Nocardiosis: A Neglected Disease

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Highlights of the Study

- Nocardiosis is a global problem, though more common in the tropics; disease frequency is increasing due to increases in traveling and immunocompromised patients.
- Pulmonary nocardiosis is the commonest presentation but no part of the body is exempt.
- Diagnosis can be made by simple microscopy; however, culture and molecular methods provide a definitive diagnosis.
- Nocardiae are resistant to many antibiotics, but effective chemotherapies are available in most cases.

Keywords

Pulmonary nocardiosis · Infectious disease · Tropical medicine · Antimicrobial resistance

Abstract

Nocardiosis is a neglected tropical disease. It has varied geographical presence and a spectrum of clinical presentations. This review aims to focus on the epidemiology of nocardial infections with a systematic approach to their diagnosis and treatment. *Nocardia* causes chronic infections and ailments, and may remain cryptic but progressive in its course. Unless suspected, diagnosis can be easily missed resulting in increased morbidity and mortality. Thorough knowledge of local epidemiology, demography, clinical course and presentation, diagnostic modalities, and antibiotic susceptibility patterns of the prevalent *Nocardia* species is essential to curb spread of this infection. This is a systematic review in which internet search has been done for citation indices (Embase, PubMed, Ovid, and other individual journals) till March 2020 utilizing the following key words “Nocardia,”

“taxonomy,” “prevalence,” “clinical features,” “diagnosis,” “treatment,” and “susceptibility.” We selected a total of 87 review articles, case series, and case reports all in English language.

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Introduction

Nocardial infections were first explained by Edmond Nocard, a French veterinarian and microbiologist [1]. *Nocardia* are thin, gram-positive, weak, acid-fast, aerobic, branching, filamentous, slow-growing, soil-borne bacteria. Speciation of *Nocardia* is complex from a laboratory standpoint, as the taxonomy continues to evolve with new species identification and assignment of “complexes” based on their biochemical properties and antimicrobial susceptibilities. Currently, 109 validly published and correctly named taxa of *Nocardia* are known as per the “List of Prokaryotic names with Standing in the Literature” [1]. There are around 54 *Nocardia* species known to

Table 1. Summary of *Nocardia* cases from India and abroad

Author, year	Place	Cases	Site of involvement
Andleigh [77], 1954	Jaipur, India	6 <i>Nocardia</i> cases (22), <i>N. asteroides</i> (2), <i>N. madurae</i> (1), <i>N. pelletieri</i> (1)	Mycetoma foot
Randhawa et al. [78], 1973	Patel Chest Institute, New Delhi, India	11 <i>Nocardia</i> cases (<i>n</i> = 639) All <i>N. asteroides</i>	Chronic bronchopulmonary disorders
Randhawa and Khan [79], 1977	Delhi, India	18 cases	Pulmonary and systemic nocardiosis
Malik et al. [80], 1980	Rohtak, India	4 cases	Bronchopulmonary dysplasia
Malik et al. [81], 1985	Rohtak, India	30 <i>Nocardia</i> cases (<i>n</i> = 2,450)	Chronic bronchopulmonary disorders
Singh et al. [28], 2000	Amritsar, India	16 cases out of 1,016 patients with pulmonary tuberculosis All <i>N. asteroides</i> species complex	Pulmonary involvement
Shivaprakash et al. [82], 2007	Chandigarh, India	12 cases over a period of 26 months: <i>N. asteroides</i> complex (6), <i>N. brasiliensis</i> (5), <i>N. otitidiscaviarum</i> (1)	Pulmonary (5), CNS (3), subcutaneous tissue (1), anterior mediastinum (1), disseminated (2)
Lalitha et al. [83], 2012	Madurai, India	55 cases (11%) of 500 due to <i>Nocardia</i>	Corneal ulcers
Jain et al. [5], 2013	BLK Hospital, Delhi, India	7 cases: <i>N. farcinica</i> (2), <i>N. otitidiscaviarum</i> (2), <i>N. brasiliensis</i> (1)	Pulmonary (4), esophageo-mediastinal fistula (1), paraspinal abscess (1), blood (1)
Singh et al. [84], 2016	Ludhiana, India	44 cases	Pulmonary nocardiosis
Dawar et al. [85], 2016	Apollo, Delhi, India	12 cases: <i>N. asteroides</i> (5), <i>N. brasiliensis</i> (3), <i>N. farcinica</i> (2), <i>N. transvalensis</i> (1), <i>N. nova</i> (1).	Pulmonary nocardiosis
Shariff and Gunasekaran [86], 2016	VP Chest Institute, Delhi, India	5 cases	Pulmonary nocardiosis
Ninan et al. [32], 2016	South India	131 cases: <i>N. asteroides</i> (73), <i>Nocardia</i> spp. (32), <i>N. brasiliensis</i> (2), aerobic actinomycetes (24)	Skin (36), eye (36), pulmonary (35), immunosuppressive therapy (48)
Nampoory et al. [47], 1996	Kuwait	6 cases: <i>N. asteroides</i> (4), <i>N. otitidiscaviarum</i> (1), <i>N. farcinica</i> (1)	Pulmonary nocardiosis (3: with 2 cerebral abscess, 1 meningitis); perinephric abscess (1), thigh abscess (1), ischiorectal abscess (1)
Lai et al. [65], 2011	Taiwan	138 isolates: <i>N. brasiliensis</i> (58), <i>N. cyriacigeorgica</i> (22), <i>N. farcinica</i> (11), <i>N. beijingensis</i> (8), <i>N. otitidiscaviarum</i> (7), <i>N. nova</i> (6), <i>N. puris</i> (6), <i>N. asiatica</i> (5), <i>N. flavorosea</i> (5), <i>N. abscessus</i> (3), others (7)	Skin and soft-tissue, respiratory tract specimens, brain biopsy, blood, lymph node
Hardak et al. [67], 2012	Israel	53 isolates of <i>Nocardia</i> spp.	Immunodeficient (43): pulmonary (60%), skin and soft tissue (21%), bacteremia (11%), peritonitis (5%)
Bambace et al. [18], 2013	Montreal, Canada	11 cases in 440 HSCT recipients: <i>N. nova</i> commonest (27%)	HSCT recipients: pulmonary involvement with nodular infiltrates in 91%
Anagnostou et al. [87], 2014	Massachusetts, USA	5 cases: <i>N. asteroides</i> (2), <i>N. farcinica</i> (1), <i>N. nova</i> (1), unidentified species (1)	Neurological cases
Wang et al. [17], 2014	Texas, USA	132 cases, 138 strains: <i>N. nova</i> (27), <i>N. cyriacigeorgica</i> (25), <i>N. farcinica</i> (19), <i>N. abscessus</i> (11), others (56)	Cancer patients: respiratory samples (101), skin and soft tissue (16), blood (13), others (8)
Li et al. [20], 2015	Beijing, China	24 cases of autoimmune disease, 33 isolates of <i>Nocardia</i> spp.	Pulmonary (25), skin (7), brain (1)
Hashemi-Shahraki et al. [68], 2015	Iran	127 cases: <i>N. cyriacigeorgica</i> (35), <i>N. asteroides</i> (30), <i>N. farcinica</i> (26), <i>N. otitidiscaviarum</i> (12), <i>N. abscessus</i> (10)	Pulmonary (66) and extrapulmonary (61)
Valdezate et al. [61], 2017	Spain	1,119 <i>Nocardia</i> strains: <i>N. cyriacigeorgica</i> (25.3%), <i>N. nova</i> (15.0%), <i>N. abscessus</i> (12.7%), <i>N. farcinica</i> (11.4%), <i>N. carnea</i> (4.3%), <i>N. brasiliensis</i> (3.5%), <i>N. otitidiscaviarum</i> (3.1%), <i>N. flavorosea</i> (2.6%), <i>N. rhamsosiphila</i> (2.6%), <i>N. transvalensis</i> (2.4%)	Pulmonary (961), bone and soft tissue (66), blood (52), CNS (13), others (27)
Shannon et al. [19], 2016	Florida, USA	15 cases in 10 years	HSCT recipients: pulmonary (87%), disseminated (47%), blood (27%)
Tan et al. [57], 2020	Australia	270 cases: <i>N. nova</i> complex (80), <i>N. cyriacigeorgica</i> (61), <i>N. brasiliensis</i> (52), <i>N. farcinica</i> (38), <i>N. pseudobrasiliensis</i> (8), <i>N. beijingensis</i> (6), <i>N. transvalensis</i> complex (4), <i>N. abscessus</i> complex (4), other <i>Nocardia</i> spp. (17)	Respiratory (164), skin and soft tissue (82), CNS (13), blood (6), others (5)
Lebeaux et al. [62], 2019	France	793 isolates: <i>N. farcinica</i> (20.2%), <i>N. abscessus</i> complex (19.9%), <i>N. nova</i> complex (19.5%)	Pulmonary (53.8%)
Huang et al. [63], 2019	China	53 isolates: <i>N. farcinica</i> (13), <i>N. cyriacigeorgica</i> (11), <i>N. terpenica</i> (8), <i>N. abscessus</i> (5), <i>N. otitidiscaviarum</i> (4)	Lower respiratory tract (31), superficial infection (15), pleural effusion (3), CSF (2), bone marrow (1), synovial fluid (1)
Hamdi et al. [56], 2020	USA	2,091 strains of <i>Nocardia</i> : <i>N. nova</i> complex, <i>N. cyriacigeorgica</i> , <i>N. farcinica</i> complex, 25 others	2,091 clinical isolates over 7 years
HSCT, hematopoietic stem cell transplant.			

cause infections in humans [2], the majority being *N. nova* complex, *N. abscessus* complex, *N. transvalensis* complex, *N. farcinica*, *N. asteroides* type VI (*N. cyriaci-georgica*), *N. brevicatena*/*N. paucivorans* complex, and *N. brasiliensis* [2, 3]. Nocardial infections are transmitted either by inhalation, ingestion, or inoculation; inhalation is considered the most common route. It has been broadly classified as pulmonary, central nervous system (CNS), cutaneous, or disseminated nocardiosis depending on the location and extent of involvement though no site in the body is exempted [4]. 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 [5, 6]. 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 L-forms, which cause serious infections and relapses [7]. Diagnosis is usually clinical, radiological, or by laboratory isolation. Clinical symptoms and signs are subtle and nonspecific, and diagnosis is bacteriological. Good clinical specimens, meticulous microscopy, prolonged cultures, and now polymerase chain reaction (PCR) are the way to make a correct and early diagnosis. Awareness of this disease is of paramount importance especially in an era of immunosuppression due to various causes.

Nocardiosis: A Global Problem

Nocardiosis is a disease of global concern (Table 1). Annually 500–1,000 cases are reported in the USA [8] and 90–130 in Italy [9]. It is also prevalent in various parts of the world including tropical areas like India, Pakistan, Iran, and also Canada, Spain, and Australia in significant numbers [10]. The incidence of nocardiosis seems to be increasing every year due to increases in the population and immunocompromised patients, and also because of better awareness [10]. A Canadian study done over 2 decades revealed an increase in the annual incidence of *Nocardia* infection/colonization from 0.33 (1997–1998) to 0.87 (2007–2008) per 100,000 inhabitants ($p = 0.001$) [11], whereas a multicentric European study found no increase in hospitalization rates due to pulmonary nocardiosis between 2005 and 2011 [12]. Risk factors in the majority of patients (11 out of 13 diagnosed) in an Indian study included immunosuppressive etiology, e.g., solid organ transplantation (SOT), carcinoma, autoimmune

diseases, use of steroids, and immunosuppressive drugs [6]. In a review of 1,000 cases of nocardiosis over 40 years, 38% of patients studied were neither immunocompromised nor had a risk factor [13]. Pulmonary and disseminated nocardiosis is more prevalent among immunosuppressed patients, while cutaneous infections are more common in immunocompetent patients [3, 14]. The disease manifests as a result of impaired phagocyte function and is an opportunistic pathogen in patients of chronic granulomatous disease, Chédiak-Higashi syndrome, and leukocyte adhesion deficiency [7].

Nocardiosis in SOT patients was recently reviewed in 5,126 patients. Nocardiosis was reported in transplant patients: 3.5% of lung, 2.5% of heart, 1.3% in intestinal, 0.2% of renal, and 0.1% hepatic transplant patients [15]. A multicentric European study found 5 main risk factors for nocardiosis in SOT cases. These included patient age, high calcineurin inhibitor trough levels 1 month before diagnosis, use of tacrolimus or corticosteroid at the time of diagnosis, and length of stay in the intensive care unit after transplantation [16]. The disease has also been reported to occur in cancer patients. Wang et al. [17] reported 132 patients during 2002–2012 in the USA. It has also been reported to occur more often in patients with autoimmune diseases and stem cell transplant patients. Of 440 stem cell transplant cases reviewed between 2007 and 2011, 11 had developed nocardiosis [18]. In another review of nocardiosis in stem cell recipients between 2003 and 2015, 15 cases of nocardiosis were recorded [19]. In a review of 52 cases of pulmonary nocardiosis, 24 patients had autoimmune disease, and all were HIV negative [20]. Mean time for nocardiosis infection after glucocorticoid therapy in 11 nephrotic syndrome patients was 11.5 ± 14.8 months, and all patients recovered [21].

Nocardiosis in HIV-positive patients is reported to occur in 3.4–16.7% of cases in various centers [22–24]. The course of the disease may be localized or disseminated; in a Spanish study on HIV and nocardia coinfection, 44% of cases had disseminated nocardiosis [25]. In HIV patients with lung or pericardial involvement and CD4+ T-cell counts $<50/\mu\text{L}$, suspicion of nocardiosis should be strong [25]. Coinfections with *M. tuberculosis* and nontuberculous mycobacteria, especially in HIV-positive patients, have been reported occasionally [26]. In pulmonary infection with nontuberculous mycobacteria, concomitant infection with *Nocardia* spp. was seen in around 6% cases in a Chinese study [27]. Singh et al. [28] found 16 (1.4%) cases of tuberculosis and nocardial coinfection among 1,016 cases examined. Coinfections have been seen in HIV patients as well. In an African

study evaluating the presence of HIV, *Mycobacterium tuberculosis*, and *Nocardia* spp. [24], nocardiosis was significantly higher in HIV-positive patients, and about 6.7% of the study group had a coinfection involving all 3. In fact, in HIV-positive patients, coinfection with *Nocardia* was more common than tuberculosis. Looking at the high incidence of pulmonary nocardiosis in some African studies, screening for *Nocardia* in patients with respiratory symptoms nonresponsive to antituberculous treatment is warranted [24, 29].

Clinical Presentation

The hallmark of nocardiosis is abscess formation and chronic progression irrespective of anatomic barriers with a tendency to recur or relapse despite adequate treatment [30]. The hosts are often immunocompromised, onset is insidious, and clinical course is indolent. One to two thirds of nocardiosis patients have pulmonary involvement. Pulmonary nocardiosis may manifest as pneumonia, endobronchial inflammatory mass, lung abscess, or cavitary disease with contiguous extension into surface and deep structures. Pleural effusions and empyema have been recorded in up to 50% of patients with nocardiosis, 90% of these are caused by members of the *N. asteroides* complex [7]. This scenario is very similar to cases of mycobacteriosis. Diagnosis is laboratory based, and treatment differs. The clinical picture may also resemble malignancy, *Rhodococcus equi* (in HIV-infected patients), or fungal infections, especially *Aspergillus* pneumonia [3]. Due to this, *Nocardia* is described as a “great masquerader.”

The brain, bone, skin, eyes, heart, joints, and kidneys are the most common extrapulmonary sites. *Nocardia* spp. have been isolated from various sites from various geographical locations. The CNS is the most commonly involved extrapulmonary nocardiosis site [13]. In the CNS, nocardiosis may manifest as a result of local effects of granulomas or abscess formation in the brain, spinal cord, or meninges. CNS invasion may be cryptic, and *Nocardia* can persist with signs and symptoms varying from acute with rapid progression to a broad range of neurological deficits and may be misinterpreted as tumor, brain abscess, vascular infarction, or space-occupying lesions caused by infectious agents like toxoplasmosis, cysticercosis, tuberculosis, and mycotic agents [31]. Hematogenous spread leads to bacteremia and involvement of any organ, especially CNS (cerebral abscess or meningitis), kidneys, bones, and joints. During systemic infections, nocardiosis behaves like any other pyogenic bacteria causing septicemia [13].

Being ubiquitous in soil, cutaneous inoculation is common. This may result in cellulitis, pyoderma, and abscess formation, and resemble staphylococcal or streptococcal infections. The infection may spread through lymphatics to the regional lymph nodes, which leads to lymphocutaneous nocardiosis, whence it may be confused with *Sporothrix schenckii*. From a localized injury, *Nocardia* may proceed to form mycetomas on the feet, legs, arms, hands, or any other body site. *N. brasiliensis* is the most frequently implicated species in skin-related nocardiosis and causes progressive cutaneous and lymphocutaneous (sporotrichoid) disease, whereas cutaneous infections due to *N. asteroides* are mostly self-limited [13].

A study from South India has reported predominance of nocardiosis from the eye with a history of trauma or intraocular lens implantation [32]. In an agricultural area with hot dry climate, dispersal of *Nocardia* from soil sources remains a strong possibility. Such cases may be missed by health professionals lacking the epidemiological knowhow of the clinical cases. Relapses and multiple episodes have been known to occur in cases of nocardiosis, especially in patients with underlying immunosuppression. In a study on 132 patients of nocardiosis, 4 patients had at least 2 episodes at the same or different sites over a gap of 6–26 months with similar or different *Nocardia* isolates [17].

In some cases, nocardial infections have been described as “health care associated.” Corroborative findings indicate their link to dust in the hospital environment, food, water, or medical instruments resulting in infections consequent to direct inoculation, ingestion, or inhalation [3]. *N. asteroides* has been linked to an outbreak involving 6 patients in a renal transplant unit [33]. Another cluster of nosocomial transmission of *N. farcinica* was noted from postoperative sternal wounds, and identical isolate was found on the hands and in the home of a health care worker [34]. *Nocardia* may form heavy biofilms and has been recorded as a causative agent of central venous catheter-associated infections. In a study of 17 cancer patients, bacteremia was attributed to the catheter in 10 cases; for the other 7, it was a disseminated *Nocardia* infection, and *N. nova* complex was the commonest cause [35]. Evidence has been gained in these cluster outbreaks by comparing susceptibility patterns of patient and source isolates, randomly amplified polymorphic DNA analysis, rRNA gene restriction patterns (ribotyping), pulsed-field gel electrophoresis, and DNA fingerprinting in various studies [3, 7, 36].

Laboratory Diagnosis of Nocardia

The microbiology laboratory plays a very important role in the diagnosis of nocardiosis. Confirmation of *Nocardia* infection can be only laboratory based as the clinical presentation mimics other common infections, and epidemiological data of nocardial infections are inconclusive [37]. Specimens submitted for diagnosis include respiratory secretions like bronchial washings, bronchioalveolar lavage fluid, sputum samples, abscesses, aspirates, wound drainages, tissue or skin biopsies, or cerebrospinal fluid. The specimen should be visibly inspected for the presence or absence of granules. Granules are most commonly seen with *N. brasiliensis*. If present, they should be carefully washed in sterile saline, crushed, and examined microscopically [4]. Laboratory diagnosis depends on the quality of the sample, rigorous microscopy, paraffin bait technique, and extended incubation. Microbiology laboratories should always be alerted on the possibility of nocardiosis as special staining techniques and media may be required for diagnosis. On Gram stain, *Nocardia* appears as thin ($<1\ \mu\text{m}$ in diameter) and long bacilli with right-angle branching and with tiny noncontiguous gram-positive beads of varying sizes. It may be difficult to visualize on Gram stain or may appear as negative images. Hence, a modified acid-fast smear should always be done in case nocardiosis is suspected. *Nocardia* may easily be identified on routine Papanicolaou stain, too [38]. *Nocardia* spp. can grow on routine laboratory media like blood agar, but from specimens like sputum containing normal flora, specialized medium such as buffered charcoal yeast extract agar or traditional mycobacterial media should be used. Prolonged incubation for up to 3 weeks may be required, although most isolates grow within 3–5 days. Colonies of *Nocardia* are powdery and whitish because of aerial mycelia with a tan to orange reverse. Organisms stained from culture may be morphologically less distinct compared to those visualized directly in specimens. Histopathology of biopsy samples reveals necrosis with microabscesses; branching filaments may be visible by methenamine silver or Grocott stains [39].

There are more than a hundred species of *Nocardia* [2], but only a half of these are pathogenic. Other *Nocardia* species are rare; they may be found as colonizers or as laboratory contaminants. In one study, *Nocardia* were found as colonizers and persisters in patients with cystic fibrosis, and their treatment did not aid in improving their pulmonary function [40]. A majority of clinical isolates were grouped as *N. asteroides* complex as they were relatively inert biochemically [41]. Based on 65-kDa heat

shock protein 65 (hsp65) and 16S rRNA sequence analysis, this complex has been divided into 6 taxa [3]; therefore, the term *N. asteroides* complex is not used any longer. Subsequently, *N. asteroides* ceased to be the most commonly isolated *Nocardia* species from humans [2] though it still remains the type species [1]. It is believed that the majority of the clinically significant isolates previously identified as *N. asteroides* complex belonged to drug pattern type VI, which have been further speciated as *N. cyriacigeorgica* [42]. Gas-liquid chromatography has also been used by some researchers for the identification of *Nocardia* [43]. Kjelstrom and Beaman [44] evaluated a battery of antigens and tested various serologic methods for the detection of antibodies against *N. asteroides*. They professed the use of multiple antigens to increase sensitivity and specificity of diagnosis. ELISA using *N. brasiliensis* purified antigens has been recommended by researchers for diagnosis in mycetoma patients [45]. Enzymatic analysis has also been attempted to type *Nocardia* spp. for soil as well as clinical isolates [46]. In a study conducted on 513 cases of renal transplantation at Kuwait between 1989 and 1994 [47], 6 developed nocardiosis, 4 of which were identified as *N. asteroides*, 1 each as *N. farcinica* and *N. otitidiscaviarum*. Further, a soil study involving 102 samples from the same geographical area revealed presence of *N. asteroides* from 42 positive samples [48]. Seven common enzymatic patterns were seen in over 90% of these soil isolates based on the API-ZYM system comparable with the clinical isolates of *N. asteroides* [46]. Whereas all above diagnostic methods are time consuming and labor intensive, proteomic profile analysis using MALDI-TOF MS (matrix-assisted laser desorption ionization-time of flight mass spectrometry) quickly identifies *Nocardia* spp. and hence aids in early diagnosis and empiric therapy. Even with a limited database, successful identification has been achieved for common species, like *N. brasiliensis*, *N. cyriacigeorgica*, *N. farcinica*, *N. nova*/*N. nova* complex, and *N. otitidiscaviarum* [49]. Rapid molecular identification of *Nocardia* spp. is aided by PCR, DNA probes, DNA sequencing, pyrosequencing, ribotyping, and restriction fragment length polymorphism analyses [50]. Molecular targets include multilocus sequence analysis of *Nocardia* housekeeping genes: 16S rRNA gene region, hsp65 gene, essential secretory protein gene secA1, DNA gyrase gene gyrA, and RNA polymerase B gene rpoB. A cocktail of 2–3 of these genes can reasonably identify *Nocardia* species in many laboratories [2]. These can directly detect *Nocardia* in paraffin-embedded tissue and clinical samples also [51].

Table 2. Activity of various antibiotics in the treatment of *Nocardia* spp.

	Sulfon-amides	Ampi-cillin	Amoxycillin-clavulanic acid	Ceftri-axone	Line-zolid	Ami-kacin	Imi-penem	Fluoro-quinolones	Clarithro-mycin
<i>N. asteroides</i> (<i>N. cyriacigeorgica</i>)	+	–	+/-	+	+	+	+	–	–
<i>N. abscessus</i> (formerly <i>N. asteroides</i> type I drug susceptibility pattern)	+	+	+	+	+	+	–	–	–
<i>N. nova</i> complex (formerly <i>N. asteroides</i> type III drug susceptibility pattern)	+	+/-	–	+	+	+	+	–	+
<i>N. brasiliensis</i>	+	–	+	+/-	+	+	–	–	–
<i>N. otitidiscaviarum</i>	+/-	–	–	–	+	+	–	+	–
<i>N. farcinica</i> (formerly <i>N. asteroides</i> type V drug susceptibility pattern)	+/-	–	–	–	+	+	+	+	–

+, usually susceptible; –, usually resistant; +/-, susceptibility may be strain dependent.

Radiological investigations such as X-rays, CT, and MRI help in localizing the lesions and defining extent of organ/space involvement. In a study involving cases of pulmonary nocardiosis, the most common radiological abnormality on CT scan was nodules with or without cavitation [52]. In another study, isolated, scattered nodules and masses, mostly subpleural or near lung hilum, were seen in a majority of patients radiologically [21]. Follow-up scans at 1, 3, 6, or 12 months may be done for some deep-seated infections to assess response to therapy.

Antimicrobial Resistance

The choice of antibiotics varies with geographical variation and species of the pathogen (Table 2). Antimicrobial drug susceptibility is typically recommended for all *Nocardia* infections – as there can be variation depending upon species as well as antimicrobial tolerance by the patient. On the basis of AST profiles, *Nocardia* isolates can be grouped into 3 major complexes: the *N. nova* complex (*N. nova*, *N. elegans*, *N. veterana*, *N. kruczakiae*, and *N. africana*), *N. transvalensis* complex (*N. blacklockiae* and *N. wallacei*, unnamed *Nocardia* sp.), and the *N. brevicatena*/*N. paucivorans* complex [2]. Resistance to antimicrobials is mediated by various genes like tet (K) for tetracycline resistance; mphA, mphB, and mphC for macrolide resistance, *bla*_{TEM-1}, *bla*_{SHV}, *bla*_Z, oxa, AmpC for β-lactam resistance; sul genes for sulfonamide resistance, aac, aph, ant, rmtA, rmtB, rmtC, rmtD, armA for aminoglycoside resistance; gyrA for fluoroquinolones; 23S rRNA, and cfr for linezolid resistance [7, 53].

In a 10-year retrospective study conducted on epidemiology and identification of *Nocardia* by the Centers for Disease Control and Prevention (USA), analysis of 765 isolates revealed 61% resistance to sulfamethoxazole and 42% to co-trimoxazole or trimethoprim-sulfamethoxazole (TmSu); *N. nova* was the commonest isolate (28%) [54]. In another prospective study from 6 centers in the USA, of 552 isolates collected between 2005 and 2011, only 2% resistance to TmSu was noted [55]. Another recent study from a tertiary hospital and reference center in the USA on 2,091 clinical isolates of *Nocardia* from 2011 to 2017 found *N. nova* complex, *N. cyriacigeorgica*, and *N. farcinica* complex to be the most common isolates, and amikacin, linezolid, and TmSu as the most effective antibiotics in vitro [56]. A surveillance study on epidemiology and resistance rates of 270 *Nocardia* isolates was done in Australia in which 59.3% isolates were resistant to imipenem (mainly *N. pseudobrasiliensis*, *N. brasiliensis*, *N. farcinica*, and *N. cyriacigeorgica*), 9.3% to TmSu (highest among *N. farcinica*), and 0.7% to amikacin, whereas all isolates remained susceptible to linezolid. *N. nova* was the commonest isolate and the least resistant [57, 58]. A study from Canada in 2015 also showed 100% susceptibility to linezolid, ceftriaxone, and amoxycillin-clavulanic acid among its 112 isolates; TmSu was 97%, imipenem 22%, and moxifloxacin 10% susceptible [59]. In studies from Spain in 2011 [60] and 2017 [61], *Nocardia* were 100% susceptible to linezolid, amikacin, and TmSu, and *N. cyriacigeorgica* was the commonest isolate (25%). In a recent study from a *Nocardia* reference laboratory in France, *N. farcinica* was the commonest isolate, and its prevalence increased over a 5-year study period. Of 793 isolates tested, susceptibility results

were the best for linezolid (zero resistance), amikacin, TmSu, minocycline, and imipenem in that order [62]. From China, *N. farcinica* was reported as the most common species [63]. *N. farcinica* was also the commonest species isolated in Belgium [64], whereas *N. brasiliensis* was the most common species in Taiwan [65]. Cases of co-trimoxazole resistance have also been observed and range from 2 to up to 43% in various studies [11, 14, 53–55]. PCR and sequencing of 76 SXT-resistant *Nocardia* strains with MIC ≥ 32 $\mu\text{g/mL}$ revealed that they belonged to 12 species, *N. farcinica* (32%), *N. flavorosea* (6.5%), *N. nova* (11.8%), *N. carnea* (10.5%), *N. transvalensis* (10.5%), and other uncommon *Nocardia* spp. (6.5%) [53]. Hence species identification and antibiotic susceptibility is essential for appropriate treatment. *Nocardia* isolates need to be speciated, followed by susceptibility testing by recognized techniques for determination of MIC, MBC, and time kill studies [66]. To be effective, MICs of commonly used antibiotics should be within the range prescribed: linezolid (2–4 $\mu\text{g/mL}$), nalidixic acid (≤ 128 $\mu\text{g/mL}$), norfloxacin or pefloxacin (≤ 64 $\mu\text{g/mL}$), ciprofloxacin (1.4– ≥ 25 $\mu\text{g/mL}$), ofloxacin, (2.6– ≥ 25 $\mu\text{g/mL}$), and moxifloxacin (8 $\mu\text{g/mL}$) [57].

Treatment of Nocardiosis

Due to their slow replication and intracellular cryptic presence, *Nocardia* have a tendency to relapse; hence, prolonged antimicrobial therapy for a few to several months is required. Therapy should be closely monitored to assess response and control drug toxicity, if any. Clinical improvement starts within 2 weeks of initiation of therapy; others should be re-evaluated for possible antimicrobial resistance, inappropriate dosage to achieve serum or onsite concentration, or presence of a focal abscess [67]. Even though antimicrobial susceptibility changes with severity, site of infection, and geographical location, resistance to linezolid has hardly been observed in studies across the globe. However, cost and toxicity (myelosuppression, neurotoxicity, and lactic acidosis) with prolonged therapy of about 6–12 months limit its widespread use. Response to co-trimoxazole or sulfamethoxazole alone seemed good in earlier times with limited resistance, and hence it was considered the first-choice agent. Prior to its use in the 1940s, pulmonary nocardiosis was considered 100% fatal. From Iran in 2015 [68], all 127 isolates were susceptible to linezolid, 95% to TmSu. Hence therapy with TmSu or linezolid for 6–12 months is recommended. Swiss guidelines recommend the use of TmSu in routine, less severe cases; if toxicity or no response is obtained, then shift to imipenem is recom-

mended [69]. The dose of co-trimoxazole should be high (25–50 mg/kg body weight) to achieve a serum level of 100–150 $\mu\text{g/mL}$. The American Society for Transplantation recommends sulfonamide as first-line treatment for nocardiosis in SOT patients and initial therapy with at least 2 antimicrobials in disseminated or severe disease; co-trimoxazole prophylaxis may also be useful [70]. Alternative drugs are minocycline, imipenem, and ceftriaxone. Minocycline has excellent activity; tigecycline also appears to be active in vitro. Imipenem is also very active against some species and has better efficacy than other carbapenems [31, 71, 72].

For CNS nocardiosis, based on recommendations by various authors [73–75], it can be concluded that for clinically stable patients with a brain abscess < 2 cm, treatment should be initiated with a long-term antibiotic, initially intravenously for 4–6 weeks followed by oral therapy for 6–12 months with or without aspiration of abscess. Aspiration helps to relieve mass effect and aid in definitive laboratory diagnosis. In case of a bigger abscess, craniotomy should be considered along with antibiotic cover. The most commonly used antibiotic for such a scenario is co-trimoxazole; other alternatives are meropenem, ceftriaxone, ampicillin, linezolid, vancomycin, or amikacin – either alone or in combination. Some clinicians recommend a multidrug therapy consisting of 3 drugs for these patients: sulfonamide, amikacin, and a carbapenem or third-generation cephalosporin [7]. Co-trimoxazole maintenance therapy for a period of 6–24 months may be required [76].

Conclusion

Nocardiosis is not an uncommon entity. A high index of clinical suspicion is required especially in immunocompromised patients and patients with chronic bronchopulmonary disease. Clinicians must alert the laboratory should such a suspicion arise. More prospective studies from India are required to study the clinical features, pathogenesis, diagnosis, and treatment modalities better. While traditional techniques like paraffin bait are time consuming, the role of good-quality specimens and diligent microscopy cannot be overemphasized. In most clinical laboratories, diagnosis up to genus level can be established based on microscopic and phenotypic culture characteristics. Further, these isolates should be sent to a reference laboratory for detailed identification and antimicrobial sensitivity. Also, a national reference center for nocardiosis is the need of the hour.

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