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Prevalence, Antibiotic Susceptibility, and Modifiable Risk Factors for Secondary Bacterial Infection in Active Pulmonary Tuberculosis: A Cross-Sectional Study from Western Rajasthan

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ABSTRACT

Background: Persistent or worsening respiratory symptoms during anti-tubercular therapy may reflect superadded bacterial infection rather than tuberculosis alone. Local pathogen distribution, antimicrobial susceptibility, and preventable host factors are insufficiently characterized in high-burden settings.
Aims: To estimate the prevalence and spectrum of secondary bacterial infection in active pulmonary tuberculosis, describe antibiotic susceptibility, as identify modifiable risk factors associated with culture positivity.
Materials and Methods: This hospital-based cross-sectional study included 90 adults with bacteriologically confirmed pulmonary tuberculosis who had received anti-tubercular therapy for more than two weeks and had clinical deterioration or inadequate improvement. Sputum was evaluated for pyogenic bacterial culture and antibiotic susceptibility. Associations with culture positivity were assessed by Fisher exact/chi-square testing, odds ratios, and multivariable logistic regression.
Results: Secondary bacterial culture was positive in 27 patients (30.0%; 95% CI 21.5-40.1). Gram-negative organisms accounted for 66.7% of isolates. Klebsiella pneumoniae was most frequent (33.3%), followed by Pseudomonas aeruginosa and Staphylococcus aureus (18.5% each). Isolate-level multidrug resistance was present in 22.2%. High susceptibility was observed to meropenem (94.4%), ertapenem (91.7%), piperacillin-tazobactam (88.9%), amikacin (73.9%), and doxycycline (71.4%), while resistance was high to ampicillin (91.7%), ciprofloxacin (73.9%), ceftazidime (66.7%), and levofloxacin (59.3%). Current/former smoking independently predicted culture positivity (adjusted odds ratio 4.07, 95% CI 1.36-12.18; p=0.012). Poor oral hygiene showed a strong borderline adjusted association (adjusted odds ratio 2.76, 95% CI 1.00-7.66; p=0.051).
Conclusion: One in three clinically non-improving patients with active pulmonary tuberculosis had culture-positive secondary bacterial infection. Gram-negative pathogens and resistance to commonly used antibiotics were prominent. Culture-guided treatment, smoking cessation, and improved oral hygiene should be integrated into the evaluation of such patients.

Keywords: *Antibiotic resistance, bacterial co-infection, oral hygiene, pulmonary tuberculosis, smoking, sputum culture.*

INTRODUCTION

Tuberculosis remains a major global health problem. The World Health Organization estimated that 10.7 million people developed tuberculosis in 2024, and India continued to contribute the largest national share of the global burden.[1] Pulmonary tuberculosis is particularly important because it drives transmission and may produce extensive airway and parenchymal injury even after effective anti-tubercular therapy.[2]

The consequences of pulmonary tuberculosis extend beyond mycobacterial infection. Cavitation, bronchiectasis, fibrosis, airway distortion, impaired mucociliary clearance, and persistent immune dysregulation can create a respiratory environment that favors colonization and superadded infection by pyogenic bacteria.[3] Contemporary work on post-tuberculosis lung disease has reinforced the concept that structural damage and altered host defense may begin during active disease and persist after microbiological cure.[4]

Secondary bacterial infection may be difficult to distinguish from persistent tuberculosis activity. Fever, productive cough, dyspnea, leukocytosis, and new or progressive radiographic opacities may occur in either condition. Failure to recognize a superadded infection may delay recovery, prolong hospitalization, and encourage repeated empirical antibiotic exposure. Conversely, indiscriminate antimicrobial treatment may select resistant organisms and obscure the true cause of clinical deterioration. Culture-based evaluation is therefore most valuable in patients with persistent or worsening symptoms despite an adequate initial period of anti-tubercular treatment.

Published estimates of bacterial co-infection in pulmonary tuberculosis vary widely because of differences in case selection, specimen type, definitions, and healthcare setting. A recent tertiary-care study reported culture-proven bacterial co-infection in 10.9% of pulmonary samples,[5] whereas a Cambodian study identified co-infection in 33% of patients with confirmed tuberculosis who underwent both mycobacterial and bacterial testing.[6] Gram-negative bacilli, including Klebsiella and Pseudomonas species, are repeatedly reported and may show substantial antimicrobial resistance.[7] Bacterial co-infection has also been associated with early mortality in hospitalized pulmonary tuberculosis.[8]

Data from Western Rajasthan are limited, particularly regarding antibiotic susceptibility and potentially modifiable host factors. The present study was undertaken to determine the prevalence and spectrum of secondary bacterial infection among clinically non-improving patients with active pulmonary tuberculosis, characterize the susceptibility profile of recovered isolates, and assess demographic and clinical factors associated with culture positivity.

MATERIALS AND METHODS

Study design and setting

A hospital-based cross-sectional observational study was conducted in the Department of Respiratory Medicine at Kamla Nehru Chest Hospital, Dr. S. N. Medical College, Jodhpur, a tertiary referral centre serving Western Rajasthan. Recruitment began after Institutional Ethics Committee approval and continued until the required sample size was achieved.

Participants

Adults older than 18 years with bacteriologically confirmed active pulmonary tuberculosis were eligible when they had received anti-tubercular therapy for more than two weeks and showed clinical deterioration or inadequate improvement. Written informed consent was obtained. Moribund patients, patients with multidrug-resistant tuberculosis, people living with HIV, and patients already receiving antibacterial antibiotics before the hospital visit were excluded.

Sample size

The sample size was calculated for an expected prevalence of 32% based on an earlier study,[12] using a 95% confidence level and 10% absolute precision. The minimum calculated sample was 83; this was rounded to 90 participants.

Clinical and microbiological assessment

Demographic characteristics, socioeconomic and educational status, smoking exposure, oro-dental hygiene, nutritional status, comorbidities, treatment history, respiratory symptoms, sputum characteristics, and radiological findings were recorded in a structured proforma. Sputum samples were examined for acid-fast bacilli and cartridge-based nucleic acid amplification testing and were also submitted for pyogenic bacterial culture. Bacterial identification and antimicrobial susceptibility testing were performed in the institutional microbiology laboratory using its standardized routine methods. Results were recorded only for antibiotics tested against the relevant isolate; consequently, denominators differed between drugs.

For analysis, a culture-positive secondary bacterial infection was defined operationally as recovery of a non-mycobacterial bacterial pathogen from sputum in an eligible patient with clinical deterioration or inadequate response during anti-tubercular therapy. Isolate-level multidrug resistance was recorded as reported by the microbiology laboratory.

Statistical analysis

Data were entered in Microsoft Excel and analyzed using SPSS. Continuous variables were summarized as mean and standard deviation, and categorical variables as frequency and percentage. Associations between culture positivity and selected factors were evaluated with the chi-square test or Fisher exact test, as appropriate. Unadjusted odds ratios with 95% confidence intervals were calculated. Clinically relevant variables and those showing evidence of association were entered into a multivariable logistic regression model. A two-sided p value below 0.05 was considered statistically significant. The 95% confidence interval for prevalence was calculated using the Wilson method.

Ethical considerations

The study was approved by the Institutional Ethics Committee, Dr. Sampurnanand Medical College, Jodhpur (SNMC/IEC/2026/Plan/1239; approval dated 9 January 2026). Participation was voluntary, confidentiality was maintained, and written informed consent was obtained from all participants.

RESULTS

Participant profile

Ninety patients were included. The mean age was 43.8 +/- 12.8 years; 75 (83.3%) were men, 56 (62.2%) were from rural areas, and 62 (68.9%) belonged to the lower socioeconomic group. Thirty patients (33.3%) were underweight. Fifty (55.6%) were current or former smokers and 37 (41.1%) had poor oro-dental hygiene. Mucopurulent sputum was reported by 55 patients (61.1%). Bilateral radiographic disease was present in 53 (58.9%), cavitation in 48 (53.3%), and far advanced disease in 29 (32.2%). Key baseline characteristics are summarized in Table 1.

Table 1. Selected demographic, clinical, and radiological characteristics (n=90)

Characteristic	Value
Age, years, mean +/- SD	43.8 +/- 12.8
Male sex	75 (83.3)
Rural residence	56 (62.2)
Lower socioeconomic status	62 (68.9)
BMI <18.5 kg/m2	30 (33.3)
Current/former smoker	50 (55.6)
Poor oro-dental hygiene	37 (41.1)
Mucopurulent or blood-mixed sputum	60 (66.7)
Moderate/copious sputum volume	72 (80.0)
Bilateral radiological involvement	53 (58.9)
Cavitation	48 (53.3)
Far advanced radiological disease	29 (32.2)
Diabetes mellitus	17 (18.9)
COPD or bronchiectasis	31 (34.4)

Values are n (%) unless otherwise stated. BMI: body mass index; COPD: chronic obstructive pulmonary disease; SD: standard deviation.

Prevalence and bacterial spectrum

Pyogenic bacterial culture was positive in 27 of 90 patients, yielding a prevalence of 30.0% (95% CI 21.5-40.1). Gram-negative organisms constituted 18 of 27 isolates (66.7%). Klebsiella pneumoniae was the most frequent isolate, followed by Pseudomonas aeruginosa and Staphylococcus aureus. Six isolates (22.2%) were categorized as multidrug resistant (Table 2).

Table 2. Spectrum of secondary bacterial isolates among culture-positive patients (n=27)

Bacterial isolate	n	%
Klebsiella pneumoniae	9	33.3
Pseudomonas aeruginosa	5	18.5
Staphylococcus aureus	5	18.5
Streptococcus pneumoniae	4	14.8
Escherichia coli	3	11.1
Acinetobacter baumannii	1	3.7
Gram-negative isolates (total)	18	66.7
Isolate-level multidrug resistance	6	22.2

Percentages for individual organisms are calculated among 27 culture-positive patients.

Antibiotic susceptibility

Susceptibility varied substantially across drug classes. Among frequently tested agents, meropenem, ertapenem, piperacillin-tazobactam, amikacin, doxycycline, and cefoperazone-sulbactam retained comparatively high activity. Vancomycin and clindamycin were active against all nine isolates for which they were tested. In contrast, resistance was marked to ampicillin, norfloxacin, ciprofloxacin, ceftazidime, amoxicillin-clavulanate, moxifloxacin, and levofloxacin. The complete drug-wise profile is shown in Table 3.

Table 3. Drug-wise antibiotic susceptibility of secondary bacterial isolates

Antibiotic	Tested, n	Sensitive, n (%)	Resistant, n (%)
Gentamicin	23	13 (56.5)	10 (43.5)
Amikacin	23	17 (73.9)	6 (26.1)
Tetracycline	9	4 (44.4)	5 (55.6)
Doxycycline	21	15 (71.4)	6 (28.6)
Ampicillin	12	1 (8.3)	11 (91.7)
Amoxicillin-clavulanate	12	4 (33.3)	8 (66.7)
Piperacillin-tazobactam	18	16 (88.9)	2 (11.1)
Cefoperazone-sulbactam	17	12 (70.6)	5 (29.4)
Ceftriaxone	16	9 (56.2)	7 (43.8)
Cefotaxime	16	7 (43.8)	9 (56.2)
Ceftazidime	15	5 (33.3)	10 (66.7)
Cefepime	18	10 (55.6)	8 (44.4)
Norfloxacin	9	0 (0.0)	9 (100.0)
Ciprofloxacin	23	6 (26.1)	17 (73.9)
Levofloxacin	27	11 (40.7)	16 (59.3)
Moxifloxacin	13	5 (38.5)	8 (61.5)
Erythromycin	9	4 (44.4)	5 (55.6)
Azithromycin	9	5 (55.6)	4 (44.4)
Clindamycin	9	9 (100.0)	0 (0.0)
Vancomycin	9	9 (100.0)	0 (0.0)
Meropenem	18	17 (94.4)	1 (5.6)
Faropenem	9	8 (88.9)	1 (11.1)
Ertapenem	12	11 (91.7)	1 (8.3)

Testing was organism-specific; therefore, denominators differ by antibiotic. Drug-wise resistance counts are not additive and should not be interpreted as separate multidrug-resistant isolates.

Factors associated with culture positivity

Culture positivity was significantly more frequent among current/former smokers than non-smokers (42.0% vs 15.0%; unadjusted OR 4.10, 95% CI 1.46-11.54; p=0.006). It was also higher among patients with poor oral hygiene than among those with good or fair hygiene (45.9% vs 18.9%; unadjusted OR 3.65, 95% CI 1.42-9.40; p=0.010). Far advanced disease, lower socioeconomic status, mucopurulent or blood-mixed sputum, and moderate/copious sputum showed numerically higher positivity but did not reach statistical significance. Cavitation and diabetes were not associated with culture positivity in this sample.

In multivariable analysis, current/former smoking remained an independent predictor (adjusted OR 4.07, 95% CI 1.36-12.18; p=0.012). Poor oral hygiene retained a strong borderline association (adjusted OR 2.76, 95% CI 1.00-7.66;

p=0.051). Far advanced disease showed a positive but non-significant trend (adjusted OR 2.41, 95% CI 0.82-7.09; p=0.111) (Table 4).

Table 4. Association of selected factors with secondary bacterial culture positivity

Predictor	Culture positive exposed/unexposed	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Current/former smoking	21/50 vs 6/40	4.10 (1.46-11.54)	0.006	4.07 (1.36-12.18)	0.012
Poor oral hygiene	17/37 vs 10/53	3.65 (1.42-9.40)	0.010	2.76 (1.00-7.66)	0.051
Far advanced disease	12/29 vs 15/61	2.16 (0.84-5.55)	0.140	2.41 (0.82-7.09)	0.111
Prior drugs other than ATT	Data analyzed as yes/no	Not retained as significant	0.074*	0.33 (0.07-1.47)	0.145
Lower socioeconomic status	22/62 vs 5/28	2.53 (0.84-7.59)	0.136	-	-
Mucopurulent/blood-mixed sputum	21/60 vs 6/30	2.15 (0.76-6.09)	0.222	-	-
Moderate/copious sputum	24/72 vs 3/18	2.50 (0.66-9.48)	0.251	-	-
Diabetes mellitus	6/17 vs 21/73	1.35 (0.44-4.12)	0.573	-	-
Cavitation	14/48 vs 13/42	0.92 (0.37-2.27)	1.000	-	-

*ATT: anti-tubercular therapy; CI: confidence interval; OR: odds ratio. *The p value of 0.074 was from correlation screening; this variable was entered into the adjusted model because of clinical relevance.*

DISCUSSION

This study found culture-positive secondary bacterial infection in 30.0% of adults with active pulmonary tuberculosis who were clinically deteriorating or not improving after more than two weeks of anti-tubercular therapy. Two-thirds of isolates were Gram-negative, K. pneumoniae was the leading organism, and more than one-fifth of isolates were multidrug resistant. Resistance to several commonly used beta-lactams and fluoroquinolones was substantial. Smoking was the most consistent independent predictor, while poor oro-dental hygiene showed a strong association that narrowly missed conventional statistical significance after adjustment.

The observed prevalence should be interpreted in the context of deliberate selection of symptomatic, clinically non-improving patients rather than an unselected tuberculosis population. Nevertheless, it is comparable to the 33% co-infection rate among confirmed tuberculosis patients in the Cambodian study by Attia et al.[6] and to the 29.7% frequency of dual infections reported by Lin et al.[9] It is higher than the 10.9% reported by Bir et al. in a tertiary-care microbiology cohort.[5] Differences in clinical threshold for sampling, specimen mix, hospitalization status, prior healthcare contact, and operational definitions probably account for much of this variability.

The predominance of Gram-negative organisms is consistent with previous work. Iliyasu et al. reported that Gram-negative bacilli were as frequent as S. pneumoniae among pulmonary tuberculosis patients with secondary pneumonia.[7] In the Cambodian cohort, Klebsiella and Pseudomonas species were the most frequent bacteria among culture-positive samples.[6] Bir et al. also identified P. aeruginosa as the commonest isolate in their series.[5] The prominence of K. pneumoniae in the present study may reflect the combination of advanced structural lung disease, malnutrition, repeated healthcare exposure, and local antimicrobial ecology.

The susceptibility profile has immediate clinical implications. Carbapenems and piperacillin-tazobactam retained high in-vitro activity, but these agents should not be interpreted as routine empirical choices. Their use should remain selective and guided by severity, likely acquisition setting, specimen quality, and culture results. High resistance to ampicillin, amoxicillin-clavulanate, ceftazidime, and fluoroquinolones suggests that repeated empirical treatment with commonly available drugs may be ineffective. Morgan et al. similarly documented high resistance among non-tuberculous bacterial isolates recovered from tuberculosis patients, including marked resistance to ampicillin and other commonly used agents.[10] The finding supports local antibiogram surveillance and early de-escalation once susceptibility results are available.

Smoking was associated with more than fourfold higher adjusted odds of culture positivity. Tobacco smoke impairs mucociliary clearance, injures the airway epithelium, alters macrophage function, and contributes to chronic airflow obstruction. These mechanisms are particularly relevant in a lung already affected by cavitation, fibrosis, or

bronchiectasis. Population-based data also show that tuberculosis survivors have an increased risk of subsequent chronic obstructive pulmonary disease and respiratory hospitalization.[15] Although the cross-sectional design cannot establish causality, the magnitude and consistency of the association make smoking cessation a practical component of comprehensive tuberculosis care.

Poor oral hygiene was the second clinically important modifiable factor. The unadjusted association was statistically significant, and the adjusted estimate remained large but was borderline significant. The oral cavity can act as a reservoir for potential respiratory pathogens, and microaspiration may be more consequential in patients with impaired airway clearance. Oro-dental assessment is rarely prioritized during tuberculosis treatment, yet it represents a low-cost target for counseling and supportive care. Larger studies should evaluate whether structured oral hygiene interventions reduce bacterial colonization, symptomatic exacerbations, or antibiotic use.

Cavitation was common but was not associated with culture positivity in this dataset. This apparently counterintuitive finding may reflect the high overall prevalence of structural abnormalities in both culture-positive and culture-negative groups, limited statistical power, and the inability of plain radiography to characterize the full burden of bronchiectasis and destroyed lung. Post-tuberculosis cohorts have shown substantial persistent radiological and functional impairment,[13] and reviews emphasize that structural lung disease creates a long-term substrate for recurrent infection.[14] Thus, absence of a statistically significant association should not be interpreted as absence of biological relevance.

Several limitations merit emphasis. The study was conducted at a single tertiary referral centre and included only patients with clinical deterioration or inadequate improvement; the 30% prevalence therefore cannot be generalized to all patients with active pulmonary tuberculosis. The sample size limited precision for subgroup analyses. Expectorated sputum may represent colonization or upper-airway contamination, and protected lower-airway specimens were not routinely obtained. Detailed molecular characterization of resistance mechanisms was unavailable. Drug testing denominators differed because susceptibility panels were isolate-specific. Finally, the cross-sectional design did not evaluate response to directed antibiotics, length of stay, recurrence, or mortality. Despite these limitations, the study provides clinically relevant regional data and identifies actionable factors that can be incorporated into routine assessment.

CONCLUSION

Secondary bacterial infection was detected in one-third of clinically non-improving adults with active pulmonary tuberculosis at a tertiary care centre in Western Rajasthan. Gram-negative organisms predominated, with K. pneumoniae as the leading isolate, and resistance to several commonly used antibiotics was frequent. Current or former smoking was an independent predictor of culture positivity, and poor oral hygiene showed a strong association. Sputum bacterial culture and susceptibility testing should be considered early when symptoms persist or worsen during anti-tubercular therapy. Antibiotic selection should be culture guided whenever possible, while smoking cessation and improvement of oro-dental hygiene should be integrated into comprehensive tuberculosis care.

CLINICAL IMPLICATIONS

- Persistent fever, purulent sputum, or clinical deterioration during anti-tubercular therapy should prompt evaluation for a secondary bacterial infection rather than automatic attribution to tuberculosis treatment failure.
- Empirical antibiotic choices should reflect local resistance patterns; carbapenems and other reserve agents should be protected and used only when clinically justified.
- Smoking cessation and basic oro-dental hygiene counseling are low-cost, potentially modifiable interventions that can be incorporated into routine tuberculosis services.

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