RESEARCH PAPER

Assessment of Secondary Bacterial and Fungal Co-infections in Patients with Clinically Suspected Tuberculosis and Their Microbiological Spectrum.

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Abstract

Background: Tuberculosis (TB) remains a major public health challenge in India, often complicated by secondary bacterial and fungal infections. These co-infections can worsen clinical outcomes, increase morbidity and mortality, and complicate treatment, particularly in the presence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) pathogens.

Objectives: To assess the prevalence and microbiological spectrum of secondary bacterial and fungal co-infections in patients with clinically suspected pulmonary tuberculosis and to analyze their antimicrobial resistance patterns.

Methods: This hospital-based cross-sectional study included 289 patients clinically suspected of pulmonary TB. Respiratory specimens (sputum and bronchoalveolar lavage) were collected and processed for microbiological analysis. Bacterial and fungal isolates were identified using standard culture techniques, and antimicrobial susceptibility testing was performed according to CLSI guidelines. Demographic, clinical, and risk factor data were recorded.

Results: Among 289 patients, male predominance was observed (59.9%). Secondary bacterial infections were detected in 128 isolates, predominantly Gram-negative bacteria, including Klebsiella pneumoniae (27.3%) and Pseudomonas aeruginosa (21.9%), and Gram-positive bacteria, mainly Staphylococcus aureus (15.6%). Fungal isolates included Candida albicans (55.6%) and Aspergillus spp. (44.4%). MDR strains accounted for 39.1%, XDR 5.5%, and ESBL producers 14.8% of bacterial isolates, while 60% of S. aureus were MRSA. Gram-negative bacteria were highly susceptible to colistin, meropenem, and amikacin, and Gram-positive isolates were universally susceptible to vancomycin and linezolid. Significant risk factors associated with secondary infections included smoking, alcohol use, diabetes, and HIV infection ($p < 0.05$).

Conclusion: Secondary bacterial and fungal co-infections are common in TB-suspected patients, with a high prevalence of MDR pathogens. Early microbiological diagnosis and targeted antimicrobial therapy are essential to reduce morbidity, prevent complications, and improve treatment outcomes.

Keywords: Tuberculosis, Secondary Bacterial Infection, Fungal Co-infection, Multidrug-Resistant (MDR), Extensively Drug-Resistant (XDR), Antimicrobial Susceptibility, Pulmonary Samples, India

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Introduction:

Tuberculosis (TB) continues to be a major public health problem in India, contributing to more than a quarter of the global TB burden. Although the main emphasis is on controlling Mycobacterium tuberculosis, secondary bacterial infections remain an important but often neglected complication. These co-infections can worsen the patient's condition, complicate treatment, and lead to poorer outcomes, including increased morbidity and mortality. The relationship between TB and secondary infections is closely linked, as active TB weakens the immune system, especially the local defenses of the lungs. In addition, TB causes structural damage to lung tissue, such as cavity formation, which creates a favorable environment for opportunistic bacteria to colonize and grow.[1] This makes TB patients highly susceptible to co infections, which can manifest as pneumonia, empyema, or sepsis. This challenge is particularly acute in settings like India.[2] where delayed diagnosis and the high prevalence of drug resistance in both TB and other bacterial pathogens complicate treatment. Recent studies from tertiary care centers in India highlight the significant prevalence of these co infections. Prevalence rates vary, with studies reporting figures ranging from around 10% to over 50%. Furthermore, fungal co-infections are increasingly being recognized as an important complication in patients with suspected or confirmed tuberculosis. Opportunistic fungi such as *Aspergillus* spp. and *Candida* spp. can colonize pre-existing lung cavities or damaged pulmonary tissue, leading to conditions like chronic pulmonary aspergillosis or invasive fungal disease, particularly in immunocompromised individuals. [4] These infections often mimic the clinical and

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radiological features of TB, making diagnosis challenging and potentially leading to mismanagement. The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis further complicates the clinical scenario, as prolonged illness and repeated antibiotic exposure predispose patients to secondary microbial infections. [5] in addition, the widespread and often irrational use of broad-spectrum antibiotics contributes to the development of resistant bacterial strains, thereby limiting therapeutic options. [6] Accurate and timely identification of these co-infecting organisms is therefore crucial for appropriate patient management. Microbiological evaluation, including culture and sensitivity testing, plays a key role in detecting the spectrum of bacterial and fungal pathogens associated with TB. Understanding the prevalence and pattern of these co-infections can help clinicians initiate targeted therapy, reduce complications, and improve overall treatment outcomes. [7] Despite growing evidence, there remains a paucity of comprehensive data on the microbiological spectrum of secondary infections among clinically suspected TB patients, particularly in resource-limited settings. Hence, this study aims to assess the prevalence of secondary bacterial and fungal co-infections and to analyze their microbiological profile in patients with clinically suspected tuberculosis. [8]

Material and Methods:

This study will be conducted in the Department of Microbiology at a tertiary care hospital, over of study period of January to December 2025. It will be a hospital-based, cross-sectional study involving patients clinically suspected of pulmonary tuberculosis.

Inclusion Criteria

1. Patients clinically suspected of pulmonary tuberculosis based on symptoms such as cough, fever, weight loss, and hemoptysis
2. Patients of either sex and all age groups
3. Patients providing respiratory specimens (sputum/bronchoalveolar lavage) for microbiological analysis
4. Patients who provide written informed consent

Exclusion Criteria

1. Patients already diagnosed with tuberculosis and receiving anti-tubercular treatment
2. Patients who have received antibiotic or antifungal therapy within the preceding 7–14 days
3. Patients with inadequate or contaminated clinical samples
4. Patients unwilling to participate or not providing informed consent

Study Population

Patients presenting with clinical features suggestive of pulmonary tuberculosis, such as persistent cough, fever, weight loss, and hemoptysis, will be included based on the predefined inclusion and exclusion criteria.

Sample Size

The sample size will be determined based on the prevalence of secondary co-infections reported in previous studies and calculated using appropriate statistical methods.

Specimen Collection

Respiratory samples, including sputum and bronchoalveolar lavage (BAL), will be collected under aseptic conditions. Early morning sputum samples will be preferred. All specimens will be transported promptly to the microbiology laboratory and processed without delay.

Laboratory Processing

- **Direct Microscopy:** Smears will be prepared and stained using Ziehl–Neelsen staining for detection of acid-fast bacilli (AFB).
- **Culture for Mycobacterium tuberculosis:** Samples will be cultured on Lowenstein–Jensen (LJ) medium or in liquid culture systems as per standard protocols.
- **Bacterial Isolation:** Samples will be inoculated onto blood agar, MacConkey agar, and chocolate agar and incubated under appropriate conditions. Identification of bacterial isolates will be done using standard biochemical tests.
- **Fungal Isolation:** Specimens will be cultured on Sabouraud dextrose agar (SDA) and incubated at appropriate temperatures. Fungal isolates will be identified based on colony morphology and microscopic examination using lactophenol cotton blue mount.
- **Antimicrobial Susceptibility Testing:** Bacterial isolates will be subjected to antimicrobial susceptibility testing using the Kirby–Bauer disk diffusion method as per CLSI guidelines.

Data Collection

Relevant demographic, clinical, and laboratory data will be recorded in a structured proforma

Result:

Table 1 Gender-wise distribution of the patients

Gender	Number of Patients	Percentage (%)
Male	173	59.9
Female	116	40.1
Total	289	100

Among the 289 patients, males (59.9%) outnumbered females (40.1%), indicating a male predominance in the study population.

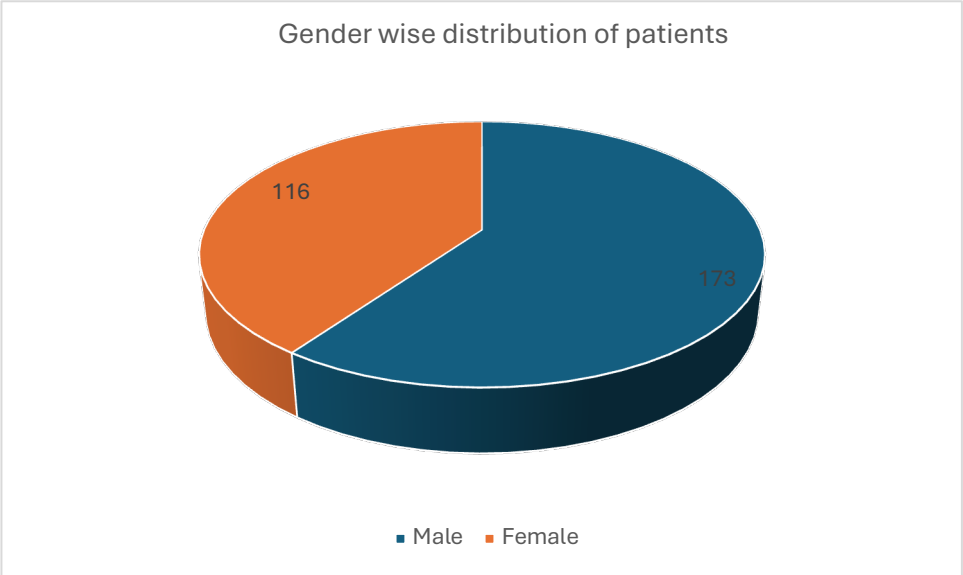

Figure 1: Graphical represents gender wise distribution of the patients

Table 2: Age wise distribution of the patients.

Age Group (years)	Number of Patients	Percentage (%)	
18–27		42	p < 0.0001
28–37	64	22.1	
38–47	70	24.2	
48–57	56	19.4	
58–67	45	15.6	
68–70	12	4.2	
Total	289	100	

The study population (n = 289) included patients aged 18–70 years, with the majority between 28–47 years (46.3%). The age distribution is statistically significant ($p < 0.0001$), indicating that certain age groups were more represented in the study population

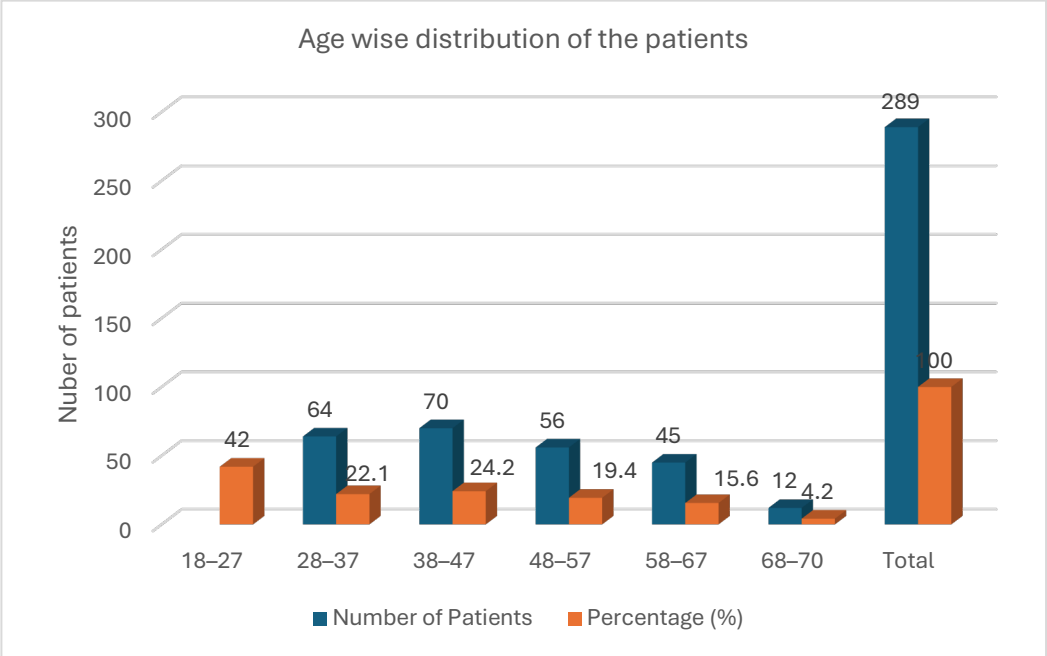

Figure 2: Graphical represents gender distribution of the patients.

Table: 3 Risk Factors Associated with Secondary Bacterial Infection (n = 289)

Risk Factor	Total n (%)	Occurrence of Secondary Bacterial Infection n (%)	χ^2 Value	p-value
Male Gender	173 (59.9)	75 (43.3)	0.56	0.45
Female Gender	116 (40.1)	53 (45.7)		
Smoking	120 (41.5)	60 (50.0)	7.12	0.008*
Alcohol Use	85 (29.4)	40 (47.1)	4.58	0.032*
Diabetes Mellitus	65 (22.5)	35 (53.8)	8.24	0.004*
Hypertension	40 (13.8)	18 (45.0)	0.12	0.73
HIV Infection	18 (6.2)	12 (66.7)	6.15	0.013*
Chronic Lung Disease (COPD/Asthma)	30 (10.4)	15 (50.0)	3.21	0.073
No Comorbidities	136 (47.1)	50 (36.8)	2.14	0.14
	*Statistically significant at $p < 0.05$			

Among the 289 patients, smoking, alcohol use, diabetes mellitus, and HIV infection were significantly associated with secondary bacterial infection ($p < 0.05$). Other factors, including gender, hypertension, chronic lung disease, and absence of comorbidities, did not show a statistically significant association with secondary bacterial infection.

Table 4: Types of Pulmonary Samples Collected (n = 289)

Type of Sample	Number of Patients	Percentage (%)
Sputum (spontaneous/early morning)	210	72.7
Induced Sputum	25	8.7
Bronchoalveolar Lavage (BAL)	40	13.8
Endotracheal Aspirate / Other	14	4.8
Total	289	100

Among the 289 patients, sputum (spontaneous/early morning) was the most commonly collected sample (72.7%), followed by bronchoalveolar lavage (13.8%). Induced sputum (8.7%) and endotracheal aspirate/other samples (4.8%) were less frequent, reflecting standard pulmonary sampling practices.

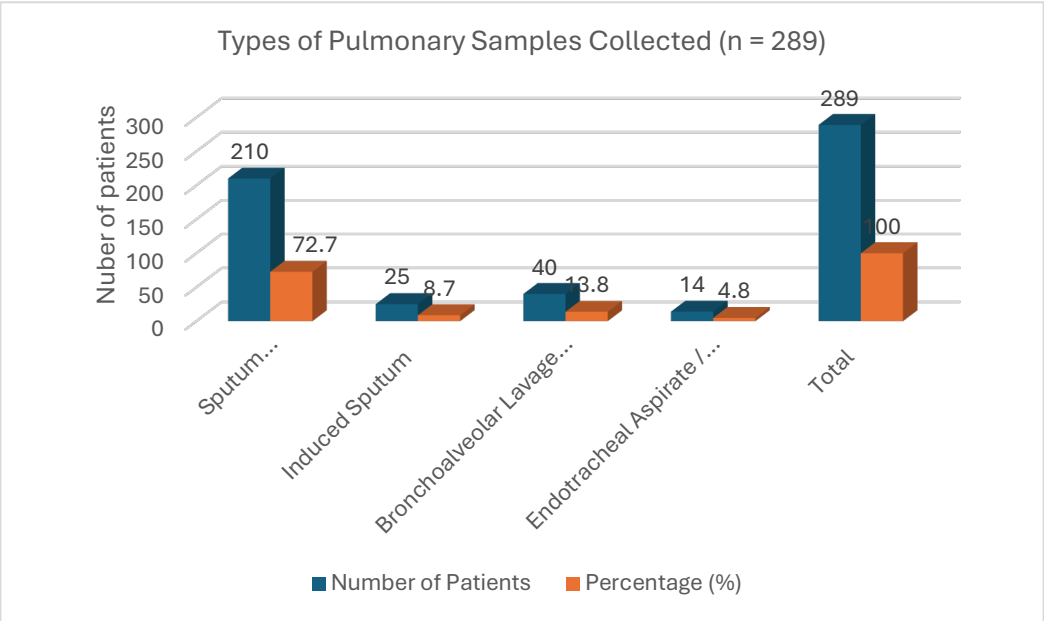

Figure 3: Graphical represents types of Pulmonary Samples Collected (n = 289

Table 5: Distribution of Microorganisms Isolated in Secondary Bacterial Infection (n = 289)

Microorganism	Type of Sample	Number of Isolates	Percentage (%)
Bacteria			
Klebsiella pneumoniae	Sputum / BAL	35	27.3
Pseudomonas aeruginosa	Sputum / BAL	28	21.9
Staphylococcus aureus	Sputum / BAL	20	15.6
Escherichia coli	Sputum / BAL	15	11.7
Acinetobacter spp.	Sputum / BAL	10	7.8
Other Gram-negative bacilli	Sputum / BAL	8	6.3
Other Gram-positive cocci	Sputum / BAL	12	9.4
Total Bacterial Isolates		128	100
Fungi			
Candida albicans	Sputum / BAL	10	55.6
Aspergillus spp.	Sputum / BAL	8	44.4
Total Fungal Isolates		18	100

Among the 128 bacterial isolates from secondary infections, Klebsiella pneumoniae (27.3%) and Pseudomonas aeruginosa (21.9%) were the most common, followed by Staphylococcus aureus (15.6%) and Escherichia coli (11.7%). Gram-negative bacteria accounted for the majority of isolates, for fungal co-infections (n = 18), Candida albicans (55.6%) was slightly more frequent than Aspergillus spp. (44.4%). All isolates were obtained from sputum or BAL samples. This highlights that Gram-negative bacteria are the predominant pathogens in secondary pulmonary infections among TB patients, with a smaller contribution from fungi.

Table 6: Distribution of MDR, XDR, and ESBL-Producing Isolates in Secondary Bacterial Infection (n = 128 bacterial isolates)

Microorganism	**Total Isolates (n)**	**MDR n (%)**	**XDR n (%)**	**ESBL n (%)**
Klebsiella pneumoniae	35	15 (42.9)	2 (5.7)	12 (34.3)

Pseudomonas aeruginosa	28	10 (35.7)	3 (10.7)	0 (0)
Staphylococcus aureus	20	8 (40.0)	0 (0)	NA
Escherichia coli	15	6 (40.0)	0 (0)	5 (33.3)
Acinetobacter spp.	10	5 (50.0)	2 (20.0)	0 (0)
Other Gram-negative bacilli	8	2 (25.0)	0 (0)	2 (25.0)
Other Gram-positive cocci	12	4 (33.3)	0 (0)	NA
Total	128	50 (39.1)	7 (5.5)	19 (14.8)

Among the 128 bacterial isolates, 39.1% were multidrug-resistant (MDR) and 5.5% were extensively drug-resistant (XDR). Extended-spectrum β-lactamase (ESBL) production was observed in 14.8% of isolates, primarily in Klebsiella pneumoniae (34.3%) and Escherichia coli (33.3%). MDR and XDR strains were most common in Gram-negative bacteria, with Acinetobacter spp. showing the highest XDR proportion (20%). Among Gram-positive bacteria, 40% of Staphylococcus aureus isolates were MRSA (MDR), while ESBL was not applicable, This indicates a significant burden of antimicrobial resistance among secondary bacterial infections in TB patients, highlighting the need for targeted antibiotic therapy.

Table 7: Antimicrobial Susceptibility Pattern of Gram-Negative Bacteria (n = 96 isolates)

Antibiotic	Klebsiella pneumoniae (n = 35)	Pseudomonas aeruginosa (n = 28)	Escherichia coli (n = 15)	Acinetobacter spp. (n = 10)	Other GNB (n = 8)
Ampicillin	5 (14.3%)	3 (10.7%)	4 (26.7%)	2 (20.0%)	2 (25.0%)
Amoxicillin-Clavulanic Acid	20 (57.1%)	15 (53.6%)	8 (53.3%)	4 (40.0%)	5 (62.5%)
Piperacillin-Tazobactam	28 (80.0%)	22 (78.6%)	12 (80.0%)	6 (60.0%)	6 (75.0%)
Ceftriaxone	18 (51.4%)	10 (35.7%)	7 (46.7%)	3 (30.0%)	3 (37.5%)
Ceftazidime	17 (48.6%)	12 (42.9%)	6 (40.0%)	3 (30.0%)	3 (37.5%)
Ciprofloxacin	22 (62.9%)	16 (57.1%)	9 (60.0%)	5 (50.0%)	5 (62.5%)
Amikacin	30 (85.7%)	24 (85.7%)	13 (86.7%)	7 (70.0%)	6 (75.0%)
Meropenem	33 (94.3%)	26 (92.9%)	14 (93.3%)	8 (80.0%)	7 (87.5%)
Colistin	35 (100%)	28 (100%)	15 (100%)	10 (100%)	8 (100%)

Gram-negative bacteria showed high resistance to ampicillin and moderate resistance to other beta-lactams, while amikacin, meropenem, and colistin were highly effective, indicating MDR strains are common but carbapenems and colistin remain reliable treatment options.

Table 8: Antimicrobial Susceptibility Pattern of Gram-Positive Bacteria (n = 32 isolates)

Antibiotic	Staphylococcus aureus (n = 20)	Other Gram-Positive Cocci (n = 12)
Penicillin	6 (30.0%)	4 (33.3%)
Oxacillin / Cefoxitin (MRSA detection)	12 (60.0%)	NA
Erythromycin	10 (50.0%)	6 (50.0%)
Clindamycin	14 (70.0%)	8 (66.7%)
Vancomycin	20 (100%)	12 (100%)
Linezolid	20 (100%)	12 (100%)
Ciprofloxacin	12 (60.0%)	7 (58.3%)
Gentamicin	15 (75.0%)	9 (75.0%)

Among Gram-positive bacteria, 60% of Staphylococcus aureus isolates were MRSA, showing resistance to beta-lactams. Most isolates were susceptible to vancomycin and linezolid (100%). Moderate susceptibility was observed for erythromycin, ciprofloxacin, and gentamicin, while clindamycin remained effective in 66–70% of isolates. This indicates MRSA prevalence is high, but glycopeptides and linezolid are reliable treatment options.

Discussion:

This study highlights a high prevalence of secondary bacterial and fungal co-infections among patients with clinically suspected tuberculosis. Gram-negative bacteria, particularly Klebsiella pneumoniae and Pseudomonas aeruginosa, were the predominant pathogens, while Staphylococcus aureus was the most common Gram-positive isolate. Fungal co-infections, mainly Candida albicans and Aspergillus spp., were less frequent but clinically significant.

A substantial proportion of bacterial isolates were multidrug-resistant (39.1%), with XDR and ESBL-producing strains observed, underscoring the challenge of antimicrobial resistance. Among Gram-positive bacteria, MRSA prevalence was 60%, while all isolates remained susceptible to vancomycin and linezolid.[9] Gram-negative bacteria showed high resistance to ampicillin but retained susceptibility to carbapenems, amikacin, and colistin, highlighting the importance of targeted therapy, Significant risk factors for secondary infections included smoking, alcohol use, diabetes, and HIV infection, emphasizing the need for careful monitoring of these patients.[10] Overall, these findings reinforce the need for early microbiological evaluation, antimicrobial stewardship, and tailored treatment to reduce morbidity and improve outcomes in TB patients with secondary infections.

Conclusion:

Secondary bacterial and fungal co-infections are common among patients with clinically suspected tuberculosis, with Gram-negative bacteria being the predominant pathogens. A significant proportion of isolates are multidrug-resistant, including MRSA, ESBL, and XDR strains, complicating management and limiting treatment options. Risk factors such as smoking, alcohol use, diabetes, and HIV infection increase susceptibility to these infections. Early microbiological diagnosis and targeted antimicrobial therapy are crucial to improve patient outcomes and reduce morbidity and mortality in this vulnerable population.

Conflict of Interest:

The authors declare no conflict of interest related to this study.

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