Infection and Drug Resistance

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Epidemiology and Drug Resistance of Pulmonary Nocardiosis Among Tuberculosis-Suspected Patients in Guangzhou, China

Chunming Luo[1], Guimin Zou[1], Yigang Tan[2], Jialou Zhu[1], Xin Liu[1], Cairong Zou[1], Kun Li[1]

[1]State Key Laboratory of Respiratory Disease, Guangzhou Key Laboratory of Tuberculosis Research, Department of Medical Laboratory, Guangzhou Chest Hospital, Institute of Tuberculosis, Guangzhou Medical University, Guangdong, 510095, People's Republic of China; [2]State Key Laboratory of Respiratory Disease, Guangzhou Key Laboratory of Tuberculosis Research, Department of Tuberculosis Internal Medicine, Guangzhou Chest Hospital, Institute of Tuberculosis, Guangzhou Medical University, Guangdong, 510095, People's Republic of China

Correspondence: Kun Li, Email 489601028@qq.com

Objective: This study aimed to analyze the detection characteristics, species distribution, and drug susceptibility profiles of *Nocardia* isolates among tuberculosis-suspected patients in the Guangzhou region, thereby offering valuable insights for clinical diagnosis and treatment.

Methods: This study included 77,550 clinical samples collected from tuberculosis-suspected patients. All specimens were cultured using the MGIT 960 liquid culture system. Culture-positive isolates underwent acid-fast staining, followed by *Nocardia* subculture purification and species identification using mass spectrometry. Subsequently, antimicrobial susceptibility testing was performed on 97 *Nocardia* clinical isolates against 13 antimicrobial agents.

Results: Species identification revealed *Nocardia farcinica* as the predominant species, accounting for 95.36% of all isolates. Morphological analysis following cultivation showed that *Nocardia farcinica* exhibited partial acid-fast staining characteristics, with a light blue background and faint red or pink filamentous bacilli displaying definitive branching structures. These features clearly distinguished it from the cord-like arrangements typically observed in mycobacteria. Antimicrobial susceptibility testing demonstrated that co-trimoxazole, amikacin, moxifloxacin, and linezolid were reliable therapeutic options. In contrast, the tested β-lactams, macrolides, and tetracyclines exhibited high resistance rates, limiting their clinical utility.

Conclusion: This study elucidates the epidemiological characteristics and antimicrobial susceptibility profiles of *Nocardia* infections in Guangzhou, providing valuable insights for early diagnosis and targeted therapy. Morphological analysis serves as an effective tool for reducing the risk of misdiagnosing *Nocardia* as mycobacteria. Future efforts should focus on optimizing detection methods and conducting multicenter studies to better understand prevalence trends and resistance mechanisms, ultimately improving clinical management.

Keywords: *Nocardia* infections, antimicrobial susceptibility, tuberculosis, acid-fast morphology

Introduction

Nocardia species are ubiquitous Gram-positive actinomycetes found in nature, commonly inhabiting soil, decomposing organic matter, and aquatic environments.[1] As opportunistic pathogens, they mainly infect immunocompromised individuals, such as patients living with HIV, organ transplant recipients, those with chronic pulmonary diseases, and long-term users of glucocorticoids or immunosuppressants. In recent years, the global incidence of nocardiosis has increased due to the growing population of immunocompromised individuals and advancements in diagnostic techniques.

These bacteria can invade multiple organ systems, most commonly affecting the lungs, followed by the central nervous system, skin and soft tissues.[2,3] Their diverse and non-specific clinical manifestations often lead to misdiagnosis as other diseases, particularly tuberculosis (TB), resulting in delayed diagnosis and clinical deterioration. A significant diagnostic challenge arises from the substantial overlap between nocardiosis and TB in terms of clinical symptoms, radiological features,

Received: 25 June 2025
Accepted: 21 October 2025
Published: 27 October 2025

and laboratory findings. This similarity not only increases the risk of misdiagnosis but may also cause treatment delays or inappropriate therapy, severely impacting patient prognosis. Specifically, both diseases present with persistent cough, low-grade fever, night sweats, and weight loss.[4] Radiologically, pulmonary infiltrates, cavitation, fibrosis, or nodular changes are common to both. Nocardial cavities are frequently mistaken for classic TB manifestations.[5] Given the insufficient specificity of radiological features, clinicians must rely on laboratory testing for differentiation. However, laboratory diagnosis remains challenging, as *Nocardia* may yield false-positive results in acid-fast staining,[6] a routine TB screening method, leading to misdiagnosis as TB and unnecessary anti-TB treatment. Diagnostic difficulties are further compounded by *Nocardia*'s slow growth and insufficient clinician awareness.

Despite the rising global incidence of nocardiosis, clinical recognition and prioritization remain inadequate, posing persistent diagnostic and therapeutic challenges. In China, particularly in high TB-burden regions like Guangzhou, unrecognized *Nocardia* infections may disproportionately affect immunocompromised TB-suspected patients.[7] The subtropical climate of Guangzhou, characterized by heat and humidity, along with its dense population and concentration of immunocompromised individuals, heightens the risk of nocardial infections. Nevertheless, systematic screening and analysis of *Nocardia* in TB-suspected patients remain scarce. Epidemiological characteristics, species distribution, and resistance patterns of *Nocardia* are poorly documented, with limited data regarding region-specific resistance profiles or prevalent strains. These knowledge gaps impede early recognition and precision therapy while potentially contributing to unnecessary anti-TB treatment and the emergence of drug resistance. Therefore, this study aims to explore the detection patterns and antimicrobial resistance profiles of *Nocardia* through culture-based identification and drug susceptibility testing of clinical isolates from TB-suspected patients in Guangzhou, thereby providing a scientific basis for guiding clinical practice.

This retrospective study analyzed 77,550 clinical samples collected from TB-suspected patients in Guangzhou between 2022 and 2024. All specimens were initially cultured in the MGIT 960 system, with acid-fast staining conducted on positive cultures. Suspected *Nocardia*-positive cultures were subcultured for purification, followed by species identification via matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Confirmed *Nocardia* isolates subsequently underwent antimicrobial susceptibility testing.

As the systematic assessment of *Nocardia* detection patterns and drug resistance among a large-scale TB-suspected population in Guangzhou, this study reveals regional epidemiological trends and their clinical significance. By delineating species distribution and antimicrobial susceptibility profiles, our findings enhance clinicians' recognition of nocardiosis, reduce the risk of misdiagnosis as TB or other infections, and provide a data-driven foundation for empirical therapy and personalized treatment regimens in Guangzhou and potentially across Southern China.

Materials and Methods

Collection of Clinical Specimens

This retrospective study enrolled TB-suspected patients admitted to Guangzhou Chest Hospital between January 2022 and December 2024. The inclusion criteria were as follows: (1) presence of typical TB symptoms (persistent cough lasting ≥2 weeks, low-grade fever, night sweats, or hemoptysis); (2) radiographic evidence of pulmonary abnormalities; (3) provision of qualified sputum specimens during the initial visit (sputum volume ≥3 mL with a purulent portion >50%). The exclusion criteria included: (1) previously diagnosed TB patients undergoing anti-TB therapy; and (2) administration of broad-spectrum antibiotics within 24 hours prior to sampling. Clinical specimens primarily consisted of morning sputum collected via deep coughing, supplemented by bronchoalveolar lavage fluid (BALF). Sample collection adhered to strict aseptic protocols to minimize contamination risks. Specimens were transported to the hospital laboratory within 2 hours of collection for prioritized acid-fast staining and subsequent MGIT 960 culture.

MGIT 960-Based Cultivation

Sputum or BALF samples (~5 mL) were placed in 50 mL centrifuge tubes. A volume of 2% N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) decontamination solution, ranging from 1 to 3 times the sample volume, was added. For viscous samples, a 3-fold volume was used. After vortex mixing for 20 seconds and standing for 15 minutes, sterile phosphate-buffered saline (PBS, pH 6.8) was added to the 50 mL mark. The tubes were tightly capped and centrifuged at

3,000 rpm for 15 minutes. The supernatant was discarded, and the sediment was resuspended in 1–3 mL PBS (pH 6.8) to adjust the pH to neutrality. Subsequently, 0.5 mL of the suspension was inoculated into BBL MGIT culture tubes and incubated in the BACTEC MGIT 960 system (Becton Dickinson, Sparks, MD, USA). The instrument automatically monitored fluorescence daily to detect mycobacterial growth. If growth was detected within 15 days, acid-fast staining was performed to identify *Nocardia*.

Acid-Fast Bacilli Staining

For sputum samples, purulent or caseous portions were evenly smeared onto glass slides, air-dried, and heat-fixed. For MGIT 960 culture-positive samples, approximately 30 μL of bacterial suspension was carefully aspirated from the bottom of the tube using a sterile 2 mL pipette. The suspension was then uniformly smeared onto a glass slide, air-dried, heat-fixed, and subsequently analyzed using acid-fast staining. Smears were flooded with 0.8% basic fuchsin (Baso, Zhuhai, China) and heated over an alcohol lamp until completely dry. The slides were then gently rinsed under running water. Decolorization was performed using a 5% acid-alcohol solution for approximately 1 minute with gentle agitation, followed by rinsing and air-drying. Counterstaining was conducted with 0.06% methylene blue for 1 minute, after which the slides were rinsed and dried. Microscopic examination under oil immersion revealed a light blue background; acid-fast bacilli appeared as bright red, slender rods occurring singly, in clusters, or with branching; weakly acid-fast *Nocardia* appeared as faint red/pink bacilli or filaments, occasionally exhibiting beaded or branching forms.

Subculture and Purification of Nocardia

Inoculated MGIT 960 tubes were incubated in the system for up to 15 days. *Nocardia*-positive cultures, which typically yielded signals within 7–10 days, were aseptically transferred using inoculation loops or swabs for streak inoculation onto Columbia Blood Agar plates (Baso, Zhuhai, China). These plates were then incubated at 37°C for 5–7 days. Colonies appeared as small, white formations on the blood agar and developed orange pigmentation with prolonged incubation. Acid-fast staining of smears from these colonies confirmed the distinct characteristics of *Nocardia*.

Species Identification of Nocardia by MALDI-TOF MS

Single colonies from the blood agar plates were picked with inoculation loops and transferred into 1.5 mL microcentrifuge tubes containing 0.5 mm glass beads and 500 μL of 70% ethanol. After vortexing for 15 minutes and inactivating at room temperature for 10 minutes, the entire suspension was transferred to 2 mL clear-bottom centrifuge tubes and centrifuged at 12,000 rpm for 2 minutes. The supernatant was completely discarded, and 10 μL of 70% formic acid (Sigma-Aldrich, St. Louis, MO, USA) was added to thoroughly resuspend the pellet by vortexing. Subsequently, 10 μL of acetonitrile was added, followed by repeated vortex mixing. The mixture was centrifuged under the same conditions for 2 minutes, after which 1 μL of the supernatant was transferred onto a target plate. After air-drying, the samples were overlaid with 1 μL of CHCA matrix solution (α-cyano-4-hydroxycinnamic acid, Sigma-Aldrich, St. Louis, MO, USA) and dried at room temperature. The target plates were analyzed using the VITEK MS system (bioMérieux, France). Species identification was performed using the Knowledge Base version 3.0, with identification confidence ≥99.9% based on protein spectral profile matching.

Antimicrobial Susceptibility Testing of Nocardia

Colonies from blood agar plates were picked using inoculation loops, vortex-mixed with 0.5 mm glass beads in saline until fully dispersed and adjusted to a 0.5 McFarland turbidity standard. A 50 μL aliquot was transferred to cation-adjusted Mueller–Hinton broth (Sensititre, Thermo Fisher Scientific, USA) tubes to achieve an inoculum concentration of 5×10^5 CFU/mL (range: 1×10^5–1×10^6 CFU/mL). After thorough mixing, 100 μL of the suspension was inoculated into antimicrobial susceptibility testing microplates (Sensititre, Thermo Fisher Scientific, USA). The sealed plates were incubated at 35°C for 72 hours. The microplates contained predefined two-fold serial dilution gradients of the following agents: moxifloxacin (MXF), amikacin (AMK), amoxicillin/clavulanate (AMC), cefepime (FEP), ceftriaxone (CRO), doxycycline (DOX), imipenem (IPM), linezolid (LZD), minocycline (MIN), tobramycin (TOB), co-trimoxazole (SXT), ciprofloxacin (CIP), and clarithromycin (CLR). Interpretive breakpoints for susceptible (S), intermediate (I), and resistant (R) categories followed

the Clinical and Laboratory Standards Institute (CLSI) guidelines (M24-A2, 2011).[8] Detailed MIC testing ranges and breakpoints are provided in Table S1.

Results

Characteristics of Collected Samples

Among 77,550 TB-suspected patient samples screened for *Nocardia* (Figure 1), analysis of 259 isolates revealed that infections predominantly affected older adults (Mann–Whitney test, $p > 0.05$) (Table 1, Table S2). The highest proportion was observed in the 45–64 age group (41.7%, 108/259), followed by those aged ≥65 years (32.8%, 85/259), 25–44 years

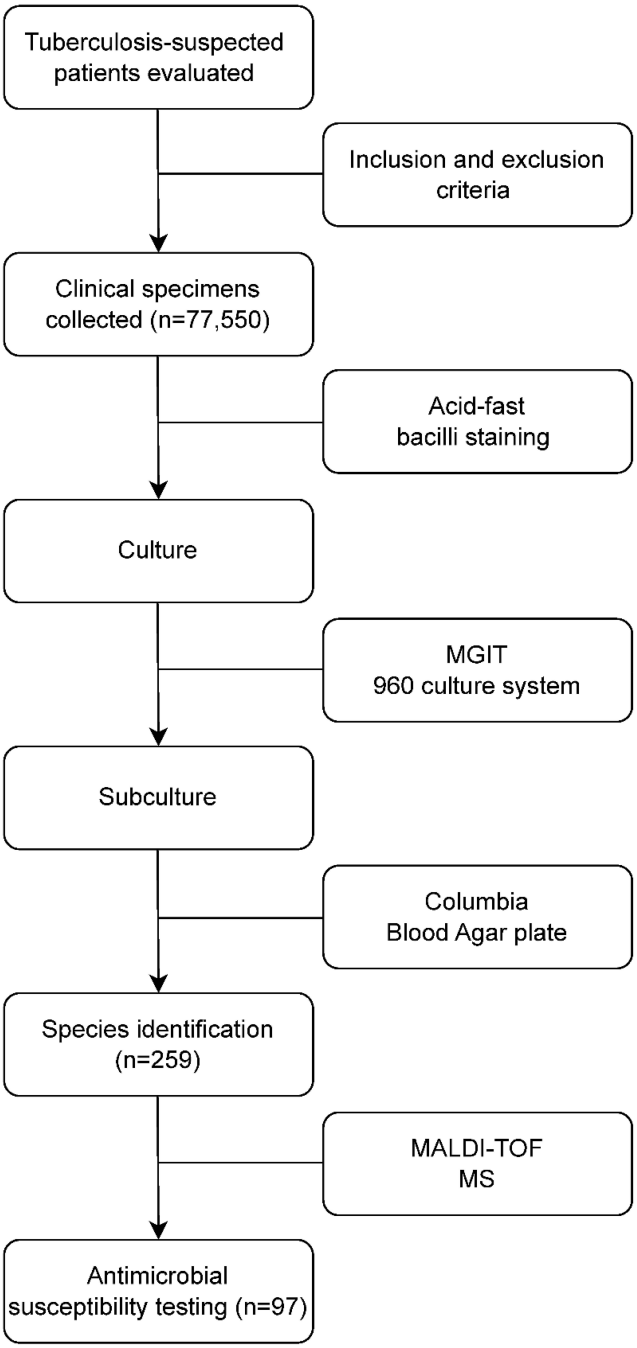

Figure 1 Study design flowchart.
Abbreviation: MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

Table 1 Demographic and Clinical Characteristics (n = 259)

Characteristics	n	%
Age		
≤24	16	6.2
25-44	50	19.3
45-64	108	41.7
≥65	85	32.8
Gender		
Female	135	52.1
Male	124	47.9
Sample type		
Sputum	254	98.1
BALF	5	1.9
Year	*Nocardia*-positive	
2022	23 (22909)[a]	0.10
2023	111 (26,453)	0.42
2024	125 (28,188)	0.44
Species		
N. farcinica	247	95.36
N. pseudobrasiliensis	1	0.39
N. cyriacigeorgica	1	0.39
Other[b]	10	3.86

Notes: [a]the total number of different patients suspected of having TB who were tested using MGIT960 liquid culture that year; [b]unable to identify the specific *Nocardia* species.
Abbreviation: BALF, bronchoalveolar lavage fluid.

(19.3%, 50/259), and ≤24 years (6.2%, 16/259). This age distribution suggests a potential association with age-related immune decline, particularly in older adults with chronic comorbidities or immunosuppression. Females slightly outnumbered males (52.1% vs 47.9%, Chi-square test, $p > 0.05$), though the difference was not statistically significant.

Sputum constituted 98.1% of positive samples (254/259), while BALF accounted for only 1.9% (5/259) (Table 1, Table S2). These findings align with *Nocardia*'s respiratory tropism and confirm sputum as the primary diagnostic specimen. BALF remains indicated for sputum-negative cases with high clinical suspicion.

Annual detection rates increased from 0.10% (23/22,909) in 2022 to 0.42% (111/26,453) in 2023 and 0.44% (125/28,188) in 2024 (Table 1, Table S2). This trend likely reflects improved clinical recognition, although the slower growth rate post-2023 warrants continued surveillance to define long-term epidemiological patterns.

Species distribution demonstrated the absolute dominance of *Nocardia farcinica* (*N. farcinica*, 95.36%, 247/259), with *N. pseudobrasiliensis* and *N. cyriacigeorgica* each representing 0.39% (1/259). Additionally, 3.86% (10/259) of isolates remained unidentified (Table 1, Table S2). The high prevalence of *N. farcinica* may relate to its enhanced pathogenicity and adaptability. The low proportion of unidentified isolates (3.86%) indicates robust species-level identification with current methods, though integration of 16S rRNA gene sequencing may further improve the detection of rare species.

Post-Culture Smear Staining and Morphology

Cultivation of TB-suspected patient samples from Guangzhou revealed distinct characteristics of *N. farcinica* in the MGIT 960 system. After approximately 15 days of incubation, characteristic flocculent precipitates formed (Figure 2A), concentrating at the tube bottom without wall adherence while maintaining overall broth clarity. On Columbia Blood Agar plates, 5-day cultures produced milky-white colonies measuring 1–2 mm in diameter (Figure 2B). These colonies

https://doi.org/10.2147/IDR.S549436

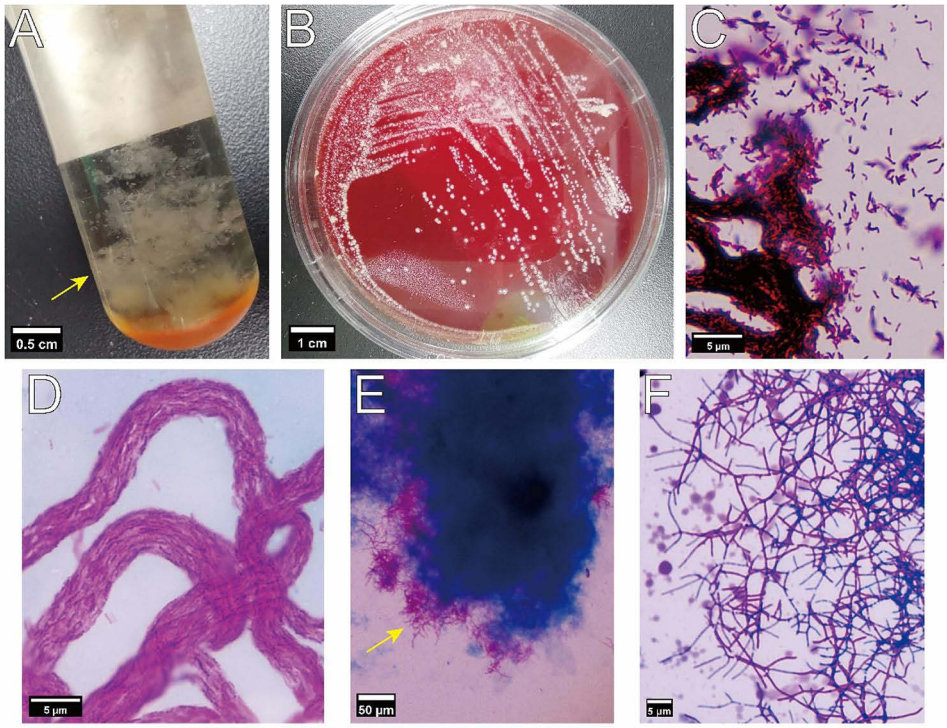

Figure 2 Morphological and staining characteristics of *Nocardia farcinica* and other mycobacteria cultured under different conditions. (**A**) *Nocardia farcinica* observed at the bottom of the MGIT960 culture tube after incubation in the MGIT960 system (yellow arrow; scale bar = 0.5 cm); (**B**) *Nocardia farcinica* colonies grown on a Columbia Blood Agar plate, showing characteristic morphology (scale bar = 1 cm); (**C**) Acid-fast staining of MTB cultured in the MGIT960 system (1000×), demonstrating uniformly acid-fast bacilli (scale bar = 5 μm); (**D**) Acid-fast staining of non-tuberculous mycobacteria (NTM, *Mycobacterium avium-intracellulare* complex) cultured in the MGIT960 system (1000×), exhibiting typical acid-fast properties (scale bar = 5 μm); (**E**) Acid-fast staining of *Nocardia farcinica* cultured in the MGIT960 system, visualized under 100× magnification (yellow arrow; scale bar = 50 μm); (**F**) Acid-fast staining of *Nocardia farcinica* cultured in the MGIT960 system, visualized under 1000× magnification, highlighting finer structural details (scale bar = 5 μm).

appeared uniformly circular, convex, and smooth-surfaced, with no hemolytic zones observed. Acid-fast staining of mycobacteria cultured for approximately 10 days showed *Mycobacterium tuberculosis* (MTB) forming tightly bundled cords with prominent dark granules (Figure 2C), whereas nontuberculous mycobacteria (NTM) exhibited loose cord formation without granules (Figure 2D). For *N. farcinica* following 15-day MGIT 960 culture, acid-fast staining demonstrated a light blue background with faint red/pink filamentous bacilli displaying definitive branching structures (Figures 2E and F). Crucially, a partial acid-fast property was observed: while some bacilli retained red/pink staining, others decolorized to blue. This partial acid-fast characteristic distinctly differs from the uniformly acid-fast nature of MTB and NTM.

Drug Sensitivity Profile of Nocardia Isolates

Antimicrobial susceptibility testing of 97 *Nocardia* clinical isolates against 13 agents revealed that SXT showed high susceptibility, with 96.9% of isolates being susceptible (94/97) and 3.1% resistant (3/97), confirming its important role in the treatment of nocardiosis (Table 2 and Table S3). AMK exhibited 99.0% susceptibility (96/97), with one intermediate isolate (1.0%) and no resistance, underscoring its critical therapeutic utility. MXF demonstrated 91.7% susceptibility (89/97) and 3.1% resistance (3/97), supporting its use as an alternative or adjunct to SXT. LZD showed 100.0% susceptibility (97/97), establishing it as a vital salvage agent. CIP had 83.5% susceptibility (81/97) and 5.2% resistance (5/97), warranting consideration as a secondary option. MIN displayed 79.4% intermediate rates (77/97) and only 11.3% susceptibility (11/97), suggesting potential intrinsic resistance. DOX showed limited efficacy, with 9.3% susceptibility (9/97) and 61.9% intermediate results (60/97), indicating constrained utility among tetracyclines.

High resistance was observed for the tested β-lactams, FEP at 89.6% (87/97) and CRO at 56.7% (55/97), confirming intrinsic resistance (Table 2 and Table S3). IPM resistance reached 80.4% (78/97), with only 10.3% susceptibility (10/97),

Table 2 Antibiotic Susceptibility Testing Results (n = 97)

Sensitivity	MXF	AMK	AMC	FEP	CRO	DOX	IPM	LZD	MIN	TOB	SXT	CIP	CLR
S (n, %)	89 (91.7)	96 (99.0)	37 (38.2)	5 (5.2)	13 (13.4)	9 (9.3)	10 (10.3)	97 (100.0)	11 (11.3)	7 (7.2)	94 (96.9)	81 (83.5)	3 (3.1)
I (n, %)	5 (5.2)	1 (1.0)	24 (24.7)	5 (5.2)	29 (29.9)	60 (61.9)	9 (9.3)	0 (0.0)	77 (79.4)	15 (15.5)	0 (0.0)	11 (11.3)	3 (3.1)
R (n, %)	3 (3.1)	0 (0.0)	36 (37.1)	87 (89.6)	55 (56.7)	28 (28.8)	78 (80.4)	0 (0.0)	9 (9.3)	75 (77.3)	3 (3.1)	5 (5.2)	91 (93.8)

Abbreviations: MXF, moxifloxacin; AMK, amikacin; AMC, amoxicillin/clavulanic acid; FEP, cefepime; CRO, ceftriaxone; DOX, doxycycline; IPM, imipenem; LZD, linezolid; MIN, minocycline; TOB, tobramycin; SXT, co-trimoxazole; CIP, ciprofloxacin; CLR, clarithromycin; S, susceptible; R, resistant; I, intermediate.

reflecting poor carbapenem applicability. CLR resistance was 93.8% (91/97), demonstrating minimal clinical utility. TOB showed only 7.2% susceptibility (7/97) and 15.5% intermediate results (15/97), contrasting sharply with AMK's high activity and highlighting variability within the aminoglycoside spectrum. Thus, SXT, AMK, MXF, and LZD represent the most reliable therapeutic options; β-lactams (FEP, CRO) and carbapenems (IPM) show pervasive resistance; macrolides (CLR) and tetracyclines (MIN, DOX) exhibit limited clinical value.

Discussion

Our study provides three clinically actionable insights for managing pulmonary nocardiosis in TB-suspected patients in Guangzhou. First, the overwhelming dominance of *N. farcinica* (95.36%), a high-virulence species with distinct environmental adaptability, necessitates region-specific empirical therapy. Second, the partial acid-fast, branching morphology of *N. farcinica* in MGIT 960 cultures serves as a rapid, cost-effective tool to differentiate it from uniformly acid-fast mycobacteria, reducing misdiagnosis. Third, the near-universal susceptibility to SXT, AMK, MXF, and LZD, contrasted with high intrinsic resistance to β-lactams and macrolides, defines a clear, evidence-based treatment hierarchy for our region. These findings directly address our study's objective to improve early diagnosis and targeted therapy.

Species identification revealed *N. farcinica* as the predominant species, accounting for 95.36% (247/259) of all isolates in our cohort. This striking dominance is likely attributable to the enhanced environmental adaptability and intrinsic pathogenicity of *N. farcinica*. As a high-virulence species, it frequently causes severe pulmonary infections in immunocompromised hosts.[9,10] Its pathogenic success may be driven by several key mechanisms: (1) the ability to survive and proliferate intracellularly within diverse human cell types; (2) immune evasion through the production of catalase and superoxide dismutase; (3) inhibition of phagosome-lysosome fusion and reduction of macrophage acid phosphatase levels; and (4) the secretion of toxins and hemolysins.[11] Critically, the Mce1E protein has been identified as a major virulence factor that mediates host cell invasion, potentially explaining its high isolation rate in our patient population.[12] Furthermore, the subtropical, humid climate of Guangzhou may favor the environmental proliferation and aerosolization of *N. farcinica*, facilitating inhalation exposure, the primary route of infection.

Our finding that *N. farcinica* constitutes 95.36% of pulmonary nocardiosis isolates in Guangzhou must be interpreted within the context of significant geographical variation in species prevalence. While *N. farcinica* is a globally recognized pathogen, its dominance is far from universal. For instance, a large multicenter study in China reported *N. farcinica* as the most common species, yet it accounted for only 29.1–34.1% of isolates,[13] substantially lower than our regional rate. In stark contrast, a Spanish study identified *N. cyriacigeorgica* as the predominant species (24.5%), with *N. farcinica* representing a mere 13.6% of cases.[14] Similarly, in a US cohort of non-transplant patients, *N. cyriacigeorgica* was the most frequently isolated species (38.0%), whereas *N. farcinica* accounted for only 16.1% of isolates.[3] This marked geographical heterogeneity likely arises from a confluence of factors, including environmental reservoirs (Guangzhou's subtropical, humid climate), host population characteristics (different local prevalence of specific immunocompromising conditions), and potentially, methodological differences in species identification. Critically, this regional variation has direct clinical implications. Empirical treatment guidelines developed in Europe or North America,[3,14] where different *Nocardia* species predominate, may not be optimal for Guangzhou. Our data provide a region-specific evidence base confirming that first-line agents effective against *N. farcinica*, namely SXT, AMK, MXF, and LZD, should be prioritized

in our local setting. This underscores the necessity of tailoring diagnostic and therapeutic strategies to local epidemiology rather than applying a one-size-fits-all global approach.

Following MGIT 960 culture, *N. farcinica* exhibited distinct morphological characteristics in acid-fast staining that are critical for differentiating it from other acid-fast pathogens like MTB and NTM (Figure 2). Specifically, *N. farcinica* appeared as faint red/pink, branching filamentous bacilli with partial acid-fastness, a hallmark feature where some bacilli retain red/pink staining while others decolorize to blue. In contrast, MTB formed tightly packed, uniformly acid-fast cords with prominent granular inclusions (Figure 2C), and NTM exhibited looser cords without granules (Figure 2D). This partial acid-fastness likely stems from structural differences in the *Nocardia* cell wall. Although *Nocardia* possesses mycolic acids like MTB, variations in their saturation, chain length, or content, combined with the possible presence of arabinogalactan, may reduce acid-fast stability and hinder stain retention.[15–17] Clinically, this atypical staining pattern serves as a rapid, cost-effective clue to suspect nocardiosis in TB-suspected samples, prompting confirmatory testing and reducing the risk of misdiagnosis. It is important to note that the morphological distinctions described herein, particularly the partial acid-fastness and branching filaments of *Nocardia*, versus the uniformly acid-fast, cord-forming morphology with granular inclusions observed in MTB, are based on empirical observations accumulated by clinical microbiologists over decades. While the exact biochemical nature of the granular structures in MTB remains to be definitively characterized, their consistent microscopic appearance serves as a valuable, rapid, and cost-effective tool for initial differential diagnosis in the MGIT 960 system.

Antimicrobial susceptibility analysis of 97 *Nocardia* isolates from Guangzhou delineated a clear regional profile, heavily influenced by the predominance of *N. farcinica* (95.36%). We confirmed SXT (96.9% susceptible), AMK (99.0%), MXF (91.7%), and LZD (100.0%) as the most reliable therapeutic options (Table 2). SXT susceptibility varies across studies. A Chinese multicenter study (2010–2020) reported 98.8% susceptibility,[18] whereas a US study (1995–-2004) indicated 61% sulfamethoxazole resistance and 42% SXT resistance,[19] underscoring the impact of geographical and species-specific factors.[20,21] Methodological variations, such as inoculum size and incubation duration, may also affect SXT susceptibility results.[22] Resistance mechanisms are likely associated with mutations in the *dfr* (dihydrofolate reductase) and *sul* (dihydropteroate synthase) genes.[23] Similarly, AMK's efficacy is paramount (99.0%), which is consistent with literature reports ranging from 99 to 100%,[24,25] but its parenteral route limits use. MXF presents a valuable oral alternative or adjunct to SXT, with in vitro synergy reported for certain *Nocardia* species.[26] Among these core agents, LZD serves as an excellent salvage option due to its oral bioavailability, though cost and toxicity concerns reserve it for complex cases.[27]

Conversely, our findings caution against the empirical use of several drug classes. High resistance was pervasive among β-lactams, including FEP (89.6%) and CRO (56.7%), attributable to constitutive β-lactamase production. While β-lactamase inhibitors, such as avibactam, can restore antibiotic activity by inhibiting these enzymes and reducing MICs when combined with amoxicillin,[28] our study revealed that 37.1% of isolates were resistant to amoxicillin/clavulanate (AMC), indicating the potential emergence of resistance to inhibitor combinations. The high macrolide resistance rate (CLR 93.8%) is consistent with data from Yantai, China,[29] which reported 100% non-susceptibility in *N. farcinica*. This resistance may be linked to point mutations in domain V of the 23S rRNA gene,[30] which reduce drug binding efficiency. Collectively, these findings underscore the necessity for region-specific empirical guidelines in areas where *N. farcinica* is prevalent.

This study has several limitations. First, while mass spectrometry provides robust species-level identification for most *Nocardia* isolates, 3.86% remained unclassified, highlighting the need for supplemental molecular methods, such as 16S rRNA gene sequencing, to accurately identify rare species. Second, the susceptibility testing covered only 13 antimicrobial agents, precluding a comprehensive evaluation of all potential therapeutic options, particularly newer agents and combination regimens. Furthermore, as a single-center retrospective study conducted in Guangzhou, our findings may not fully reflect the epidemiological characteristics and resistance patterns in other regions. Multicenter studies with larger cohorts are needed to validate these conclusions. Finally, the absence of a detailed investigation into resistance mechanisms, including mutations in the *dfr* and *sul* genes linked to sulfonamide resistance, hinders our ability to fully understand the factors driving resistance and refine targeted therapeutic strategies.

Conclusion

This study characterizes *Nocardia* infections among TB-suspected patients in Guangzhou, revealing key epidemiological features, species distribution, and antimicrobial susceptibility patterns. *N. farcinica* emerged as the predominant pathogen (95.36%), likely due to its heightened pathogenicity and environmental adaptability. The partial acid-fast staining characteristic of the species serves as a key diagnostic marker for differentiation. Susceptibility testing confirmed SXT, AMK, MXF, and LZD as reliable therapeutic options, while β-lactams, macrolides, and tetracyclines demonstrated high resistance rates. These findings provide critical guidance for the early diagnosis and precision therapy of *Nocardia* infections.

Ethics Approval and Consent to Participate

The study protocols were reviewed and approved by the Human Ethics Committee of Guangzhou Chest Hospital. The research was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their inclusion in the study. Clinical trial number: not applicable.

Funding

The authors declare that this research did not receive any external funding.

Disclosure

The authors declare that there are no conflicts of interest regarding the publication of this article.

References

1. Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ. Clinical and laboratory features of the Nocardia spp. based on current molecular taxonomy. *Clin Microbiol Rev*. 2006;19(2):259–282. doi:10.1128/CMR.19.2.259-282.2006
2. Wang H, Zhu Y, Cui Q, et al. Epidemiology and antimicrobial resistance profiles of the nocardia species in China, 2009 to 2021. *Microbiology Spectrum*. 2022;10(2):e0156021. doi:10.1128/spectrum.01560-21
3. Gupta S, Grant LM, Powers HR, et al. Invasive nocardia infections across distinct geographic regions, United States. *Emerg Infect Dis*. 2023;29 (12):2417–2425. doi:10.3201/eid2912.230673
4. Wilson JW. Nocardiosis: updates and clinical overview. *Mayo Clin Proc*. 2012;87(4):403–407. doi:10.1016/j.mayocp.2011.11.016
5. Steinbrink J, Leavens J, Kauffman CA, Miceli MH. Manifestations and outcomes of nocardia infections: comparison of immunocompromised and nonimmunocompromised adult patients. *Medicine*. 2018;97(40):e12436. doi:10.1097/MD.0000000000012436
6. Muricy EC, Lemes RA, Bombarda S, Ferrazoli L, Chimara E. Differentiation between nocardia spp. and mycobacterium spp.: critical aspects for bacteriological diagnosis. In: *Revista Do Instituto de Medicina Tropical de Sao Paulo*. Vol. 56. 2014;397–401. doi: 10.1590/s0036-46652014000500005
7. Kandi V. Human nocardia infections: a review of pulmonary nocardiosis. *Cureus*. 2015;7(8):e304. doi:10.7759/cureus.304
8. Clinical and Laboratory Standards Institute. *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes; Approved Standard*. 2nd ed. Wayne (PA): Clinical and Laboratory Standards Institute; 2011.
9. Xu N, Li L, Lei W, Qian W.Nocardia farcinica brain abscess in a multiple myeloma patient treated with proteasome inhibitor: a case report and review of the literature.11;9. doi:10.3390/brainsci11091204
10. Wang X, Liang Y, Cheng Q, Nong W, Hu L. Abdominal abscesses caused by nocardia farcinica in an immunocompromised patient: a case report and literature review. *Infection Drug Resistance*. 2023;16:7447–7454. doi:10.2147/idr.s441117
11. Mehta H, Shamoo Y. Pathogenic Nocardia: a diverse genus of emerging pathogens or just poorly recognized? *PLoS Pathogens*. 2020;16(3): e1008280. doi:10.1371/journal.ppat.1008280
12. Ji X, Tan X, Hou X, et al. Cloning, expression, invasion, and immunological reactivity of a mammalian cell entry protein encoded by the mce1 operon of nocardia farcinica. *Front Microbiol*. 2017;8:281. doi:10.3389/fmicb.2017.00281
13. Wang C, Sun Q, Yan J, et al. The species distribution and antimicrobial resistance profiles of Nocardia species in China: a systematic review and meta-analysis. *PLoS Negl Trop Dis*. 2023;17(7):e0011432. doi:10.1371/journal.pntd.0011432
14. Carrasco G, Valdezate S, Garrido N, Villalón P, Medina-Pascual MJ, Sáez-Nieto JA. Identification, typing, and phylogenetic relationships of the main clinical Nocardia species in Spain according to their gyrB and rpoB genes. *J Clin Microbiol*. 2013;51(11):3602–3608. doi:10.1128/JCM.00515-13
15. Beaman BL, Moring SE, Ioneda T. Effect of growth stage on mycolic acid structure in cell walls of Nocardia asteroides GUH-2. *J Bacteriol*. 1988;170(3):1137–1142. doi:10.1128/jb.170.3.1137-1142.1988
16. Bafghi MF, Heidarieh P, Soori T, et al. Nocardia isolation from clinical samples with the paraffin baiting technique. *Germs*. 2015;5(1):12–16. doi:10.11599/germs.2015.1066
17. Lechevalier H, Lechevalier MP, Becker B. Comparison of the chemical composition of cell-walls of nocardiae with that of other aerobic actinomycetes. *Int J Syst Evol Microbiol*. 1966;16(2):151–160. doi:10.1099/00207713-16-2-151
18. Wei M, Xu X, Yang J, et al. MLSA phylogeny and antimicrobial susceptibility of clinical Nocardia isolates: a multicenter retrospective study in China. *BMC Microbiol*. 2021;21(1):342. doi:10.1186/s12866-021-02412-x

19. Uhde KB, Pathak S, McCullum I Jr, et al. Antimicrobial-resistant nocardia isolates, United States, 1995–2004. *Clin Infect Dis*. 2010;51(12):1445–1448. doi:10.1086/657399
20. Schlaberg R, Fisher MA, Hanson KE. Susceptibility profiles of Nocardia isolates based on current taxonomy. *Antimicrob Agents Chemother*. 2014;58(2):795–800. doi:10.1128/AAC.01531-13
21. Hashemi-Shahraki A, Heidarieh P, Bostanabad SZ, et al. Genetic diversity and antimicrobial susceptibility of Nocardia species among patients with nocardiosis. *Sci Rep-Uk*. 2015;5:17862. doi:10.1038/srep17862
22. Bennett JE, Jennings AE. Factors influencing susceptibility of Nocardia species to trimethoprim-sulfamethoxazole. *Antimicrob Agents Chemother*. 1978;13(4):624–627. doi:10.1128/AAC.13.4.624
23. Valdezate S, Garrido N, Carrasco G, Villalon P, Medina-Pascual MJ, Saez-Nieto JA. Resistance gene pool to co-trimoxazole in non-susceptible Nocardia strains. *Front Microbiol*. 2015;6:376. doi:10.3389/fmicb.2015.00376
24. Larruskain J, Idigoras P, Marimon JM, Perez-Trallero E. Susceptibility of 186 Nocardia sp. isolates to 20 antimicrobial agents. *Antimicrob Agents Chemother*. 2011;55(6):2995–2998. doi:10.1128/AAC.01279-10
25. McTaggart LR, Doucet J, Witkowska M, Richardson SE. Antimicrobial susceptibility among clinical Nocardia species identified by multilocus sequence analysis. *Antimicrob Agents Chemother*. 2015;59(1):269–275. doi:10.1128/aac.02770-14
26. Tripodi MF, Durante-Mangoni E, Fortunato R, et al. In vitro activity of multiple antibiotic combinations against Nocardia: relationship with a short-term treatment strategy in heart transplant recipients with pulmonary nocardiosis. *Transpl Infect Dis*. 2011;13(4):335–343. doi:10.1111/j.1399-3062.2010.00588.x
27. Tanioka K, Nagao M, Yamamoto M, et al. Disseminated Nocardia farcinica infection in a patient with myasthenia gravis successfully treated by linezolid: a case report and literature review. *J Infect Chemother*. 2012;18(3):390–394. doi:10.1007/s10156-011-0315-1
28. Lebeaux D, Ourghanlian C, Dorchène D, et al. Inhibition activity of avibactam against nocardia farcinica β-lactamase FAR(IFM10152). *Antimicrob Agents Chemother*. 2020;64(2). doi:10.1128/aac.01551-19
29. Yi M, Wang L, Xu W, et al. Species distribution and antibiotic susceptibility of nocardia isolates from yantai, China. *Infect Drug Resist*. 2019;12:3653–3661. doi:10.2147/IDR.S232098
30. Taylor DE. Pathophysiology of antibiotic resistance: clarithromycin. *Can. J. Gastroenterol. Hepatol = J canad de gastroenterol*. 2000;14(10):891–894. doi:10.1155/2000/140806