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Nocardiosis: Overview, Diagnosis, and Clinical Implications

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Abstract

Nocardiosis is an uncommon but serious infection caused by aerobic, gram-positive bacteria from the genus *Nocardia*. These bacteria are ubiquitously found in soil and the environment, and more than 30 species can infect humans. Infections often occur in immunocompromised patients, involving organs such as the lungs, brain, and skin. Diagnosis typically relies on microbiological cultures and imaging techniques. Recent advances include metagenomic next-generation sequencing (mNGS) of bronchoalveolar lavage fluid (BALF), enabling rapid and precise identification of *Nocardia* species. Treatment is guided by species identification and antibiotic susceptibility, with trimethoprim-sulfamethoxazole commonly used. Among *Nocardia* species, *N. farcinica* and *N. brasiliensis* are notable pathogens causing severe infections like brain abscesses and cutaneous lesions, respectively. *N. farcinica* can also cause rare complications such as septic embolism and endocarditis. The temperature-dependent growth and colony morphology vary, affecting laboratory recognition. Early detection is essential as *Nocardia* infections can mimic other diseases and often require prolonged antibiotic therapy. The widespread environmental presence of *Nocardia* and its opportunistic nature necessitate heightened clinical awareness, especially in vulnerable populations. Timely and accurate diagnosis, including molecular techniques like mNGS, improves treatment outcomes and reduces mortality. Continued research on species-specific pathogenesis and antibiotic resistance will further enhance management strategies for nocardiosis.

Key words: *Nocardia* , Immunocompromised , Diagnosis , mNGS , Treatment , *N.farcinica*

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Introduction

Population-based descriptions of the disease nocardiosis and its etiologic agent, the bacterial genus *Nocardia*, in the United States are scarce, despite a substantial body of research . Nocardiosis is a medically important disease that more frequently affects immunocompromised patients. There are varied clinical presentations that can make clinical recognition challenging, and as a soilborne opportunistic pathogen, prevention methods are limited. The disease is difficult to treat, requiring months or years of antimicrobial therapy , and contributes to mortality in patients with underlying conditions. *Nocardiae* belong to the class *Actinobacteria* in the order Corynebacteriales . Other notable pathogenic genera in this group include *Mycobacterium* and *Corynebacterium*. The genus was first described in 1889 by Trevisan (Sneath, 1980), and reports on the clinical relevance of *Nocardia* began to appear in the literature in the early 1900s. In the 1940s, case reports appear implicating *Nocardia* species in invasive pulmonary infections. A review of nocardiosis cases published at that time notes that with the introduction of sulfonamide therapy in the late 1930s, fatalities from pulmonary nocardiosis decreased. However, a high mortality rate remained in patients with disseminated disease . Bacteria that belong to the genus *Nocardia* are high GC, aerobic, Gram-positive, partially acid-fast, lysozyme resistant, and catalase positive with a characteristic beaded branching cell morphology. On blood agar, *Nocardiae* form distinctive colonies with white aerial mycelium giving a chalky appearance to mature growth. A few species, most notably *Nocardia farcinica*, can appear as raised and wet (or muccoid) when young and then begin producing the characteristic aerial hyphae with age (Traxler et al., 2022). Nocardia cells exist ubiquitously in soil,organic matter, or water and help in decayingorganic matter. As an oral microflora, they are found innormal gingiva and periodontal pockets along withother species such as Actinomyces, Arthromyces andStreptomyces .

Among 85 species till identified, thehuman disease *Nocardiosis* is associated with *N.asteroides, N. brasiliensis, N. otitidiscaviarum, N.farcinica, N. abscessus, N. nova, N. transvalensis, N.pseudobrasiliensis and N. africana* . Nocardiaasteroides is the predominant human pathogen followedby *N. brasiliensis*, *Nocardia farcinica* and Nocardianova. When pulmonary infection is usually causedby *N. asteroides,* the skin infection is commonlyassociated with *N. brasiliensis.* The organisms aregram-positive, branching filamentous, weakly acid –fast, strictly aerobic, slow growing bacilli which mayfragment into bacillary and coccoid elements. Thedisease is most common in men than women without apparent racial predilection. All ages from infants to old are susceptible (Sethy et al., 2016). Nocardia is a genus of bacteria that is gram-positive, filamentous in structure (resembling fungi), and obligately aerobic. These bacteria are of significant medical interest due to their ability to cause severe diseases in both humans and livestock. Despite being Gram-positive, staining *Nocardia* for this characteristic can be challenging. Additionally, *Nocardia* is catalase-positive, bacillary in shape, and classified as relatively acid-fast, though it maintains a fungal-like appearance despite being bacterial in nature. There are around 100 species of *Nocardia* and about half are known to cause human infection, with the four most common isolates being *N. brasiliensis, N. cyriacigeorgica, N. farcinica, and N. nova complex*. The most common site of infection is pulmonary (62–86%), followed by central nervous system (CNS) (up to 44%), skin and soft tissue (8–31%), and disseminated infection (12–50%). *Nocardia* bacteraemia is usually associated with concurrent pulmonary, cutaneous or CNS infection but blood may be the only positive specimen in 38% of cases (McKinney et al., 2023) There are 85 known species of *Nocardia*, some of which are non-pathogenic while others are capable of causing the disease Nocardiosis. A key feature in microbial identification is acid-fastness. Bacteria are generally categorized as either gram-positive (stained purple) or Gram-negative (stained red), but acid-fast bacteria retain their original

stain even after exposure to strong acid solutions. This property is attributed to the high concentration of mycolic acids in the cell walls of acid-fast bacteria, including *Mycobacterium* and *Nocardia*. However, *Nocardia* is considered partially acid-fast, as its acid-fastness is comparatively weaker than true *mycobacteria*, such as those causing *tuberculosis*, due to lower concentrations of mycolic acids. Clinically, *Nocardia* serves as an opportunistic pathogen. It typically does not pose a threat to individuals with robust immune systems. However, in vulnerable populations such as organ transplant recipients, patients undergoing chemotherapy, or those with *HIV/AIDS* it can lead to severe infections. This underscores the importance of timely testing and ongoing research to understand how this bacterium breaches the body's defensive barriers and causes disease, which is critical for protecting at-risk individuals (McKinney et al., 2023). Nocardiae are part of the aerobic actinomycetes belonging to the class *Actinobacteria* in the order *Corynebacteriales* along with other clinically important genera, including *Corynebacterium* and *Mycobacterium*. They are opportunistic soil pathogens that are emerging as uncommon causes of invasive infections, primarily pulmonary, central nervous system (CNS), bloodstream (BSI), and skin and soft tissue infections (SSTIs) in immunocompromised patients(Church et al., 2025). Pulmonary nocardiosis is often confused with *tuberculosis*, fungal *pneumonia* such as aspergillosis, or bacterial infections. Late diagnosis due to similarities reduces the patient's chances of survival. The diagnosis of *Nocardia* requires certain colors (such as Nelsen Zil or warm colors) and long-lasting cultures . Research can be diagnostic methods such as pcr or safety methods . *Nocardia* is an embankment bacterium. Activities such as agriculture, construction, or climate change caused by dust displacement can increase environmental pollution. *N. farcinica* a causative agent of nocardiosis or of in immunocompromised patients. Strains of this species have been isolated from human brain abscesse.

Epidemiology and risk factors for nocardiosisNocardiosis is most commonly seen in immunocompromisedpatients, but may occur in immunocompetent persons. In recentstudies, apparently immunocompetent persons generally represented 18e45% of all cases of nocardiosis. Comorbiditiesassociated with impaired immunity (e.g. alcoholism, diabetes) arepresent in some infected patients classified as immunocompetentAmong immunocompromised hosts, solid organ transplant(SOT) recipients represent the largest group of patients withnocardiosis . Among them, the highest incidence ofnocardiosis is seen in lung and heart recipients, and in patients witha particularly high level of immunosuppressio.Other causes of immunodeficiency that have been associatedwith nocardiosis include hematopoietic stem cell transplantation(HSCT) , cancer and immunosuppression for autoimmunediseases. *Nocardia* infection is rare among human immunodeficiency virus (HIV)-infected persons, whorepresented 10% ofall cases of nocardiosis in recent series . Rareimmunodeficiencies possibly complicated by nocardiosis includechronic granulomatous disease, autoantibodies againstgranulocyte-macrophage colony-stimulating factor andectopic adrenocorticotropic hormone syndrome. Other potential risk factors for nocardiosis include the presenceof chronic lung disease, which has been reported in 10e58% ofinfected patients. Traumatic inoculation isanother well described risk factor which may cause skin nocardiosis(Margalit et al., 2021). Despite numerous *Nocardia* species have been characterized both phenotypically and genotypically within the genus, the genotype remains heterogeneous and continues to evolve .In addition, the genus of *Nocardia* is rapidly expanding and the species distribution varies with different geographical locations. Reports about *Nocardia* species in China are limited to a few case reports, case series, and research studies .Particularly, there is only limited information about the species distribution and drug susceptibility of *Nocardia* .

Thus, the present study was designed to identify *Nocardia* species using 16SrRNA and mass spectrometry (MS) in two tertiary hospitals in China and to investigate the species distribution, clinical manifestations, microbiological characteristics, and antimicrobial susceptibility of the *Nocardia* species. In addition, we retrospectively analyzed the therapeutic effects and prognosis of patients with *Nocardia* infection (Lu et al., 2020) . Cerebral Nocardiosis is a rare clinical entity representing only 2% of all cerebral abscesses. It usually results in intraparenchymal, poorly encapsulated abscess formation. One of the major risk factor for the occurrence of cerebral Nocardiosis is the host immunodeficiency following immunosuppressive drugs, organ transplant, *HIV*, diabetes mellitus, chronic infection and cancer. Although Nocardiosis can occur in immunocompetent hosts, symptomatic human infection needs careful evaluation of clinical conditions causing immunosuppression. 86% of all Nocardial infection in humans is caused by members of the *Nocardia* asteroides complex. Infection of the central nervous system can rarely be caused by N. cyriacigeorgica, brasiliensis, farcinica. (Baldawa et al., 2014).

Results

There are currently over a hundred known species of *Nocardia*, but around four or five of these account for 80 to 90 percent of nocardiosis cases in humans. These primary species include the *Nocardia asteroides complex, Nocardia farcinica, Nocardia nova, and Nocardia otitidiscaviarum*. In addition, other species are occasionally linked to nocardiosis infections. The first case was a 73-year-old man, from a poor socioeconomic background, with a 25-pack-year smoking history and alcohol dependence. Two years previously he had received radiotherapy for locally advanced non-keratinising, undifferentiated carcinoma of the nasopharynx. At a follow-up appointment, low serum titres of *Epstein–Barr virus (EBV)* DNA were found, raising suspicion for recurrence of nasopharyngeal carcinoma, an *EBV*-associated malignancy(Punjabi et al., 2024). Infections are often seen in immunocompromised hosts, especially in those with deficient cell-mediated immunity such as persons with organ transplantation, malignancy, diabetes mellitus, *AIDS*, autoimmune diseases, and prolonged corticosteroid therapy. Neutrophils form the first line of defense in early stages, and abscess formation is the hallmark of such infections. Protective immunity is largely cell mediated,but more virulent strains of *N. asteroides* may resist neutrophil-mediated killing while some others inhibit phagolysosome fusion to remain unabated as dormant Lforms, which cause serious infections and relapses(Mehta & Shamoo, 2020) . It is important to note that *Nocardia* species may colonize the airways, particularly among patients with chronic pulmonary disease . Identification of *Nocardia* in respiratory culture or by molecular methods does not prove pulmonary infection in isolation and needs to be considered in the context of the patient's symptomatology, radiographic findings, and comorbid conditions.Primary cutaneous nocardiosis takes 3 main forms: mycetoma, lymphocutaneous disease, and superficial skin infections.Mycetoma is most common in tropical regions and presentsas a chronic, progressive, destructive lesion that may involvedeeper structures or form fistulae . Lymphocutaneous infection leads to nodules or pustules tracking proximally along lymphatic channels, usually on an extremity . This is alsoreferred to as sporotrichoid spread and is seen with multiplefungal and mycobacterial pathogens. Superficial skin infectionshave been described as nonspecific skin abscess or cellulitis.These may be clinically indistinguishable from typical bacterial cellulitis or abscess and should be considered among patients who do not respond to first-line therapy(Yetmar et al., 2025).

Table1: Examining the history of identifying these species reveals that the discovery and classification of *Nocardia* have been a continuous process, with new species consistently being added to this genus as molecular techniques advance. Data regarding the year of discovery and geographical distribution (specific countries/locations) indicate that these bacteria are found across various environments worldwide. Acknowledging these temporal and spatial differences in identification is essential for a deeper understanding of the ecology and epidemiology of nocardiosis (Traxler et al., 2022) .

Species of Nocardia	Year	Source	Country of origin	Type strain in culture Collection
Nocardia aciditolerans	2013	Non-human	UKa	KACC 17155 / DSM 45801
Nocardia altamirensis	2008	Non-human	Spain	CIP 109315 /DSM 45049/ JCM 14670
Nocardia amamiensis	2007	Non- human	Japan	DSM 45066/JCM 14877 /KCTC 19208/NBRC 102102
Nocardia amikacinitolerans	2013	Human	USA	CCUG 59655 / DSM 45539
Nocardia arizonensis	2015	Human	USA	DSM 45748/CCUG 62754 /NBRC 08935
Nocardia artemisiae	2011	Non- human	China	CTCCAA 209038 / DSM 45379100526 /GU367157
Nocardia aurantia	2020	Non-human	Germany	NRRL B65542/ VKM Ac-2842/ KY558730
Nocardia aurantiaca	2020	Non-human	Thailand	JCM 33775 /TISTR 2838
Nocardia aurea	2019	Non-human	China	DSM 103986 /KCTC 39849 /MH091575
Nocardia barduliensis	2021	Human	Spain	CECT 9924 /DSM 109819
Nocardia bovistercoris	2021	Non-human	Chin	EML 1451/DSM 101696 /KU131666
Nocardia boironii	2016	Human	France	ATCC 70035/DSM 45135
Nocardia camponoti	2016	Non-human	China	DSM 100526/CGMCC 4.7278/ KP784782

Nocardia brasiliensis is primarily responsible for localized infections of the skin and underlying tissues, often reported in tropical regions. Compared to systemic species like *N. farcinica,* it generally exhibits greater susceptibility to antibiotics. Many strains respond well to trimethoprim-sulfamethoxazole, though isolates of *Nocardia farnesina* tend to show resistance to this drug. Effective treatment options often include antibiotics such as amikacin, amoxicillin-clavulanic acid, cefepime, cefotaxime, ceftriaxone, ciprofloxacin, doxycycline, gentamicin, imipenem, minocycline, moxifloxacin, trimethoprim, and vancomycin. On the other hand, *Nocardia farcinica* is a common cause of pulmonary infections, particularly in immunocompromised individuals. This species is frequently resistant to certain antibiotics like penicillin, making precise identification crucial This species is frequently resistant to certain antibiotics like penicillin, making precise identification crucial for appropriate diagnosis and treatment planning. *Nocardia farcinica* is a rare *Nocardia* species causing localised and disseminated infections. A case of *Nocardia farcinica* infection is presented, and 52 cases previously reported in the literature are reviewed. The hosts usually had predisposing conditions (85%), and acquired the infection through the respiratory tract or skin; the infection then often spread to the brain, kidney, joints, bones and eyes. Pulmonary or pleural infections (43%), brain abscesses (30%) and wound infections (15%) which failed to respond to conventional antimicrobial therapy were the more frequent forms of infection. *Nocardia farcinica* was frequently isolated from pus (100% of samples), bronchial secretions (41%) and biopsy specimens (63%), but isolation from blood and urine, as in the case presented here, is rare(Torres et al., 2000). *Nocardia brasiliensis* is primarily responsible for localized infections of the skin and underlying tissues, often reported in tropical regions. Compared to systemic species like *N. farcinica*, it generally exhibits greater susceptibility to antibiotics.

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Methods

Diagnosing Nocardiosis requires close cooperation between physicians and clinical microbiologists. Early suspicion of the disease can often be achieved through direct examination, primarily via Gram staining. *Nocardia* are weakly Gram-stained and typically appear as dotted or striped Gram-positive bacteria. They are usually arranged in thin, branched filaments that fragment into

However, similar findings may also occur in other *Actinobacteria* such as *Streptomyces* spp. or *Actinomyces* spp. *Nocardia* can be cultured on a variety of standard media, including blood agar, chocolate agar, buffered charcoal-yeast extract (BCYE), or Sabouraud agar. Cultures should be incubated for 14 to 21 days at 37°C under aerobic conditions enriched with 5% carbon dioxide and maintained in a humid atmosphere to prevent desiccation. *Nocardia* colonies are typically dome-shaped, slightly raised, and may exhibit a chalky appearance with a distinctive potting soil odor. The colonies may also vary in pigmentation, appearing beige-yellow, white, orange, or red-pink.The morphological and color variations of *Nocardia* on different culture media illustrate significant differences among strains. These differences, including features such as size, shape (spherical or puffed), and coloration (ranging from white to light brown), can complicate identification processes and limit the utility of selective media for differentiation. Diagnosing nocardiosis presents significant challenges due to its nonspecific clinical symptoms and the slow-growing nature of *Nocardia* in culture. Identification of *Nocardia* species may take several days to weeks, resulting in delays in initiating targeted treatment. Misdiagnosis is frequent, particularly in resource-limited areas without access to specialized diagnostic tools. Laboratory confirmation relies on microbiological culture, typically using selective media such as buffered charcoal yeast extract or Sabouraud dextrose agar. Molecular techniques, such as 16S rRNA gene sequencing and polymerase chain reaction (PCR), provide greater sensitivity and specificity for species identification. Histopathological examination, employing modified acid-fast staining, can assist in detecting the characteristic acid-fast branching filamentous bacteria. Additionally, radiological imaging like computed tomography (CT) or magnetic resonance imaging (MRI) can be useful for assessing disseminated disease.

The diagnostic process for *Nocardia* involves several steps: isolating and culturing the organism, identifying it, and confirming the species. Clinical specimens, such as cerebrospinal fluid (CSF) or tissue biopsies, are usually obtained by a surgeon or radiologist. Given Nocardia's slow growth, specimens are often treated with sodium hydroxide (NaOH) to remove normal flora and enhance isolation chances.Common culture media include Blood Agar and Chocolate Agar, but enriched or selective media may also be employed for more precise isolation. Examples include Middlebrook 7H10 or 7H11 Agar, originally developed for *Mycobacteria* but also effective for cultivating *Nocardia*. Sabouraud Dextrose Agar, commonly used for fungal cultures, is another option for growing *Nocardia*. Cultures should be incubated at 37°C for 2 to 3 weeks due to their typically slow growth rates. *Nocardia* spp. are Gram-positive, aerobic actinomycetes with a worldwide distribution in soil. At least six species are pathogenic for both humans and animals and may enter the body via inhalation of contaminated dust particles or via wounds contaminated with dust or soil. They are responsible for several infections including pulmonary, central nervous system, and cutaneous infections . An increasing number of cases have been reported since 1980, which supports the view of many investigators that the incidence of nocardiosis is on the rise.Nocardiosis is diagnosed by isolation and culture identification. However, colonial characteristics and cellular morphology are variable, and *Nocardia* spp. may be misidentified and confused with members of closely related genera such as Dietzia , *Gordona,Mycobacterium, Rhodococcus,Tsukamurella,* and even *Streptomyces* (Laurent et al., 1999). Atypical (or non-tuberculous) *mycobacteria* may have different histomorphological appearances from *Mycobacterium tuberculosis*, posing a diagnostic challenge in rare instances(Punjabi et al., 2024).

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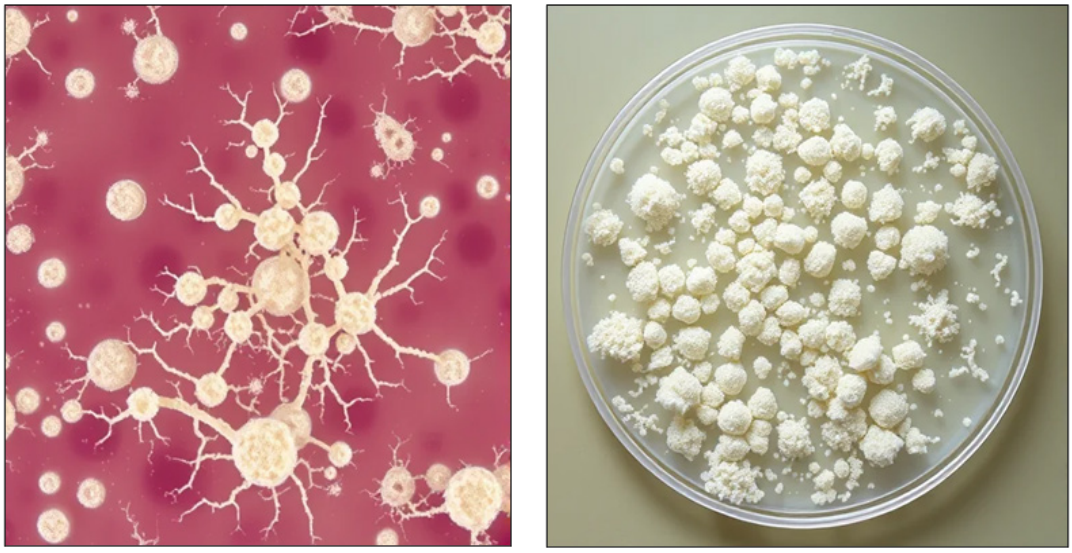
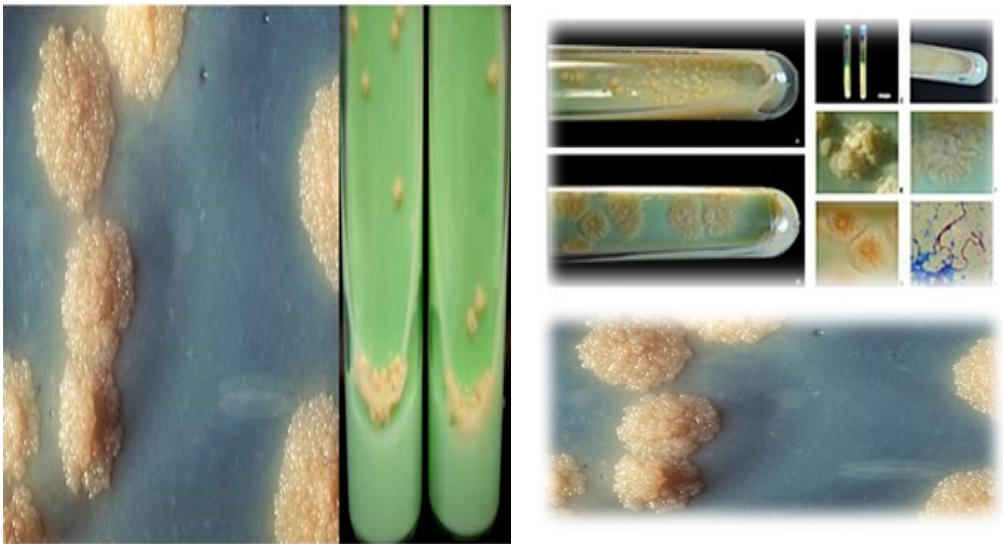
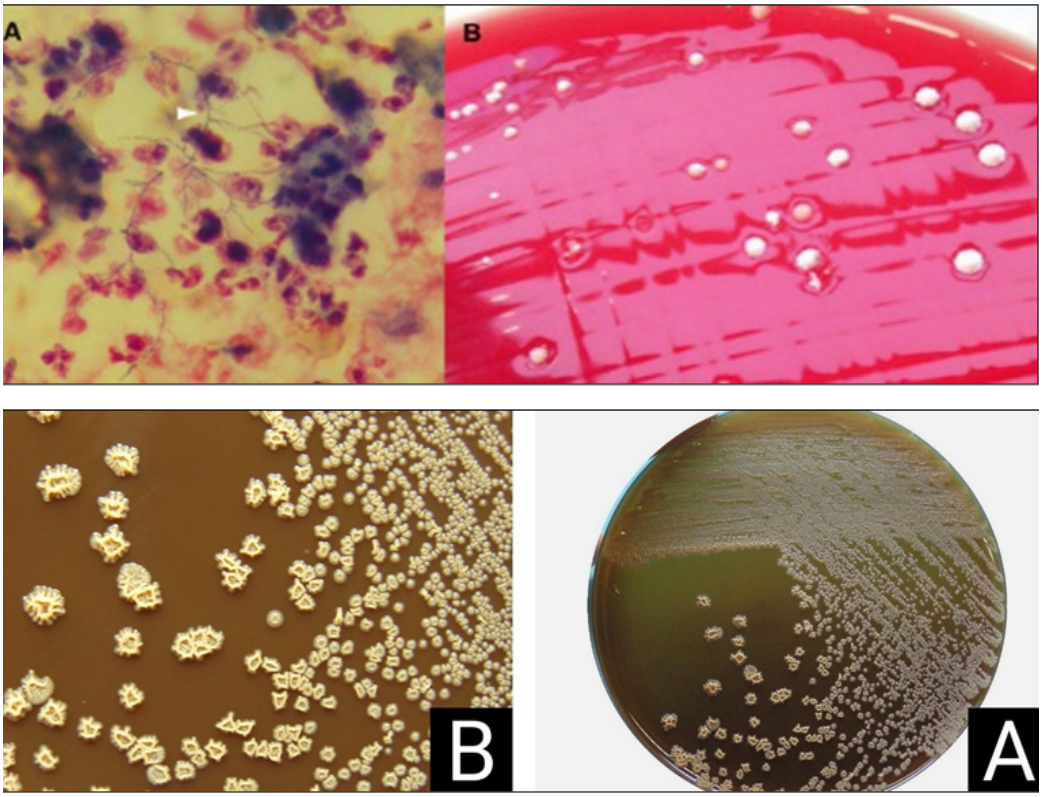


Figur 1 : Appearance: *Nocardia* typically appears as long chains of cells that resemble fungal mycelium, although with a much smaller diameter. These filamentous structures branch out, forming patterns reminiscent of trees or bush-like branches, giving rise to the term "branching."Fragmentation: A key characteristic of *Nocardia* is its tendency for these branching filaments to fragment easily into smaller, rod-like or cocci-like components. As a result, a microscopic sample will often display both long, branching filaments and smaller fragmented pieces.



Figur 2 : In culture media, *Nocardia* typically appears as dry, powdery colonies with a mold-like appearance and a relatively faster growth rate. These colonies may exhibit distinct pigmentation. In contrast, *Mycobacterium tuberculosis* (MTB) forms waxy, elevated, and wrinkled colonies, characterized by significantly slower growth. These visual differences mold-like versus waxy play a crucial role in the initial differentiation of these two acid-fast bacteria.



Figur 3: This figure shows the microscopic features and the initial and developed growth pattern of *Nocardia* colonies in culture media. In the first image (panels A and B), the microscopic view and the initial stages of colony growth are visible. In panel A, microscopic examination of a clinical specimen shows the presence of slender, branched, filamentous structures of *Nocardia,* indicated by the white arrow. This appearance is very similar to fungi and is the reason for the classification of *Nocardia* as a filamentous bacterium. Also, the relative acid-fastness of this bacterium, which is an important diagnostic indicator in the laboratory, is noteworthy. The presence of inflammatory cells, including neutrophils, also indicates the body's immune response to the infection. In panel B, the growth of *Nocardia* colonies on blood agar is shown. In the early stages of culture, colonies are small, round, convex, and creamy white in color, with a shiny appearance and soft texture, but as time passes and the colonies mature, their appearance becomes dry, chalky, and powdery.

Biochemical methods like oxidase and heximidase production tests, as well as molecular techniques such as 16S rRNA gene sequencing and whole genome sequencing (WGS), are frequently utilized as advanced tools for diagnosing *Nocardia*. When it comes to antibiotic testing for *Nocardia*, sulfonamides, including trimethoprim/sulfamethoxazole, along with macrolides, are commonly evaluated. In vitro evolution of *Nocardia* to TMP-SMXIn the absence of data identifying de novo mutations that contribute to resistance in vivo,Experimental evolution in vitro under the selective pressure from antibiotics can be used torecapitulate the paths leading to antimicrobial resistance in bacteria. Recently, ourgroup conducted in vitro experimental evolution to adapt susceptible clinical isolates of *N.nova* and *N. cyriacigeorgica* to the treatment of choice, TMP-SMX. To our knowledge,this is the first study of its kind to identify the genetic basis of de novo resistance to TMP-SMX in Nocardia. Not surprisingly, mutations were seen within genes encoding DHFR andDHPS. Some of those mutations were identical to mutations implicated in resistance in othersbacterial species like *Escherichia coli* and were involved in substrate or inhibitor binding. Inaddition to mutations affecting enzymes targeted by these drugs, changes were also seen in regulatory regions of genes encoding the folate pathway, which led to up-regulation(Mehta & Shamoo, 2020).

All *Nocardia* strains were susceptible to linezolid, followed by amikacin (99.3%; 3 of 7 *Nocardia wallacei* were amikacin-resistant) and TMP-SMX (99.1%; all 4 resistant strains belong to N. farcinica).or tetracyclines, doxycycline and minocycline-resistant Nocardia accounted for 2.0 and 0.9%, respectively, but the intermediate rates were high: 59.2% and 55.6%, respectively. Tigecycline showed low MIC values against different *Nocardia* species, with its MIC90 at 2 μg/mL. However, for macrolides, 73.9% *Nocardia* strains were resistant to clarithromycin.For β-lactam antibiotics, including imipenem, cefepime, cefoxitin, amoxicillin-clavulanic acid, and ceftriaxone, all demonstrated a poor performance against *Nocardia* spp. and high heterogeneity between *Nocardia* species, as shown in Table 3, suggesting the critical role of AST before the usage of these antibiotics(Wang et al., 2022) . The discovery of antibiotics was a great achievement for the control and treatment of infectious diseases . In spite of the fact that there are multiple classes of antibiotics available,excessiveuse of antibiotics has led to the development of drug-resistant bacteria. Moreover,antibiotic resistance genes can be transmitted from a bacterium to other strains via different ways, such asplasmids, chromosomes, or conjugative transposons, which can lead to the spread of antibiotic-resistant infections. In addition to its enormous economic burden, antibioticresistance also leads to increased morbidity and mortality in patients with bacterial infections . As a result, the identification of new and effective antibiotics that can inhibitthe growth of antibiotic-resistant bacteria is necessary. *Actinobacteria* are one of the important groups of soil microorganismsthat have an impressive role in the production of a variety of drugs that are highly essential forthe health and nutrition of humanity . Hence, natural product drugs with new chemical structuresmade from these bacteria have beneficial biological activities. Studies have revealed that almost 80% of antibiotics Studies on herbal medicine have also shown that endophytic actinomycetes surround the rootsof many herbal plants. Furthermore, endophytic actinomycetes, especially in samples isolated from the surface ofhealthy sterilized tissues, are considered a potential source for the production of substances such as secondarymetabolites, antimicrobial, antioxidants, and plant growth promoters (Seratnahaei et al., 2023). Sulfa-containing antimicrobials remain the drugs of choiceand may improve survival when used alone or in combination with other antimicrobials(Michael A. Saubolle & Den Sussland, 2003). Initial visualization of phenotypic colony coloration and morphology, together with the presence of aerial hyphae, with a dissecting microscope often provides initial clues to the genus of the isolate. Presumptive identification can be achieved if a filamentous, branched isolate stains with the carbolfuchsin modified acid-fast stain with a weak (0.5% to 1%) sulfuric acid decolorizing solution but not with the traditional Kinyoun acid-fast stain. Resistance to lysozyme differentiates *Nocardia* species from *Streptomyces* species. On occasion, examination of cell wall components by high-pressure liquid chromatography or thin-layer chromatography is needed for identification to the genus level. Identification to the species level may be more tedious and problematic.Originally, identification of the nocardial species was based on hydrolysis of casein, tyrosine, xanthine, and hypoxanthine. However, different stable susceptibility profiles among N. asteroides isolates showed that at least six unique species were identifiable . Molecular as well as further phenotypic studies of the species confirmed their disparity and uniqueness. At present, molecular methods used to successfully identify the nocardiae to the species level include restriction endonuclease analysis of an amplified portion of the 16S rRNA gene, restriction fragment length

polymorphism analysis of the amplified hsp gene, and sequencing methodologies, such as sequencing of the 16S rRNA or DNA (Michael A Saubolle & Den Sussland, 2003). Diagnosis is usually clinical, radiological, or by laboratory isolation. Clinical symptoms and signs are subtle andnonspecific, and diagnosis is bacteriological. Good clinical specimens, meticulous microscopy, prolonged cultures, and now polymerase chain reaction (PCR) are theway to make a correct and early diagnosis. Awareness ofthis disease is of paramount importance especially in an era of immunosuppression due to various causes(Duggal & Chugh, 2020).

Conclusion

This study highlights the antimicrobial susceptibility of *Nocardia* species isolated in Japan, showing that TMP-SMX remains effective against most isolates. However, resistant strains were found in *N. otitidiscaviarum, N. cyriacigeorgica*, and *N. mexicana.* While mechanisms of TMP-SMX resistance are well-studied in bacteria like *E. coli* and *Salmonella*, the resistance mechanisms in *Nocardia* remain unclear. Recent research suggests plasmid-mediated resistance genes contribute to high-level resistance, but no such strains were found in this study. In contrast, the resistant isolates exhibited low-level resistance, with no high-level TMP-SMX resistance identified. Furthermore, molecular methods like HRM and MLSA proved valuable for distinguishing *Nocardia* and other NTM species, providing accurate and rapid identification without sequencing. The findings emphasize the importance of molecular tools for diagnosing *Nocardia* infections and the need for continuous research to address antimicrobial resistance.

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