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Epidemiology and Antimicrobial Susceptibility of Slow-Growing Non-Tuberculous Mycobacteria in a TB-Endemic Setting: An 8-Year Laboratory-Based Study from North India

Puneeta Singh[1*], Shalabh Malik[1], Vandana Lal[2]

[1]Department of Microbiology and Serology, National Reference Laboratory, Dr Lal Path Labs, Delhi, India
[2]National Reference Laboratory, Dr Lal Path Labs, Delhi, India
Email: *puneeta.singh@lalpathlabs.com

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Abstract

Background: Differentiating non-tuberculous mycobacteria (NTM) from *Mycobacterium tuberculosis* remains a significant diagnostic challenge in tuberculosis (TB)-endemic settings, often leading to misdiagnosis and inappropriate therapy. Data on the epidemiology and antimicrobial susceptibility of slow-growing mycobacteria (SGM) in North India remain limited. This study aimed to characterize the prevalence, species distribution, and in vitro antimicrobial susceptibility patterns of SGM isolated from clinical specimens in a TB-burden setting. Methods: A retrospective laboratory-based study was conducted on clinical specimens processed between January 2018 and December 2025 at a tertiary reference laboratory in Delhi, India. Mycobacterial cultures were performed using liquid (MGIT 960) and solid (Lowenstein-Jensen) media. Species identification was carried out using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Antimicrobial susceptibility testing (AST) was performed on a subset of isolates using broth microdilution in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Temporal trends were assessed using the Cochran-Armitage test and Joinpoint regression analysis. Results: Of 197,943 clinical specimens processed, 34,776 (17.5%) yielded mycobacterial growth; of these, 30,343 (87.3%) were identified as members of the *Mycobacterium tuberculosis* complex, while 4433 (12.7%) were classified as NTM. Among these, 1845 (41.6%) were slow-growing mycobacteria (SGM) species. Pulmonary specimens accounted for the majority of SGM isolates (63.2%). The *Mycobacterium avium*

complex (MAC) predominated, with *M. chimaera* representing 51.7% of SGM isolates, followed by *M. simiae* (13.0%) and *M. kansasii* (12.7%). Non-chromogenic species constitute 67.8% of isolates. Antimicrobial susceptibility testing (n = 204) demonstrated high in vitro activity of amikacin (99.5%) and clarithromycin (97.5%), followed by linezolid (95.2%) and moxifloxacin (94.6%), whereas comparatively higher resistance rates were observed for doxycycline, ciprofloxacin, and minocycline. Temporal analysis showed year-to-year variability without a statistically significant trend (annual percent change +0.15%; p = 0.963), indicating a stable proportional burden of NTM over time. Conclusions: This laboratory-based study highlights the persistent and diverse presence of slow-growing NTM in a TB-endemic setting, with MAC predominance across pulmonary and extrapulmonary specimens. The findings underscore ongoing diagnostic challenges and the importance of accurate species identification. However, the absence of clinical correlation necessitates cautious interpretation. Prospective studies integrating microbiological, clinical, and radiological data are essential to define the true burden and clinical significance of NTM disease.

Keywords

Non-Tuberculous Mycobacteria (NTM), Slow-Growing Mycobacteria (SGM), Antimicrobial Susceptibility, Non-Tuberculous Mycobacterial Pulmonary Disease (NTM-PD), *Mycobacterium avium* Complex (MAC), MALDI-TOF, TB Endemic Setting

1. Introduction

Non-tuberculous mycobacteria (NTM) have emerged as clinically significant opportunistic pathogens responsible for a broad spectrum of pulmonary and extrapulmonary diseases. Their importance is particularly evident in tuberculosis (TB)-endemic settings, where substantial overlap in clinical presentation, radiological findings, and microbiological characteristics with *Mycobacterium tuberculosis* complicates accurate diagnosis, often leading to misclassification and suboptimal management [1]-[9].

The increasing global burden of NTM disease has been attributed to multiple converging factors, including an ageing population, rising prevalence of chronic lung diseases, expanded use of immunosuppressive therapies, and improvements in diagnostic methodologies [2] [4]. Despite these advances, the clinical relevance of NTM isolation remains incompletely defined, and adherence to standardized diagnostic and therapeutic guidelines continues to be variable across settings. NTM are ubiquitous environmental organisms, widely distributed in soil, water, and biofilms, with the capacity to colonize or infect susceptible hosts. Among the various clinical manifestations, non-tuberculous mycobacterial pulmonary disease (NTM-PD) represents the most common presentation and is frequently associated with underlying structural lung abnormalities, including bronchiectasis, cystic fibrosis, and chronic

obstructive pulmonary disease (COPD) [1]-[3] [5] [7]-[14] [15].

NTM are broadly classified into rapid- and slow-growing groups based on culture characteristics. Slow-growing mycobacteria (SGM), requiring more than 7 days for visible growth, comprise a diverse group of clinically relevant species. To date, over 200 NTM species have been identified, many of which are implicated in human disease [2] [7] [11] [13] [14] [16]. Among these, the *Mycobacterium avium* complex (MAC), comprising *M. avium*, *M. intracellulare*, and *M. chimaera*, is most frequently associated with pulmonary disease globally. Other important species include *Mycobacterium kansasii*, *Mycobacterium simiae*, and *Mycobacterium gordonae* [3] [7] [10] [11].

Data from North India remain limited, although available reports suggest an NTM isolation rate of approximately 12.1% in Delhi [17]. Globally, NTM-associated mortality has shown an increasing trend, particularly among elderly populations. Furthermore, significant geographic variability in species distribution has been well documented, with MAC predominating worldwide [12].

In this context, the present study was undertaken to comprehensively characterize the species distribution and antimicrobial susceptibility patterns of slow-growing mycobacteria isolated from pulmonary and extrapulmonary clinical specimens in Delhi, North India, over an eight-year period (2018-2025). Traditionally, NTM has been classified according to the Runyon classification system, which categorizes species into four groups based on growth rate and pigment production. Accordingly, Groups I-III consist of slow-growing mycobacteria, further classified as photochromogens, scotochromogen, and non-chromogens, while Group IV includes rapidly growing species that typically exhibit visible growth within seven days (Table 1).

Table 1. Runyon classification of slow-growing mycobacteria species identified in the study (2018-2025).

Runyon Group	Pigment Characteristic	Species Identified in Dataset
Group I: Photochromogens	Produce yellow-orange pigment only after exposure to light	*M. kansasii*, *M. simiae*, *M. asiaticum*, *M. marinum*
Group II: Scotochromogen	Produce pigment in both light and dark	*Mycobacterium gordonae*, *Mycobacterium scrofulaceum*, *Mycobacterium szulgai*, *Mycobacterium lentiflavum*, *M. paragordonae*
Group III: Non-chromogens	Non-pigmented colonies in light and dark	*M. chimaera*, *Mycobacterium avium*, *Mycobacterium intracellulare group*, *Mycobacterium colombiense*, *Mycobacterium parascrofulaceum*, *Mycobacterium hiberniae*, *Mycobacterium heraklionense*, *Mycobacterium paraense*, *Mycobacterium sherrisii*, *Mycobacterium riyadhense*, *Mycobacterium europaeum*, *Mycobacterium heckeshornense*, *Mycobacterium nonchromogenicum*, *Mycobacterium kumamotonense*, *M. timonense*, *M. kubicae*, *M. marseillense*, *Mycobacterium longobardum*, *Mycobacterium shimoidei*, *Mycobacterium arupense*, *M. terrae*, *Mycobacterium saskatchewanense*, *Mycobacterium minnesotense*, *Mycobacterium xenopi*, *M. vulneris*, *M. triplex*, *M. farcinogenes*

2. Materials and Methods

This retrospective study was conducted at Dr Lal Path Labs, National Reference Laboratory (NRL), New Delhi, India, over an eight-year period from January 2018 to December 2025. All patients with culture-confirmed non-tuberculous mycobacteria (NTM) isolates from pulmonary and extrapulmonary clinical specimens were included. Application of the American Thoracic Society (ATS) diagnostic criteria for NTM pulmonary disease was not feasible due to the unavailability of detailed clinical and radiological data; accordingly, the analysis was restricted to microbiological and laboratory-based characterization of NTM isolates. Species identification was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with the MALDI Bio typer Compass/IVD reference library (2023). Antimicrobial susceptibility testing was conducted by broth microdilution using the Sensititre system and interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) M24-A2 guidelines [19].

2.1. Specimen Collection and Transport

Clinical specimens were collected from patients clinically suspected of tuberculosis and included sputum, bronchoalveolar lavage (BAL), body fluids (ascitic, pleural, peritoneal, pericardial, and synovial), pus, drain fluids, gastric aspirates, tissue samples, cerebrospinal fluid, and early-morning urine specimens collected on three consecutive days. All specimens were transported in sterile, leak-proof containers and processed in accordance with standard biosafety protocols. The analytical unit of the study was the mycobacterial isolate recovered through routine laboratory processing. As this investigation was based exclusively on retrospective laboratory data, patient follow-up, clinical evaluation, and radiological correlation were not performed.

2.2. Specimen Processing and Culture

All specimens were processed using standard N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) digestion, decontamination, and concentration methods. The concentrated sediment was allocated for microscopy and culture. An aliquot was inoculated into the MGIT 960 liquid culture system (Becton Dickinson) and incubated according to the manufacturer's recommendations. Cultures flagged as positive were examined by Ziehl-Neelsen and Gram staining. Acid-fast bacilli (AFB)-positive cultures were tested for the MPT64 antigen to differentiate *Mycobacterium tuberculosis* complex (MTBC) from nontuberculous mycobacteria (NTM). MPT64-negative isolates underwent species identification, whereas MPT64-positive isolates were further assessed for cording on ZN staining and confirmed after subculture on Middlebrook agar by MALDI-TOF mass spectrometry. Cultures flagged positively in the absence of AFB were considered contaminated. MGIT cultures remain negative after 42 days were reported as negative. A second aliquot was inoculated onto Lowenstein-Jensen (LJ) medium and incubated at 37°C, with

daily examination for the first week and weekly monitoring for up to six weeks. Growth was confirmed by ZN staining, and isolates demonstrating visible colonies within ≥7 days were classified as slow-growing mycobacteria. A third aliquot was inoculated onto blood agar to detect contamination by non-mycobacterial organisms. Direct smears were prepared from the remaining sediment, stained by the ZN method, and examined for AFB.

2.3. Protein Extraction and MALDI-TOF MS Analysis

Mycobacterial isolates were processed using an extended protein extraction protocol prior to MALDI-TOF analysis.

Protein extraction was performed using a standardized bead-beating method optimized for mycobacterial cell disruption. Briefly, 2 - 3 colonies (or a 0.5-mL pellet from MGIT culture) were suspended in 300 µL of distilled water, followed by the addition of 900 µL ethanol for inactivation and lysis. After centrifugation (13,000 rpm, 2 min), the pellet was resuspended in 50 µL of 70% formic acid and 50 µL of acetonitrile and vortexed. Zirconia-silica beads (0.1 mm; 100 µL) were added, and samples were bead-beaten at 30 Hz for 3 min using a Tissue Lyser II. Following centrifugation (13,000 rpm, 2 min), 1 µL of the supernatant was spotted onto an MSP 96 polished steel target plate and overlaid with 1 µL of α-cyano-4-hydroxycinnamic acid matrix (70% acetonitrile, 0.1% trifluoroacetic acid), then air-dried.

Spectra were acquired using 240 laser shots per spectrum over a mass range of 2000 - 20,000 Da, with automatic calibration performed using the Bruker Bacterial Test Standard. Identification scores were interpreted according to Bruker criteria: log(score) ≥2.0 indicated high-confidence species-level identification; scores of 1.7 - 1.999 indicated low-confidence species- or genus-level identification requiring manual review; scores of 1.0 - 1.699 supported genus-level identification only; and scores <1.0 were considered non-identifiable and prompted repeat extraction.

Preliminary identification was based on growth characteristics and colony morphology, followed by species-level identification using MALDI-TOF mass spectrometry. Species within MAC may be misidentified due to spectral overlap; molecular confirmation was not performed.

2.4. Statistical Analysis

Descriptive statistical analysis was performed using Microsoft Excel, and the distribution of slow-growing mycobacteria (SGM) was summarized as frequencies and percentages.

3. Results

Among 197,943 clinical specimens processed during the study period, 34,776 (17.5%) yielded acid-fast bacilli in culture. Of these, 30,343 (87.3%) were identified as members of the Mycobacterium tuberculosis complex, whereas 4433 (12.7%) were classified as non-tuberculous mycobacteria (NTM). Among the

NTM isolates, 2588 (58.4%) were rapidly growing mycobacteria and 1845 (41.6%) were slow-growing species. Pulmonary specimens accounted for 63.2% of the slow-growing mycobacteria isolates, while 36.8% recovered from extrapulmonary samples. Within the slow-growing group, *Mycobacterium avium complex* is the most frequently isolated slow NTM species. Detailed APC (Annual Percent Change) and segmented trend analysis are provided in **Table 2**.

Table 2. Temporal trends in NTM isolation rates among tuberculosis-suspected patients, 2018-2025.

Year	Total AFB (n = 34776)	Positive NTM (n = 4433)	% of NTM
2018	4785	551	11.5
2019	4015	547	13.6
2020	3721	274	7.4
2021	5344	479	8.9
2022	4163	578	13.9
2023	3882	537	13.8
2024	4189	658	15.7
2025	4677	809	17.3

A total of 34,776 acid-fast bacilli (AFB) culture samples were analyzed between 2018 and 2025, of which 4433 were identified as non-tuberculous mycobacteria (NTM), yielding an overall prevalence of 12.7%. The annual proportion of NTM isolation demonstrated variability over time, ranging from 7.4% in 2020 to 17.3% in 2025. An initial increase was observed from 11.5% in 2018 to 13.6% in 2019, followed by a transient decline in 2020, and a subsequent gradual rise through 2025, suggesting apparent increase in recent years; however, this was not statistically significant.

Despite these fluctuations, trend analysis using the Cochran-Armitage Chi-square test did not demonstrate a statistically significant temporal change ($p > 0.05$), indicating the absence of a consistent directional trend. This was further supported by Joinpoint regression analysis, which showed a minimal and non-significant increase in NTM proportions over time (Annual Percent Change [APC]: +0.15% per year; $p = 0.963$). Overall, these findings suggest that, despite year-to-year variability and a modest rise in recent years, the proportional burden of NTM remained stable without statistically significant temporal variation across the study period.

The distribution of slow-growing mycobacteria species isolated from various clinical specimens during 2018-2025 demonstrated that pulmonary specimens accounted for many isolates (1166/1845; 63.2%), with sputum (37.1%) and bronchoalveolar lavage (BAL) (26%) representing the predominant sources. In contrast, extrapulmonary specimens constituted 36.8% of the isolates.

Among the non-respiratory specimens, 171 isolates (9.2%) were categorized under other specimen types, followed by pus samples (145; 7.9%), tissue specimens (126; 6.8%), and body fluids (120; 6.5%). A smaller proportion of isolates were recovered from endometrial samples (57; 3.1%), urine (31; 1.7%), and menstrual blood (29; 1.6%) (Figure 1).

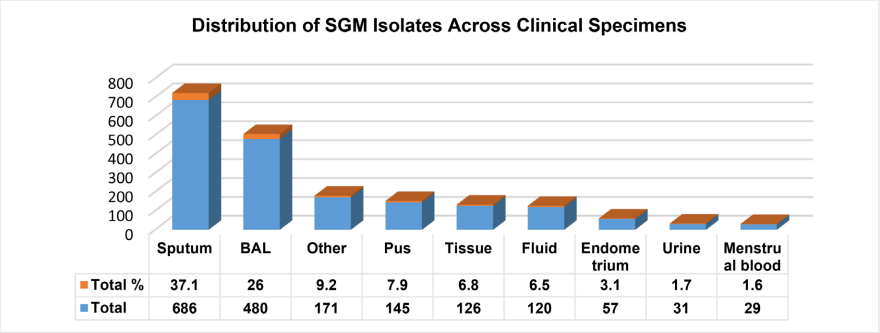

Figure 1. Distribution of slow growing mycobacteria isolates across clinical specimen types.

Slow-growing mycobacteria isolates were categorized according to the Ernest H. Runyon classification system based on pigment production. Most isolates belonged to the non-chromogenic group, accounting for approximately 67.8% (n = 1251) of all isolates. This group was predominantly represented by species such as *Mycobacterium chimaera*, *Mycobacterium avium*, *Mycobacterium intracellulare*, *M. colombiense*, and several other related species.

Photochromogens accounted for 26.7% (n = 493) of isolates, including *Mycobacterium kansasii*, *M. simiae*, *M. marinum*, and *M. asiaticum*, while scotochromogens comprised 5.5% (n = 101), represented by *M. gordonae*, *M. scrofulaceum*, *M. szulgai*, *M. lentiflavum*, and *M. paragordonae*. Overall, non-chromogenic slow-growing NTM predominated, followed by photochromogens and scotochromogens. Most isolates across all Runyon groups were derived from pulmonary specimens, with fewer from extrapulmonary sites; however, non-chromogenic species also showed notable extrapulmonary representation (Table 3).

Table 3. Pulmonary vs. extrapulmonary distribution of slow-growing mycobacteria isolates (2018-2025).

Table: Distribution of Slow-Growing mycobacteria According to Runyon Groups and Specimen Type (2018-2025)				
Runyon Group	**Representative Species**	**Pulmonary N = 1166 (63.2%)**	**Extrapulmonary N = 679 (36.8%)**	**Total N = 1845 (%)**
Group I: Photochromogens	*M. kansasii*, *M. simiae*, *M. marinum*, *M. asiaticum*	308 (16.7%)	185 (10%)	493 (26.7%)
Group II: Scotochromogens	*M. gordonae*, *M. lentiflavum*, *M. szulgai*	67 (3.6%)	34 (1.8%)	101 (5.5%)
Group III: Non-chromogens	*M. chimaera*, *M. avium*, *M. colombiense* and other related species	791 (42.9%)	460 (24.9%)	1251 (67.8%)

A total of 1845 slow-growing mycobacteria (SGM) isolates were recovered from various clinical specimens during the study period. Most isolates originated from pulmonary samples, accounting for 1166 (63.2%) of all isolates. The remaining 36.8% (679/1845) were obtained from extrapulmonary specimens, Among the identified species, members of the Mycobacterium avium complex (MAC) accounted for 55.7% of the slow-growing mycobacteria isolates. Within this group, *Mycobacterium chimaera* was the most frequently isolated species, representing 954 isolates (51.7%), followed by *Mycobacterium avium* (39 isolates) and isolates belonging to the *Mycobacterium intracellulare* (35 isolates). These closely related species were collectively analyzed as MAC due to their phylogenetic relatedness and similar clinical relevance. Most MAC isolates originated from respiratory samples, underscoring the predominance of pulmonary infection.

The next most frequently identified species were *Mycobacterium simiae* (240 isolates; 13.0%) and *Mycobacterium kansasii* (235 isolates; 12.7%), both predominantly recovered from pulmonary specimens. Less frequently encountered species included *Mycobacterium parascrofulaceum*, *Mycobacterium gordonae*, and *Mycobacterium colombiense*, along with several rare isolates such as *Mycobacterium europaeum*, *Mycobacterium heckeshornense*, *Mycobacterium xenopi*, and *Mycobacterium triplex*. It is important to note that some of these species may reflect environmental contamination or colonization rather than true clinical infection.

Notably, while most species were predominantly isolated from pulmonary samples, several organisms—including *Mycobacterium farcinogenes*, *Mycobacterium marinum*, *and Mycobacterium arupense*—were more frequently associated with extrapulmonary specimens, particularly pus and tissue, suggesting their involvement in soft-tissue or disseminated infections.

Overall, the data demonstrate that respiratory specimens represent the principal source of slow-growing mycobacteria isolates, with *Mycobacterium chimaera* emerging as the dominant species, followed by *Mycobacterium simiae* and *Mycobacterium kansasii*. The identification of multiple SGM species from both pulmonary and extrapulmonary specimens highlights the diversity and increasing clinical relevance of non-tuberculous mycobacteria in clinical settings (Table 4).

Table 4. Distribution of slow-growing mycobacteria species in different pulmonary and extrapulmonary samples.

Slow grower mycobacteria species (2018-2025)	Sputum	BAL	Pus	Fluid	Tissue	Urine	Menstrual blood	Endometrium	other	Total
M. kansasii	76	67	19	23	13	7	4	4	22	**235**
M. simiae	97	59	22	12	17	-	6	8	19	**240**
M. asiaticum	4	5	-	-	-	-	-	-	4	**13**
M. marinum			1		2				2	**5**
M. gordonae	18	9	-	4	3	2	-	-	2	**38**

Continued

M. scrofulaceum	8									**8**
M. szulgai	3	3								**6**
M. lentiflavum	9	5	-	1	5	-	-	-	5	**25**
M. Paragordonae	11	5	2	3					7	**28**
M. chimaera	353	261	79	64	53	22	14	39	69	**954**
M. avium	15	11	3	5	2	-	-	2	1	**39**
M. intracellulare group	13	11	3	1	3	-	1	2	1	**35**
M. parascrofulaceum	20	9	-	-	4	-	3	-	5	**41**
M. colombiense	5	4	3	2	5	-	-	-	2	**21**
M. heraklionense	10	7		1					4	**22**
M. hiberniae	6		2		3				5	**16**
M. paraense	3	4	-	-	2	-			3	**12**
M. sherrisii	3	5	-	-	3		-		3	**14**
M. riyadhense	5	2	-	-	-				1	**8**
M. farcinogenes	6	2	6	-	-	-	-	2	4	**20**
M. europaeum	2	2		3					1	**8**
M. heckeshornense	2				1				4	**7**
M. nonchromogenicum	5		2		2					**9**
M. timonense					3				2	**5**
M. kumamotonense		-	2	-	2		1		1	**6**
M. kubicae	3	2							1	**6**
M. marseillense	2	1								**3**
M. longobardum	-	2	-	-	-		-			**2**
M. shimoidei	4	3							2	**9**
M. arupense					2				1	**3**
M. terrae					1					**1**
M. saskatchewanense			1	1						**2**
M. minnesotense	1									**1**
M. xenopi		1								**1**
M. vulneris	1									**1**
M triplex	1									**1**
Total	**686**	**480**	**145**	**120**	**126**	**31**	**29**	**57**	**171**	**1845**

The age- and sex-wise distribution of SGM isolates is presented in Figure 2. Among the 1845 culture-positive cases, the largest proportion was observed in individuals aged ≥65 years (n = 516; 27.9%), followed by those aged 36 - 50 years (n = 496; 26.9%), 51 - 65 years (n = 452; 24.5%), and 13 - 35 years (n = 381; 20.7%). No cases were recorded in children aged 0 - 12 years (n = 0). The ≥65-year age group constituted the largest cohort and showed male predominance. Overall, males predominated in most age groups, except in the 36 - 50-year group, where females were slightly more prevalent.

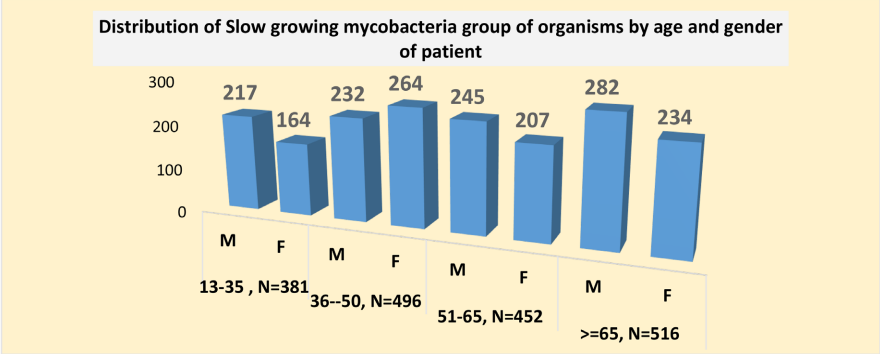

Figure 2. Distribution of slow growing mycobacteria (SGM) group of organisms by age and gender of patient.

AST was intentionally performed on a subset of 204 slow-growing mycobacteria isolates, in accordance with clinician-directed requests and real-world diagnostic practices. Given that routine susceptibility testing for all isolates is neither universally recommended nor always clinically indicated, particularly for certain NTM species, this targeted approach ensured clinically relevant and guideline-aligned testing.

Overall, most isolates showed high susceptibility to several important antimicrobial agents. Amikacin demonstrated the greatest activity, with 99.5% of isolates classified as susceptible and only 0.5% as resistant, reflecting excellent *in vitro* efficacy against slow-growing mycobacterial species. Clarithromycin also showed strong activity, with 97.5% susceptibility and 2.5% resistance, reinforcing its well-established role as a key drug in the treatment of many NTM infections.

High susceptibility rates were also observed for Linezolid (95.2% susceptible; 1.9% resistant) and Moxifloxacin (94.6% susceptible; 4.4% resistant), suggesting that these agents may serve as effective alternative or adjunct therapeutic options in selected cases. Moderately high susceptibility was observed for Cotrimoxazole (93.2% susceptible; 6.8% resistant). In contrast, Ciprofloxacin and Minocycline demonstrated 88.7% susceptibility, with resistance rates of 11.3% and 10.8%, respectively. Similarly, Doxycycline showed 86.2% susceptibility, with 2.5% intermediate susceptibility and 11.3% resistance.

Overall, the AST profile indicates that Amikacin and Clarithromycin exhibited the highest *in vitro* activity against slow-growing mycobacteria isolates, followed by Linezolid and Moxifloxacin. Conversely, comparatively higher resistance rates

were observed with Doxycycline, Ciprofloxacin, and Minocycline. These findings underscore the importance of species-level identification and antimicrobial susceptibility testing to guide appropriate therapeutic management of NTM infections (Figure 3).

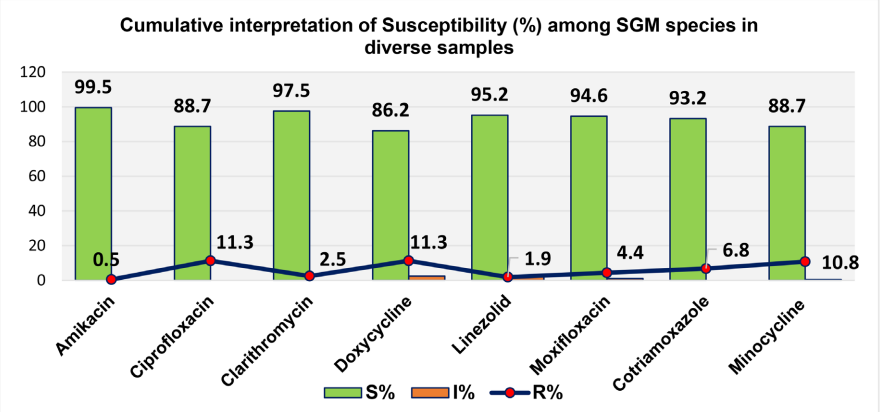

Figure 3. Cumulative interpretation of antimicrobial susceptibility (%) among slow growing mycobacteria (SGM) group of organisms in diverse samples.

Antimicrobial susceptibility testing was performed for 126 isolates of *Mycobacterium chimaera* and 78 isolates of other species of slow-growing mycobacteria (SGM).

Overall, both groups demonstrated high susceptibility to several key antimicrobials, although minor differences were observed between *M. chimaera* and other SGM species. Among *M. chimaera* isolates, Amikacin exhibited the highest activity, with 99.2% susceptibility and only 0.8% resistance, while other SGM isolates showed complete susceptibility (100%). Similarly, Clarithromycin demonstrated excellent activity, with 96.8% susceptibility in *M. chimaera* and 98.7% susceptibility among other SGM isolates, indicating its strong efficacy and clinical importance in NTM treatment regimens.

High susceptibility rates were also observed for Linezolid (95.3% in *M. chimaera* and 94.8% in other SGM) and Moxifloxacin (96.0% and 92.3%, respectively). These findings suggest that these agents may represent useful therapeutic options in the management of slow-growing mycobacteria infections.

Moderate susceptibility patterns were observed with Cotrimoxazole, showing 93.7% susceptibility in *M. chimaera* and 92.3% in other SGM isolates. In contrast, relatively higher resistance rates were noted for Ciprofloxacin, with 9.5% resistance among *M. chimaera* and 14.1% among other SGM isolates. Likewise, Doxycycline exhibited 84.9% susceptibility in *M. chimaera* and 88.5% in other SGM, with resistance rates exceeding 11% in both groups.

Among tetracycline derivatives, Minocycline demonstrated 86.5% susceptibility in *M. chimaera* compared with 92.3% in other SGM isolates, with resistance observed in 12.7% and 7.7% of isolates, respectively.

Overall, the findings indicate that Amikacin and Clarithromycin exhibited the

highest in-vitro activity against both *M. chimaera* and other slow-growing mycobacteria species, followed by Linezolid and Moxifloxacin. In contrast, comparatively higher resistance rates were observed with Ciprofloxacin, Doxycycline, and Minocycline, highlighting the importance of species-level identification and antimicrobial susceptibility testing to guide appropriate therapy for NTM infections (Table 5).

Table 5. Comparative antimicrobial susceptibility profile of *M. chimaera* and other slow-growing mycobacteria.

	M. chimaera, (N = 126)						Other Species of SGM (N = 78)					
Antibiotic	**S**	**S%**	**I**	**%**	**R**	**R%**	**S**	**S%**	**I**	**I%**	**R**	**R%**
Amikacin	125	**99.2**	**0**	**0**	1	0.8	78	**100**	0	0	**0**	0
Ciprofloxacin	114	**90.5**	**0**	**0**	12	9.5	67	**85.9**	0	0	**11**	14.1
Clarithromycin	122	**96.8**	**0**	**0**	4	3.2	77	**98.7**	0	0	**1**	1.3
Doxycycline	107	**84.9**	**5**	**3.9**	14	11.1	69	**88.5**	0	0	**9**	11.5
Linezolid	120	**95.3**	**4**	**3.2**	2	1.6	74	**94.8**	2	2.6	**2**	2.6
Moxifloxacin	121	**96**	**1**	**0.8**	4	3.2	72	**92.3**	1	1.3	**5**	6.4
Cotriamoxazole	118	**93.7**	**0**	**0**	8	6.3	72	**92.3**	0	0	**6**	7.7
Minocycline	109	**86.5**	**1**	**0.8**	16	12.7	72	**92.3**	0	0	**6**	7.7

4. Discussion

This study presents a large-scale, laboratory-based characterization of slow-growing mycobacteria (SGM) isolated from tuberculosis (TB)-suspected patients in a high-burden setting in North India over an eight-year period. NTM accounted for 12.7% of culture-positive mycobacterial isolates, reflecting a consistent presence in routine diagnostic practice. Although global studies have reported a rising prevalence of NTM, the present analysis demonstrated a stable proportional burden without a statistically significant temporal increase [2]-[6] [8] [9] [11] [12] [15] [17].

This trend is further supported by global mortality data, which show an increase in NTM-associated deaths from 0.36 per million in 2000 to 0.77 per million in 2022, particularly among older populations, underscoring the growing clinical importance of NTM infections [12]. The transient decline observed in 2020 is attributable to disruptions in healthcare access and diagnostic services during the COVID-19 pandemic [17]. The predominance of pulmonary specimens (63.2%) aligns with global data indicating that the respiratory tract remains the principal site of NTM recovery. However, the inclusion of a TB-suspected cohort inherently enriches for pulmonary samples and may overestimate the relative contribution of respiratory isolates [1] [2] [4] [8] [10] [11] [13]. The substantial proportion of extrapulmonary isolates further reinforces the broad ecological and pathogenic spectrum of NTM, encompassing soft tissue, body fluid, and genitourinary speci-

mens. Notably, certain species such as *Mycobacterium marinum*, *M. farcinogenes*, and *M. arupense* demonstrated a predilection for extrapulmonary sites, consistent with their known clinical associations [16].

A key finding of this study is the predominance of the *Mycobacterium avium* complex (MAC), which accounted for more than half of all slow-growing mycobacteria isolates. Within this group, *M. chimaera* was the most frequently identified species. While MAC dominance is consistent with global trends, the high proportion of *M. chimaera* should be interpreted cautiously. Species-level differentiation within MAC using MALDI-TOF mass spectrometry remains challenging due to spectral overlaps, and misclassification with closely related species, such as *M. intracellulare* cannot be excluded in the absence of molecular confirmation.

In contrast, several international studies have reported *M. avium* and *M. intracellulare* as the predominant MAC species [3] [6]. Nevertheless, the overall predominance of MAC observed in the present study remains aligned with global trends [3] [4] [8] [11] [13] [18]. Conversely, studies from other regions have identified *Mycobacterium kansasii* and *Mycobacterium simiae* as the leading species, highlighting substantial geographic variability in NTM species distribution [1-2] [15] [16]. These findings are in broad agreement with global data, where species such as *Mycobacterium abscessus*, *Mycobacterium fortuitum*, MAC, and *Mycobacterium kansasii* are among the most frequently encountered NTM [4] [7] [10] [11] [13] [17] [18].

The identification of thirty-seven distinct slow-growing mycobacteria species in this study highlights the expanding spectrum of mycobacteria recognized in clinical microbiology, driven by advances in diagnostic technologies. However, the clinical significance of many of these species remains uncertain. Notably, several organisms detected in this cohort, including *Mycobacterium gordonae* and other scotochromogen species, are well-recognized environmental contaminants. In the absence of correlated clinical, radiological, and repeat microbiological evidence, differentiation between true infection, colonization, and transient contamination remains challenging. Therefore, the present findings should be interpreted as reflecting patterns of laboratory isolation rather than definitive disease burden.

The *Mycobacterium tuberculosis* complex (MTBC) remained the predominant pathogen, consistent with TB-endemic settings [1] [2] [13]. In contrast, NTM predominates in low-TB-incidence regions, reflecting an epidemiological transition driven by declining TB burden and improved diagnostics [18]. The demographic distribution observed in this study, with a higher prevalence among older individuals and a slight male predominance, is broadly consistent with known epidemiological patterns of NTM isolation [5] [12] [16]. However, other studies have reported a female predominance in clinically significant NTM infections, indicating variability across populations [2]. Nonchromogenic species predominated, consistent with MAC dominance. Followed by photochromogens species such as *Mycobacterium simiae*, *M. kansasii* were identified nonchromogenic, whereas scotochromogen represent a smaller proportion, in line with international reports [4]

[7] [10].

Antimicrobial susceptibility testing revealed high in vitro activity of amikacin and clarithromycin against slow-growing NTM isolates, consistent with their established role as key agents in the treatment of NTM infections [4] [6] [7] [9] [10]. Linezolid and moxifloxacin also demonstrated favorable susceptibility profiles, indicating their potential as adjunctive therapeutic options in selected clinical settings. However, application of the American Thoracic Society (ATS) diagnostic criteria for NTM pulmonary disease was not feasible in this study due to the absence of detailed clinical and radiological data. Therefore, the findings are restricted to microbiological characterization and should not be interpreted as evidence of definitive clinical disease.

Importantly, for most NTM species, in vitro susceptibility does not consistently correlate with clinical outcomes, with the notable exception of macrolide susceptibility in *Mycobacterium avium* complex (MAC) infections. Although the clinical use of certain agents, such as linezolid, may be constrained by toxicity, they remain valuable components of treatment regimens when first-line options are unsuitable or ineffective [7] [13]. The variable susceptibility observed for fluoroquinolones and tetracyclines further underscores the intrinsic heterogeneity of NTM and the limitations of extrapolating in vitro findings to clinical decision-making. Collectively, these results reinforce current recommendations emphasizing the importance of accurate species-level identification and individualized, susceptibility-guided therapy rather than reliance on empirical treatment strategies.

This study has several notable strengths, including its large sample size, extended duration, and standardized laboratory workflow incorporating both liquid and solid culture systems, as well as MALDI-TOF-based species identification. These features provide a comprehensive overview of NTM isolation patterns in a high-volume reference laboratory.

Nevertheless, important limitations must be acknowledged. Foremost, the retrospective design and lack of clinical and radiological correlation precluded application of established diagnostic criteria for NTM disease. As a result, differentiation between true infection, colonization, and environmental contamination was not feasible. Second, species identification relied solely on MALDI-TOF mass spectrometry without molecular confirmation, which may have affected accuracy, particularly within closely related complexes such as MAC. Third, antimicrobial susceptibility testing was performed on a limited subset of isolates, potentially introducing selection bias. Finally, the study population comprised TB-suspected patients, which may limit the generalizability of findings to the broader community.

5. Conclusion

This study highlights the persistent and diverse presence of slow-growing NTM in a TB-endemic setting, with MAC predominance and substantial representation across both pulmonary and extrapulmonary specimens. The findings underscore

the diagnostic challenges posed by NTM in routine practice and the critical importance of accurate species identification. However, the absence of clinical correlation necessitates cautious interpretation, and future prospective studies integrating microbiological, clinical, and radiological data are essential to delineate the true burden and clinical significance of NTM disease in this region.

Author Contribution

Formal analysis: P.S., S.M.; Investigation: P.S.; Writing—original draft preparation: P.S.; Writing—review and editing: P.S., S.M.; Visualization: P.S., S.M., V.L. All authors have read and agreed to the published version of the manuscript.

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Ethical Approval

The study was based on anonymized laboratory data without patient identifiers; therefore, ethics committee approval was waived.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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