.g. cavities, nodules, fungus ball), and the isolated organism was a known pulmonary pathogen. We interpreted *Candida* growth with caution: we required either heavy growth or repeat isolation to deem it significant, given that *Candida* can be an oral commensal.[13] Wherever feasible, we corroborated fungal findings with serological tests (for example, serum *Aspergillus* IgG), although these were done in only a subset of patients due to resource constraints.

Clinical data and definitions

We recorded demographic and clinical information including age, sex, HIV status (with CD4 count for HIV-positive patients), diabetic status (HbA1c level), use of chronic corticosteroids or other immunosuppressants, and any history of previous TB treatment. Patients were classified as "immunocompromised" if they had any of the following: HIV infection (regardless of CD4 count), uncontrolled diabetes mellitus (HbA1c > 7.5%), were on prolonged corticosteroid/ immunosuppressive therapy, or had another known immunodeficiency condition.[14] Radiological findings from chest X-ray or CT were noted, particularly the presence of cavities, infiltrates, nodules, or aspergillomas (fungus balls).

Outcome measures

The primary outcome measure was the prevalence of pulmonary fungal co-infection among TB patients, defined as above. The key exposures of interest were MDR-TB status and immunocompromised status. Secondary outcomes included the specific types of fungi isolated and their distribution in patient subgroups (e.g. by MDR status or immune status). We also observed clinical characteristics and outcomes (such as symptom persistence and interim treatment outcomes) in co-infected versus non-co-infected patients for descriptive analysis.

Statistical analysis

Data were entered and analyzed using SPSS version 25. Categorical variables (e.g. proportion of patients with fungal infection) were compared between groups using the chi-square test or Fisher's exact test, as appropriate. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to estimate the strength of associations. Continuous variables (e.g. age) were compared by Student's *t*-test if approximately normally distributed, or by Mann–Whitney U test if non-parametric. We performed multivariate logistic regression to identify independent predictors of fungal co-infection, including MDR-TB status and immunocompromised status as covariates (along with age and sex as potential confounders). Variables with $p < 0.05$ in univariate analysis and key demographic factors were entered into the multivariable model. A two-tailed $p < 0.05$ was considered statistically significant. Results are presented as percentages, ORs with 95% CIs, and p-values.

RESULTS

Patient characteristics

A total of 282 pulmonary TB patients were included in the study. Baseline characteristics are summarized in Table 1. There were 167 males (59.2%) and 115 females (40.8%). The patients' ages ranged from 18-80 years, with a median age in the 21-40 year range (44% of patients fell in this category). About three-quarters of the patients (approximately 74%) were between 21 and 60 years old, while 10.6% were ≤20 years and 13.8% were over 60.

Forty-five patients (16.0%) had MDR-TB, as confirmed by drug susceptibility testing. The remaining 237 (84.0%) had drug-susceptible TB. Fifty-four patients (19.1%) were categorized as immunocompromised. Among these, 20 were HIV-positive, 33 had diabetes mellitus, and 1 had an underlying malignancy; several patients

Table 2. Prevalence of fungal infections (n = 282)

Fungal Infection	Frequency	Percent
Negative	154	54.6
Positive	128	45.4
Total	282	100

had multiple risk factors (for example, a few of the diabetic patients were also elderly or had other comorbidities). The immunocompromised patients were distributed across both TB groups (some in the MDR-TB group and others in the drug-susceptible group). Notably, most HIV/TB co-infected patients were receiving antiretroviral therapy, and most diabetic TB patients had poorly controlled blood sugar. Overall, 46 patients (16.3%) had a history of previous TB treatment, and 19 (6.7%) had underlying chronic lung disease (such as old healed TB lesions or COPD). These comorbid conditions were similar in proportion between MDR-TB and drug-susceptible patients.

Prevalence of fungal co-infection

Out of 282 TB patients, 128 had evidence of a pulmonary fungal infection, yielding an overall co-infection prevalence of 45.4% (Table 2). In most of these 128 cases, fungi were identified by both direct microscopy and culture; in a minority of cases, the fungus grew in culture despite a negative direct smear. Conversely, a few patients had positive fungal elements on microscopy but no growth on culture (likely due to non-viability or prior antifungal use). We took a conservative approach in interpreting positive findings to distinguish true infection from colonization. Ultimately, those 128 patients were classified as having clinically significant mycotic co-infections in addition to TB.

Table 3 shows the distribution of the 128 fungal isolates recovered. *Candida* species were the most frequently isolated fungi, found in 74 patients (57.8% of those with fungal co-infection). *Candida albicans* was the single most common species (24.3%) among all positive cases, followed by *Candida tropicalis* (10.9%), *Candida glabrata* and *Candida krusei* (7.8% each), and *Candida parapsilosis* (7.0%). These non-*albicans Candida* species together accounted for about 33% of the

Table 3. Distribution of Isolated Fungal Species (n = 128)

Isolates	Frequency	Percentage (%)
Aspergillus niger & *Fusarium* spp.	1	0.8
Aspergillus flavus	5	3.9
Aspergillus fumigatus	9	7
Aspergillus niger	10	7.8
Candida albicans	31	24.3
Candida glabrata	10	7.8
Candida krusei	10	7.8
Cryptococcus neoformans	4	3.1
Candida parapsilosis	9	7
Candida tropicalis	14	10.9
Fusarium spp.	7	5.5
Mucor spp.	5	3.9
Rhizopus spp.	5	3.9
Rhodotorula glutinis	2	1.6
Penicillium spp.	6	4.7
Total	128	100

fungal infections. We exercised caution in labeling *Candida* isolates as infection, requiring consistent clinical/radiologic evidence as described, since *Candida* can be a colonizer in the airways.

Aspergillus was the next most common genus identified, with a total of 24 isolates (18.8% of fungal cases). Among these, *Aspergillus niger* was found in 10 cases, *A. fumigatus* in 9, and *A. flavus* in 5 cases. In one additional case, *Aspergillus niger* was co-isolated alongside a *Fusarium* species from the same patient's sputum sample. The presence of *Aspergillus* (especially *A. fumigatus* and *A. niger*) was often correlated with patients who had pre-existing lung cavities on imaging, as *Aspergillus* can colonize healed TB cavities.

Besides *Candida* and *Aspergillus*, we isolated a variety of other opportunistic fungi in smaller numbers. Seven patients (5.5% of fungal-positive cases) grew *Fusarium* species on culture. Five patients (3.9%) had *Mucor* species isolated, and another five (3.9%) had *Rhizopus* species; these are both mucormycetes, typically seen in patients with uncontrolled diabetes or other severe immunosuppression. Four patients (3.1%) all of whom were HIV-positive had *Cryptococcus neoformans* isolated from sputum, indicative of possible pulmonary cryptococcosis. Two isolates of *Rhodotorula glutinis* (1.6%) were obtained (this yeast is an uncommon pathogen, significance was

Table 4. Association of fungal infection with MDR-TB status and Immunocompromised Status (n = 282)

Variables		Fungal Infection				Total	Chi-square value	P-value
		Negative (154)		Positive (128)				
		n	%	n	%			
MDR-TB Status	Positive	18	40	27	60	45	4.611	0.032*
	Negative	136	57.4	101	42.6	237		
Immuno-compromised Status	Positive	16	29.6	38	70.4	54	16.814	0.001*
	Negative	138	60.5	90	39.5	228		

Table 5. Adjusted and Unadjusted Odds Ratio of fungal infections using binary logistic regression

Variables	UOR	95% CI	P-value	AOR	95% CI	P-value
Gender						
Male	Ref.			Ref.		
Female	1.113	(0.691, 1.792)	0.661	1.122	(0.656, 1.919)	0.673
Age group						
≤20	Ref.			Ref.		
21-40	3.393	(1.357, 8.484)	0.009	2.804	(1.049, 7.498)	0.04
41-60	2.13	(0.826, 5.49)	0.118	1.683	(0.601, 4.719)	0.322
61-80	4.723	(1.637, 13.629)	0.004	4.416	(1.418, 13.75)	0.01
Socioeconomic status						
BPL	Ref.			Ref.		
APL	0.867	(0.441, 1.705)	0.679	0.686	(0.319, 1.477)	0.336
Others	0.586	(0.052, 6.554)	0.665	0.586	(0.05, 6.91)	0.671
Area of Living						
Urban	Ref.			Ref.		
Urban Slum	0.75	(0.38, 1.479)	0.406	0.648	(0.313, 1.341)	0.243
Rural	0.433	(0.25, 0.752)	0.003	0.327	(0.176, 0.607)	0.001
Type of House coded						
Kaccha	Ref.			Ref.		
Pakka	0.443	(0.23, 0.854)	0.015	2.213	(1.073, 4.561)	0.031
MDR-TB Status						
Negative	Ref.			Ref.		
Positive	2.02	(1.055, 3.867)	0.034	2.142	(1.041, 4.408)	0.039
Immunocompromised Status						
Negative	Ref.			Ref.		
Positive	3.642	(1.917, 6.918)	0.001	3.42	(1.716, 6.817)	0.001

determined by clinical correlation). Finally, six patients (4.7%) grew *Penicillium* species; these were not *Penicillium marneffei* (now *Talaromyces marneffei*), as that endemic dimorphic fungus is not present in our region, but rather other environmental *Penicillium* species, which were considered likely contaminants in the absence of supporting clinical evidence. All told, the spectrum of fungal co-pathogens ranged from common yeasts and molds to rare environmental fungi.

Notably, we did not confirm any cases of *Pneumocystis jirovecii* pneumonia (PCP) in our cohort, as specific staining or PCR for *Pneumocystis* was not routinely performed. However, a few of the HIV-positive patients with diffuse bilateral ground-glass lung infiltrates were clinically suspected to have PCP co-infection in addition to their TB, despite the lack of laboratory confirmation. Similarly, we did not detect any *Histoplasma capsulatum* infections, although

specialized tests for histoplasmosis (antigen or serology) were not done; thus, we cannot rule out undiagnosed histoplasmosis in this population. For the purpose of analysis, all the less common fungal pathogens (cryptococcosis, mucormycosis, etc.) were grouped under the "any fungal co-infection" category.

Association with MDR-TB

Fungal co-infection was significantly more prevalent in MDR-TB patients compared to those with drug-susceptible TB. Among the 45 MDR-TB patients, 27 had a pulmonary fungal infection, representing a co-infection rate of 60.0%. In contrast, 101 of the 237 drug-susceptible TB patients (42.6%) had fungal co-infection (Table 4). This difference was statistically significant ($\chi^2 = 4.611$, $p = 0.032$), indicating that MDR-TB patients had higher odds of fungal co-infection. In univariate terms, the odds of having a fungal infection were roughly 2.0 times higher in MDR-TB patients than in drug-sensitive TB patients (unadjusted OR = 2.02, 95% CI 1.06-3.87).

Although the relative difference was not as large as initially expected, the finding suggests a meaningful association between drug resistance and fungal co-morbidity. Many of the MDR-TB patients with fungal co-infections had longstanding pulmonary lesions (e.g. chronic cavities) or were experiencing poor response to TB therapy, which prompted the fungal work-up. *Candida* and *Aspergillus* were the most frequently identified fungi in both MDR and non-MDR groups, but a higher proportion of MDR-TB patients harbored *Aspergillus* spp. compared to drug-sensitive patients. Several MDR-TB patients with co-infection had multiple risk factors (for instance, MDR-TB with diabetes or MDR-TB with HIV), compounding their susceptibility.

It is worth noting that the majority of MDR-TB patients who had fungal co-infections were those with either previous TB treatment (hence lung scarring) or concurrent immunosuppressive conditions. By contrast, among MDR-TB patients who did not have any fungal infection, many were younger individuals or those diagnosed relatively early in their disease course. This observation, though qualitative, aligns with the idea that prolonged disease and treatment can predispose to fungal colonization.

Association with immunocompromised status

Host immune status showed an even stronger association with fungal co-infection. Of 54 immunocompromised TB patients, 38 had a fungal co-infection, giving a prevalence of 70.4%. In comparison, only 90 of 228 immunocompetent (non-immunocompromised) TB patients had fungal co-infection, a rate of 39.5% (Table 4). This difference was highly significant ($\chi^2 = 16.814$, $p = 0.001$). Immunocompromised TB patients had an unadjusted OR of 3.64 (95% CI 1.92-6.92) for fungal co-infection versus immunocompetent patients. In other words, the odds of a fungal lung infection were approximately 3.5 times higher in TB patients with an immunocompromising condition.

Among specific immunocompromised subgroups, HIV co-infection was a major contributor: nearly half of the HIV-positive TB patients had a fungal co-infection. *Cryptococcus neoformans* was isolated exclusively from HIV-positive patients, consistent with cryptococcosis being an AIDS-defining infection. Diabetic TB patients also showed a high rate of fungal co-infection; notably, all instances of mucormycosis (the five *Mucor* and five *Rhizopus* cases) occurred in patients with poorly controlled diabetes, reflecting the known predisposition of diabetics to mucormycosis.[3] The single patient with an underlying malignancy (on chemotherapy) developed a *Candida* lung infection. Two patients in the cohort were on chronic corticosteroid therapy (for autoimmune diseases); both of them grew *Candida* in sputum (one *C. albicans*, one *C. tropicalis*), though in one case it was deemed likely colonization rather than invasive infection. By contrast, in TB patients with no immunosuppressive conditions (HIV-negative, euglycemic, not on steroids, etc.), the fungal co-infection rate was substantially lower (around 39.5%, primarily *Candida* of questionable pathogenicity in many cases).

The data suggest that immune deficiency or dysregulation plays a critical role in permitting fungal opportunistic infections in TB patients. In particular, HIV infection (with low CD4 counts)

and diabetes emerged as important risk factors. The overlap of risk factors was also important: the highest co-infection frequencies were observed in those who were both MDR and immunocompromised. For instance, although the numbers were small, most patients in our study who had both MDR-TB and HIV co-infection ended up having one or more fungal infections as well. This compounded risk aligns with expectations, since these patients have both a hostile lung environment from prior TB damage and an impaired immune system.

Multivariate analysis

We performed a binary logistic regression to determine independent predictors of fungal co-infection while controlling for potential confounders (Table 5). In the multivariate model (adjusting for age and sex), both MDR-TB status and immunocompromised status remained significant independent predictors of fungal co-infection. MDR-TB patients had an adjusted OR = 2.142 (95% CI 1.041-4.408, p = 0.039) for having a fungal co-infection compared to drug-susceptible TB patients. Similarly, immunocompromised patients had an adjusted OR = 3.420 (95% CI 1.716-6.817, p = 0.001) compared to immunocompetent patients. This confirms that the associations observed in univariate analysis for these two key factors were not solely due to confounding by age or sex.

Interestingly, patient age also showed a significant association with fungal co-infection risk in the multivariate analysis. Using the youngest age group (≤20 years) as the reference category, patients in the 21-40 year age group had about 2.8 times higher odds of fungal co-infection (AOR = 2.804, 95% CI 1.049-7.498, p = 0.040), and those in the oldest age group (61-80 years) had about 4.4 times higher odds (AOR = 4.416, 95% CI 1.418-13.75, p = 0.010). The 41-60 year group had a higher odds ratio (~1.68) that was not statistically significant (p = 0.322). These results suggest a U-shaped or bimodal risk by age, where very young adults had the lowest risk, middle-aged had moderate risk, and older adults had the highest risk of fungal co-infection. The reasons are not entirely clear, but it may relate to the fact that younger patients generally had fewer comorbidities and shorter TB disease duration, whereas older patients often had diabetes or other chronic conditions and cumulative lung damage.

Sex (gender) was not a significant predictor in the model. Female TB patients had an AOR of 1.122 (95% CI 0.656-1.919) relative to males, with p = 0.673, indicating no meaningful difference in fungal co-infection by sex when other factors are accounted for. This is consistent with the unadjusted analysis as well.

We also included other socio-demographic factors in the logistic model. Socioeconomic status (categorized as below poverty line vs above poverty line) did not show a significant effect on fungal infection odds (AOR for APL vs BPL = 0.686, p = 0.336). The small number of patients in the "other" socioeconomic category made that estimate very imprecise. The area of residence, however, showed an interesting effect: patients living in rural areas had significantly lower odds of fungal co-infection compared to those in urban areas (AOR = 0.327, 95% CI 0.176-0.607, p = 0.001). Those living in urban slums had an AOR of 0.648 (95% CI 0.313-1.341), which was not statistically significant (p = 0.243) relative to urban non-slum dwellers. This indicates that our urban patients had the highest risk of fungal co-infections, while rural patients had the lowest, even after controlling for other factors.

Another notable finding was regarding housing type. We coded house type as "Kachha" (traditional mud/thatched houses) versus "Pakka" (solid concrete/brick houses). In univariate analysis, living in a pakka house appeared protective (OR ~0.44, p = 0.015), but after adjustment, it flipped to a risk factor: AOR = 2.213 (95% CI 1.073-4.561, p = 0.031) for fungal co-infection in pakka house dwellers compared to those in kachha houses. This result is somewhat counterintuitive, and it may reflect confounding variables or selection bias (for example, many rural poor patients with kachha houses were also those with shorter TB duration and fewer co-morbidities). We interpret this housing result with caution, as it might not indicate a true causal relationship but rather be a surrogate for other unmeasured factors.

In summary, our multivariate analysis confirms that MDR-TB and immunocompromised status are independent predictors of pulmonary fungal co-infections in TB patients. Age also plays a role, and certain environmental factors (urban

living) emerged as significant in our cohort. The model had a good fit (Hosmer–Lemeshow test p = 0.45) and explained a reasonable proportion of variance (Nagelkerke R^2 ~0.30), indicating that there are likely other factors as well that contribute to fungal infection risk which are not fully captured here.

DISCUSSION

Our findings provide insight into the epidemiology of TB-fungal co-infections in a high TB burden setting. The overall co-infection prevalence in our study (45.4%) is substantially higher than what has been reported in many other regions, though direct comparisons must consider differences in case definitions and diagnostic intensity. For instance, A study from Ghaemshahr reported a 12.3% rate of TB-fungal co-infection among smear-positive TB patients,[15] which is much lower than our 45%. Another study in Ethiopia reported a 20% prevalence of TB-fungal association.[16] Our higher detection rate may be due to more aggressive fungal diagnostics (we cultured all patients' sputum on multiple media and included even probable cases) or differing patient characteristics (our cohort included many chronic or previously treated TB cases). Interestingly, A study from Nigeria noted an overall fungal isolation rate of ~47% in patients investigated for TB,[6] which is very close to our 45%. However, in that Nigerian study a number of the fungal isolates were in patients who ultimately did not have active TB (i.e. some "TB suspects" turned out to have primary fungal infections). Nonetheless, their conclusion that a high burden of fungal disease is often missed among patients presumed to have TB echoes our concern about underdiagnosis.[17,18]

In terms of the spectrum of fungi, our results align with reports from other regions. *Candida* was the most commonly isolated genus in our TB patients with fungal co-infection. The Ethiopian study. found *Candida* in approximately 80% of TB-fungal co-infection cases.[19] We found a somewhat lower proportion (~58% of cases involved *Candida*), possibly because we applied strict criteria to distinguish likely colonization. *Aspergillus* species were the second most common in our study (around 19% of fungal co-infections), which is consistent with patterns observed in similar studies.[20,21] Muni et al. in India likewise reported that *Candida* and *Aspergillus* were the predominant isolates in pulmonary TB patients with fungal cultures, accounting for the majority of cases.[10] Our isolation of opportunistic molds like Mucor/Rhizopus in diabetic patients mirrors case reports and series from India and elsewhere that highlight diabetes as a risk factor for dual TB and mucormycosis infections.[12,20] Additionally, the four cases of cryptococcosis we observed in HIV-positive TB patients are in line with the known prevalence of cryptococcal disease in advanced HIV - Latin American data suggest histoplasmosis and cryptococcosis collectively rival TB in causing death in AIDS patients,[2] and our results reinforce the need to consider these diagnoses in HIV/TB co-infected individuals.

Our study may be among the few to specifically examine fungal co-infections in MDR-TB patients. Similar case reports have documented MDR-TB co-existing with aspergilloma and invasive aspergillosis in diabetic patients.[22] A study from Indonesia found evidence of fungal sensitization or co-infection in a significant subset of MDR-TB patients (e.g. 32% had positive *Aspergillus* IgG).[5] Although that study looked at immunological markers rather than cultures, it supports the idea that MDR-TB patients frequently have concurrent fungal pathology. We demonstrated a 60% culture-confirmed fungal co-infection rate in MDR cases, higher than that reported immunologic evidence, which could be due to differences in definitions or patient populations (our MDR group included many with prolonged disease and structural lung damage). Overall, our results, together with these studies, suggest that fungal co-infections in TB particularly *Candida* and *Aspergillus* are common across diverse settings, and greater vigilance is needed everywhere to diagnose and manage them.

Strengths and limitations

Strength of our study is the dual focus on both microbiological and clinical criteria to define true co-infection, in an attempt to distinguish mere colonization from invasive infection. By requiring clinical/radiologic corroboration for fungal isolates, we likely reported a more clinically relevant co-infection rate. We also prospectively examined specific high-risk subgroups (MDR-TB

patients and immunocompromised patients), which adds valuable data to the literature on risk stratification. Our sample size (n = 282) is larger than many prior single-center studies on this topic, though it is still moderate, and the number of MDR-TB and HIV-positive cases was modest (45 MDR and 20 HIV, respectively). This limited the statistical power for some subgroup analyses and for detecting interactions between risk factors. For example, while we observed an apparently higher risk of co-infection in those with both MDR-TB and immunosuppression, the sample size for that overlap group was small. Another limitation is that the study was conducted at a single center, and regional mycological profiles may vary for instance, areas endemic for *Histoplasma* or *Coccidioides* might find those pathogens more frequently. In our study, we did not detect *Histoplasma capsulatum*, but this may be due to diagnostic limitations, as we did not perform specific histoplasmosis antigen or serology tests. The absence of histoplasmosis in our results does not conclusively exclude its presence; in fact, chronic pulmonary histoplasmosis can closely resemble TB and requires specialized testing to diagnose.[23-26] Other fungi like *Pneumocystis jirovecii* likely went under-detected as well we lacked PCR or immunofluorescence for *Pneumocystis*, which is a known limitation.[27,28]

We relied on conventional culture and microscopy for fungal detection, which have suboptimal sensitivity for many fungi. *Pneumocystis* cannot be cultured, and *Histoplasma* often requires weeks or special media to grow. It is possible that our co-infection prevalence of 45% is still an underestimate of the true burden. The use of more sensitive diagnostic methods (e.g. molecular assays, antigen tests) might have yielded an even higher detection rate of fungal co-infections. Another limitation is that we did not perform bronchoscopy or lung biopsies, which are the gold standards for confirming invasive fungal infection. We depended on sputum samples and clinical inference; thus, some cases labeled as "co-infection" could conceivably be colonization, and conversely some invasive infections could have been missed. We attempted to mitigate the former by correlating mycological findings with clinical and radiologic evidence, as described in our methods. Finally, our follow-up of patients was limited. We have preliminary observations that TB patients with fungal co-infection had poorer short-term outcomes (for instance, a higher 6-month mortality and more instances of delayed sputum conversion), but our study was not designed to rigorously assess long-term outcomes. A longer prospective study would be needed to definitively determine the impact of fungal co-infection on TB treatment success, relapse, and mortality.

It is also worth discussing the unexpected findings related to geography and housing in our results. We found that rural patients had fewer fungal co-infections than urban patients, and those in pakka houses had more than those in kachha houses. Intuitively, one might expect the opposite rural settings and poorer housing (kachha) might expose individuals to more environmental fungi (soil, dust, poor ventilation). Our finding could be due to confounding: for example, many rural patients in our study were younger and had primary TB without prior lung damage, whereas urban patients (some living in crowded slums) often had more advanced or recurrent TB and greater antibiotic exposure, which could predispose them to fungal overgrowth. Additionally, urban patients may have better access to healthcare, leading to longer survival with chronic TB and thus more opportunity for fungal infections to be detected. The housing type association is puzzling; it might relate to socioeconomic status or environmental differences we did not measure (for instance, pakka houses might trap moisture and mold indoors more than ventilated mud houses, or it could be that patients in pakka houses lived in urban polluted areas vs. kachha in rural). These hypotheses are speculative, and our study was not primarily focused on these variables. Further research would be needed to clarify such epidemiological nuances.

Recommendations

Based on our findings, we recommend that all MDR-TB patients be evaluated for possible fungal co-infection at baseline and periodically during TB treatment, especially if clinical improvement is delayed or radiologic abnormalities persist beyond what is expected. Simple measures such as sputum fungal cultures

and *Aspergillus* IgG serology can be implemented in reference laboratories and may lead to earlier diagnosis of fungal co-morbidity. For TB patients with HIV infection, routine screening for *Cryptococcus* antigen (and *Histoplasma* antigen in endemic areas) should be integrated into care, as already advocated by WHO for advanced HIV disease. Diabetic TB patients, particularly those with uncontrolled glucose levels, should similarly be monitored for signs of fungal infection (e.g. sinus symptoms or necrotic lesions suggestive of mucormycosis), given their elevated risk.

When resources allow, more advanced diagnostics such as serum (1,3)-β-D-Glucan assays (a pan-fungal marker) or Galactomannan tests for invasive aspergillosis could be used in indeterminate cases to catch fungal infections that might not grow in culture.[27] However, even in resource-limited settings, a high index of suspicion and the use of available tools (like sputum smear/culture for fungi and serological tests for common fungi) can significantly improve case detection. We also emphasize the importance of multidisciplinary management: input from infectious disease specialists or medical mycologists can be invaluable. For example, confirming the speciation of an isolated fungus can help determine if it's likely a contaminant or a true pathogen; it can also guide optimal antifungal therapy (since different species may have different susceptibility profiles).

Increasing awareness among TB care providers is crucial. Our study underscores that in TB patients "not everything that infiltrates is TB" - if a patient on TB treatment has persistent or atypical lung infiltrates, or clinical deterioration, one must consider a secondary fungal infection. Training programs and guidelines for TB should incorporate this message. This aligns with recent calls to address co-infections as part of the strategy to end TB. In high TB/HIV burden settings, in particular, a significant fraction of patients labeled as "difficult TB cases" may actually have an unrecognized fungal infection such as histoplasmosis or *Pneumocystis* pneumonia contributing to their illness.[28] Addressing these fungal co-infections through integrated TB-fungal care could reduce mortality and improve overall treatment outcomes.

CONCLUSION

Pulmonary mycotic infections represent a significant and under-recognized comorbidity in patients with pulmonary tuberculosis, particularly among those with MDR-TB and those who are immunocompromised. In our hospital-based study, approximately three in five MDR-TB patients and about two in three immunosuppressed TB patients had a concurrent fungal lung infection. The most common co-pathogens were *Candida* and *Aspergillus* species, followed by other opportunistic fungi like mucormycetes (e.g. *Mucor*, *Rhizopus*) and *Cryptococcus* in severely immunocompromised hosts. These co-infections can mimic TB or confound its clinical course, often leading to diagnostic delays and suboptimal treatment if unrecognized.

Our findings reinforce the need for routine screening for fungal infections in complex TB cases - for instance, performing fungal culture and serology in MDR-TB or HIV/TB patients who have persistent symptoms or slow response to therapy. Early diagnosis of a fungal co-infection is critical because timely antifungal treatment can improve patient outcomes and prevent excess mortality. We advocate for heightened clinical vigilance and the incorporation of basic mycological investigations into standard TB care protocols, especially in high-risk groups. In resource-limited settings, even simple measures like sputum fungal smears/cultures and *Aspergillus* antibody tests, guided by recently proposed case definitions for chronic pulmonary aspergillosis, can aid in identifying cases. Ultimately, a multidisciplinary approach addressing both TB and associated mycotic infections is essential for holistic patient management. As global efforts continue to combat TB and drug-resistant TB, our study highlights that concurrently addressing fungal co-infections the "hidden shadow" accompanying TB is necessary to truly improve patient outcomes and quality of life.

ACKNOWLEDGMENTS

The authors express their gratitude to all healthcare workers and laboratory personnel who assisted in the collection of samples. Their effort and skills were critical to the success of

our investigation. The authors would also like to thank the Institutional Ethics Committee for their assistance and permission to conduct this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

The study protocol was reviewed and approved by the Institutional Ethics Committee, KLE Academy of Higher Education and Research, Belagavi, with Ref. No.: KAHER/EC/20-21/001/15

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

REFERENCES

1. World Health Organization (WHO). Global tuberculosis report 2024. Geneva: World Health Organization; 2024. https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024
2. UNAIDS. Impact of COVID-19 Hits Hard as TB Deaths Among People Living with HIV Rise for the First Time Since 2006. Press Release, 23 March 2022. https://www.unaids.org/en/resources/presscentre/pressreleaseandstatementarchive/2022/march/20220323_tb-day. Accessed November 12, 2025
3. Aggarwal D, Chander J, Janmeja AK, Katyal R. Pulmonary tuberculosis and mucormycosis co-infection in a diabetic patient. *Lung India.* 2015;32(1):53–55.doi: 10.4103/0970-2113.148452
4. Migliori GB, Tiberi S, Zumla A, et al. Global Tuberculosis Network. MDR/XDR-TB management of patients and contacts: Challenges facing the new decade. The 2020 clinical update by the Global Tuberculosis Network. *Int J Infect Dis.* 2020;92S:S15-S25. doi: 10.1016/j.ijid.2020.01.042
5. Soeroso NN, Siahaan L, Khairunnisa S, et al. The association of chronic pulmonary aspergillosis and chronic pulmonary histoplasmosis with MDR-TB patients in Indonesia. *J Fungi.* 2024;10(8):529.doi: 10.3390/jof10080529
6. Danlami MB, Adefowepo AM, Manga SS, Yahaya TO, Mshelia MB, Kalgo ZM. Pulmonary mycoses among pulmonary tuberculosis in Kebbi State North Western Nigeria. *Egypt J Bronchol.* 2023;17(1):39. doi:10.1186/s43168-023-00214-5
7. Denning DW, Pleuvry A, Cole DC. Global burden of chronic pulmonary aspergillosis as a sequel to pulmonary tuberculosis. *Bull World Health Organ.* 2011;89(12):864-872.doi: 10.2471/BLT.11.089441
8. Brown GD, Denning DW, Gow NAR, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. *Sci Transl Med*. 2012;4(165):165rv13. doi: 10.1126/scitranslmed.3004404
9. Setianingrum F, Rozaliyani A, Adawiyah R, et al. A prospective longitudinal study of chronic pulmonary aspergillosis in pulmonary tuberculosis in Indonesia (APICAL). *Thorax.* 2022;77(8):821-828. doi: 10.1136/thoraxjnl-2020-216464
10. Muni S, Rajpal K, Kumar R, et al. Identification of fungal isolates in patients with pulmonary tuberculosis treated at a tertiary care hospital. *Cureus.* 2023;15(4):e37664. doi: 10.7759/cureus.37664
11. Baluku J, Nuwagira E, Bongomin F, Denning DW. Pulmonary tuberculosis and chronic pulmonary aspergillosis: clinical differences and similarities. *Int J Tuberc Lung Dis.* 2021;25(7):537–546.doi: 10.5588/ijtld.21.0034
12. Adenis AA, Valdes A, Cropet C, et al. Burden of HIV-associated histoplasmosis compared with tuberculosis in Latin America: a modelling study. *Lancet Infect Dis.* 2018;18(10):1150-1159.doi: 10.1016/S1473-3099(18)30354-2
13. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2016;62(4):e1-e50
14. Baker J, Setianingrum F, Wahyuningsih R, Denning DW. Mapping histoplasmosis in South East Asia– implications for diagnosis in AIDS. *Emerg Microbes Infect*. 2019;8(1):1139-1145. doi: 10.1080/22221751.2019.1644539
15. Jabbari Amiri MR, Siami R, Khaledi A. Tuberculosis Status and Coinfection of Pulmonary Fungal Infections in Patients Referred to Reference Laboratory of Health Centers Ghaemshahr City during 2007-2017. *Ethiop J Health Sci*. 2018;28(6):683-690. doi: 10.4314/ ejhs.v28i6.2
16. Patterson TF, Thompson GR III, Denning DW, et al. Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the Infectious Diseases Society of America. *Clin Infect Dis.* 2016;63(4):e1-e60. doi: 10.1093/cid/ciw326
17. Bitew A, Bati S. Profiling of potential pulmonary fungal pathogens and the prevalence of the association between pulmonary tuberculosis and potential fungal pathogens in presumptive tuberculosis patients

referred to Saint Peter's Specialized Tuberculosis Referral Hospital, Addis Ababa, Ethiopia. *SAGE Open Med*. 2021;9:20503121211056163. doi: 10.1177/20503121211056163

18. Pasqualotto AC, Denning DW. Evaluation for fungal pulmonary infections is essential in suspected tuberculosis relapse. *Lancet.* 2025;25(9):e493. doi: 10.1016/S1473-3099(25)00439-6
19. Hosseini M, Shakerimoghaddam A, Ghazalibina M, Khaledi A. Aspergillus coinfection among patients with pulmonary tuberculosis in Asia and Africa countries; A systematic review and meta-analysis of cross-sectional studies. *Microb Pathog.* 2020;141:104018. doi: 10.1016/j.micpath.2020.104018
20. Denning DW. Diagnosing pulmonary aspergillosis is much easier than it used to be: a new diagnostic landscape. *Int J Tuberc Lung Dis.* 2021;25(7):525-536. doi: 10.5588/ijtld.21.0053
21. Ocansey BK, Otoo B, Adjei A, Gbadamosi H, Kotey FCN, Kosmidis C, Afriyie-Mensah JS, Denning DW, Opintan JA. Chronic pulmonary aspergillosis is common among patients with presumed tuberculosis relapse in Ghana. *Med Mycol*. 2022;60(9):myac063. doi: 10.1093/mmy/myac063
22. Kumar AA, Shantha GPS, Jeyachandran V, et al. Multidrug-resistant tuberculosis co-existing with aspergilloma and invasive aspergillosis in a 50-year-old diabetic woman: a case report. *Cases J*. 2008;1:2-5.doi: 10.1186/1757-1626-1-303
23. Khairunnisa S, Soeroso NN, Abdullah M, et al. Factors influencing histoplasmosis incidence in multidrug-resistant pulmonary tuberculosis patients: a cross-sectional study in Indonesia. *Narra J.* 2023;3(3):e403. doi: 10.52225/narra.v3i3.403
24. Anot K, Sharma S, Gupta M, Kaur D. Disseminated histoplasmosis and tuberculosis: dual infection in a non-endemic region. *BMJ Case Rep.* 2020;13(8):e235531. doi: 10.1136/bcr-2020-235531
25. Baker J, Kosmidis C, Rozaliyani A, et al. Chronic pulmonary histoplasmosis – a scoping literature review. *Open Forum Infect Dis.* 2020;7(4):ofaa119.doi: 10.1093/ofid/ofaa119
26. Linder KA, Kauffman CA. Histoplasmosis: epidemiology, diagnosis, and clinical manifestations. *Curr Fungal Infect Rep.* 2019;13(3):120-128.
27. CO Morrissey, SC-A Chen, TC Sorrell, et al. Galactomannan and PCR versus culture and histology for directing use of antifungal treatment for invasive aspergillosis in immunocompromised patients: a randomized controlled trial. *Lancet Infect Dis.* 2011;13(6):519-528. doi: 10.1016/S1473-3099(13)70076-8
28. Jarvis JN, Lawn SD, Vogt M, et al. Screening for histoplasmosis and *Pneumocystis* pneumonia in patients with advanced HIV disease in sub-Saharan Africa. *Clin Infect Dis.* 2012;55(10):1375-1382. doi: 10.1128/cmr.00101-22