AKADÉMIAI KIADÓ

Acta Microbiologica et Immunologica Hungarica

70 (2023) 3, 239–245

DOI:
10.1556/030.2023.02071

RESEARCH ARTICLE

Clinical and microbiological characteristics of nocardiosis: A 5-year single-center study in Crete, Greece

SOFIA MARAKI[1*†], VIKTORIA EIRINI MAVROMANOLAKI[2†], ANNA KASIMATI[1], DIMITRA STAFYLAKI[1] and EFFIE SCOULICA[3]

[1] Department of Clinical Microbiology and Microbial Pathogenesis, University Hospital of Heraklion, Crete, Greece

[2] University of Crete Medical School, Heraklion, Crete, Greece

[3] Laboratory of Clinical Microbiology and Molecular Microbiology, School of Medicine, University of Crete, Crete, Greece

Received: June 6, 2023 • Accepted: July 2, 2023
Published online: July 17, 2023

ABSTRACT

Nocardiosis is a rare disease affecting both immunocompromised and immunocompetent hosts, presented in various clinical forms ranging from localized to disseminated infection. Aim of the present study was to investigate the clinical and microbiological characteristics of nocardiosis, antimicrobial resistance profiles, treatment, and outcomes of *Nocardia* infection over the last 5 years at our institution. The medical records and microbiological data of patients affected by nocardiosis and treated at the university hospital of Heraklion, Crete, Greece, between 2018 and 2022, were retrospectively analyzed. The isolates were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and through sequencing of 16S rRNA. Antimicrobial susceptibility for 17 agents was determined by E-test and results were interpreted according to CLSI guidelines. Among the 28 *Nocardia* isolates, eight species were identified, with *Nocardia brasiliensis* being the most prevalent (32.1%), followed by *Nocardia otitidiscaviarum* (25%), and *Nocardia farcinica* (14.3%). Skin and soft tissue infections were the most common presentations, noted in 13 (50%) patients, followed by pulmonary infection presented in 10 (38.5%) patients. Fifteen patients (57.7%) had at least one underlying disease, and 11 (42.3%) were on immunosuppressive or long-term corticosteroid treatment. Susceptibility rates of linezolid, tigecycline, amikacin, trimethoprim-sulfamethoxazole, moxifloxacin, and imipenem were 100, 100, 96.4, 92.9, 82.1, and 42.9%, respectively. The 26 patients in this study were treated with various antibiotics. Mortality rate was 3.8%, and the patient who died had disseminated infection. Since epidemiology and antimicrobial susceptibility are evolving, continuous surveillance is mandatory in order to initiate appropriate treatment in a timely manner.

KEYWORDS

nocardiosis, Greece, epidemiology, underlying disease, antimicrobial susceptibility, treatment

INTRODUCTION

Nocardia species are Gram-positive, weakly acid-fast, filamentous, branching bacteria belonging to the family *Nocardiaceae*. They are ubiquitous in the environment, present as saprophytes in soil, dust, fresh and salt-water, decaying vegetation and organic matter [1, 2]. *Nocardia* species distribution varies geographically and changes over time with some species being more prevalent in different areas, mainly affected by different climatic conditions and soil characteristics. They are most commonly associated with opportunistic localized or disseminated infections in immunocompromised hosts, particularly those with impaired

†These authors contributed equally to the study.

*Corresponding author. Tel.: +30 2810 392598; fax: +30 2810 392597.
E-mail: sofiamaraki@yahoo.gr

cell-mediated immunity, such as transplant recipients, patients with solid organ or hematological malignancies, those with acquired immunodeficiency syndrome (AIDS) and patients on long-term steroid or immunosuppressant therapy [3, 4]. However, up to one-third of affected patients are immunocompetent without underlying diseases [1, 2, 5]. Human acquisition of the microorganism is exogenous. Nocardiosis arises either from inhalation leading to pulmonary infections, or from direct inoculation via the skin and soft tissues. The clinical spectrum is mainly represented by pulmonary, skin and soft tissue and cerebral infections [1, 2]. The infection may also disseminate haematogenously from the initial foci of infection to the brain, kidney, spleen, liver, heart, joints, bones, intestines, and eyes with fatal consequences [6]. Furthermore, *Nocardia* species may colonize the lower respiratory airways of patients with chronic pulmonary diseases such as cystic fibrosis, chronic obstructive pulmonary disease (COPD) and bronchiectasis [7]. Although community-acquired nocardiosis is common, some cases of hospital-acquired infection have been infrequently reported in patients undergoing surgeries or following of insertion of central venous catheters or prostheses [8, 9]. A few reports from Europe, US and Asia have also described outbreaks among immunosuppressed hospitalized individuals [10–12].

In Greece, a few cases of nocardiosis have been reported during the last decade [13, 14]. The only epidemiological study on nocardiosis in our region was published in 2009 [15]. Since species distribution and antimicrobial resistance profiles are changing over time, ongoing surveillance is essential.

This 5-year retrospective study aimed to analyze the epidemiology, the clinical and microbiological characteristics, the antimicrobial resistance profiles, treatment and outcome of patients with confirmed nocardiosis, in a Greek tertiary care hospital.

MATERIALS AND METHODS

Study design, patients and setting

This retrospective cohort study included data from all adult patients aged >18 years diagnosed with nocardiosis and cared for at the University Hospital of Heraklion, Crete, Greece, between the years 2018 and 2022. This hospital is a 760-bed, tertiary care academic institution, serving a population of approximately 750,000 inhabitants.

The study has been approved by the hospital's Ethical Review Board and met the guidelines of the Helsinki Declaration.

The demographic characteristics, including age, gender, as well as infection localization, type of clinical sample from which the isolate was identified, underlying diseases, immunocompromising conditions including hematological malignancy, solid tumor, autoimmune diseases, AIDS, use of corticosteroids and immunosuppressives, therapeutic regimen and outcome of included patients, were retrospectively reviewed from the medical records.

Definitions

Nocardiosis was defined on the basis of a positive culture for *Nocardia* species in the presence of compatible clinical and/or radiological features of the infection.

Disseminated *Nocardia* infection was defined as having 2 or more non-contiguous organs involved that yielded a *Nocardia* species.

Nocardia relapse was defined as culture growth of the same *Nocardia* species accompanied by clinical symptoms and/or abnormal imaging after completion of primary therapy

Immunosuppressed were considered patients who had HIV infection with CD4 count <200 cells mL^{-1}, solid organ or hematopoietic cell transplant (HCT) receiving medication to prevent rejection of transplanted organs, those treated with chemotherapy for solid tumor or hematologic malignancy, autoimmune disorders treated with immunosuppressive agents, or high-dose corticosteroid therapy (equivalent of ≥0.3 mg/kg/day of prednisone) given for at least 3 weeks before the diagnosis of *Nocardia* infection.

Microbiology

Isolates and identification. Clinical specimens, including blood, pus, respiratory specimens (sputum, bronchoalveolar lavage fluid [BALF], and pleural effusion) were cultured on the Columbia blood agar and chocolate agar plates and incubated at 37 °C for 3–7 days. Initial presumptive identification was based on colony morphology, on positive Gram stain (Gram-positive branching, beaded, and filamentous bacilli) and positive modified acid-fast staining. *Nocardia* species were further identified to species level by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (BioMérieux, Marcy L'Etoile, France) and through sequencing of 16S ribosomal RNA [13].

Antimicrobial susceptibility testing. Antibiotic susceptibility testing was performed by E-test (BioMérieux), as described previously [16]. Seventeen antimicrobial agents, ampicillin, amoxicillin-clavulanic acid, cefotaxime, ceftriaxone, cefepime, imipenem, clarithromycin, gentamicin, tobramycin, amikacin, minocycline, doxycycline, ciprofloxacin, trimethoprim-sulfamethoxazole, linezolid, moxifloxacin and tigecycline, were tested. Minimum inhibitory concentration (MIC) values were interpreted as susceptible (S), intermediate (I), or resistant (R) according to Clinical and Laboratory Standards Institute (CLSI) interpretative standards for *Nocardia* [17].

RESULTS

Demographic and clinical characteristics

Table 1 summarizes the demographic and clinical characteristics of the 26 patients with nocardiosis. Of them

Table 1. Demographic characteristics and clinical features of patients with nocardiosis

	No. (%) of patients (n = 26)
Age/years (mean ± SD)	62.4 ± 16.6
Age range	25–86
<65 years	12 (46.2)
≥65 years	14 (53.8)
Gender	**No. (%)**
Male	17 (65.4)
Female	9 (34.6)
Infection site	**No. (%)**
Skin and soft tissue infection	13 (50)
Pulmonary infection	10 (38.5)
Bloodstream infection	2 (7.7)
Disseminated infection	1 (3.8)
Clinical specimen	**No. (%)**
Abscess	15 (53.6)
BALF	6 (21.5)
Sputum	2 (7.1)
Pleural effusion	2 (7.1)
Blood	3 (10.7)
Underlying disease/condition	**No. (%)**
Without underlying disease	11 (42.3)
Solid organ cancer	5 (19.2)
Hematologic cancer	1 (3.8)
Diabetes mellitus	2 (7.7)
Chronic lung disease	3 (11.6)
Chronic kidney disease	2 (7.7)
Autoimmune disease	2 (7.7)
AIDS	1 (3.8)
Immunosuppressant use	7 (26.9)
Steroid use	4 (15.4)

Abbreviations: SD = standard deviation; BALF = bronchoalveolar lavage fluid; AIDS = acquired immunodeficiency syndrome

17 (65.4%) were male and 9 (34.6%) were female. The mean patient age was 62.4 ± 16.6 years (range, 25–86 years, with 14 (53.8%) being ≥65 years years and only 3 (11.5%) less than 40 years.

The most common infection site was skin and soft tissue (13/26, 50%). Ten patients (38.5%) were diagnosed with pulmonary nocardiosis, 2 (7.7%) with bloodstream infection and 1 (3.8%) with disseminated infection that included soft tissue infection and subhepatic abscess.

Fifteen patients (57.7%) had at least one underlying disease, including solid organ cancer (5/26, 19.2%), chronic lung disease (3/26, 11.6%), diabetes mellitus (2/26, 7.7%), autoimmune disease (2/26, 7.7%), chronic kidney disease (2/26, 7.7%), and acquired immunodeficiency syndrome (1/26, 3.8%). Additionally, 7 patients (26.9%) had received immunosuppressants and 4/26 (15.4%) corticosteroids.

Distribution of *Nocardia* species

During the study period, 28 *Nocardia* isolates were identified. Thirteen of them (46.4%) were cultured from skin and soft tissue, 11 (39.3%) from respiratory tract specimens, 3 (10.7%) from blood and 1 (3.6%) from subhepatic abscess. The 28 *Nocardia* isolates belonged to eight species. *Nocardia brasiliensis* was the most common species (9/28, 32.1%), followed by *Nocardia otitidiscaviarum* (7/28, 25%), *Nocardia farcinica* (4/28, 14.3%), *Nocardia cyriacigeorgica* (3/28, 10.7%), *Nocardia abscessus* (2/28, 7.1%), and *Nocardia elegans*, *Nocardia carnea* and *Nocardia transvalensis* (1/28, 3.6%). Only the isolate of *N. elegans* had been misidentified by MALDI-TOF MS as *Nocardia veterana.* The species distribution by infection site are depicted in Table 2. *N. brasiliensis* was the most frequently isolated species from skin and soft tissue (9/12, 75%), while from respiratory tract *N. cyriacigeorgica* and *N. otitidiscaviarum* were isolated in equal percentages (10.7%).

Antimicrobial susceptibility testing

Overall, the MIC ranges, MIC_{50} and MIC_{90} values, and the percentages of susceptible, intermediate and resistant isolates for each antimicrobial tested are presented in Table 3. All isolates were susceptible to linezolid and tigecycline, 96.4% to amikacin, 92.9% to trimethoprim-sulfamethoxazole (TMP-SMX) (one resistant strain belonged to *N. transvalensis*, and another one to *N. otitidiscaviarum*), and 82.1% to minocycline and moxifloxacin. Fifty percent of the isolates were susceptible to ceftriaxone and 42.9% to imipenem. Clarithromycin exhibited the lowest activity with 25% of isolates classified as susceptible, followed by ciprofloxacin and ampicillin (32.1% susceptible isolates).

The antimicrobial resistance profiles varied within different *Nocardia* species. All *Nocardia brasilensis, N. farcinica*,

Table 2. Distribution of *Nocardia* species by infection site

No. of isolates (%)					
Species	Blood	Respiratory tract	Skin and soft tissue	Subhepatic abscess	Total
N. brasiliensis	0	0	9 (32.2)	0	9 (32.1)
N. otitidiscaviarum	0	3 (10.7)	3 (10.7)	1 (3.6)	7 (25)
N. farcinica	2 (7.1)	2 (7.1)	0	0	4 (14.3)
N. cyriacigeorgica	0	3 (10.7)	0	0	3 (10.7)
N. abscessus	1 (3.6)	1 (3.6)	0	0	2 (7.1)
N. elegans	0	0	1 (3.6)	0	1 (3.6)
N. carnea	0	1 (3.6)	0	0	1 (3.6)
N. transvalensis	0	1 (3.6)	0	0	1 (3.6)
Total	3 (10.7)	11 (39.3)	13 (46.4)	1 (3.6)	28 (100)

Table 3. In vitro activity of 17 antimicrobial agents against the 28 *Nocardia* isolates

Antibiotics	MIC (μg mL^{-1})			% of isolates		
	MIC range	MIC_{50}	MIC_{90}	S	I	R
Ampicillin	0.5 – >256	16	>256	32.1	17.9	50
Amoxicillin-clavulanic acid	0.125 – >256	2	>256	75	3.6	21.4
Cefotaxime	0.094 – >32	3	>32	50	–	50
Ceftriaxone	0.25 – >32	8	>32	50	–	50
Cefepime	0.094 – >32	8	>32	50	–	50
Imipenem	0.047 – >256	>32	>32	42.9	–	57.1
Clarithromycin	0.38 – >256	12	>256	25	17.9	57.1
Gentamicin	0.125 – >256	0.75	>256	78.6	3.6	17.8
Tobramycin	0.064 – >256	1.5	>256	71.4	3.6	25
Amikacin	0.094 – 48	1	4	96.4	–	3.6
Minocycline	0.094 – 4	1	2	82.1	17.9	–
Doxycycline	0.19 – 6	1	4	64.3	28.6	7.1
Ciprofloxacin	0.5 – >32	2	>32	32.1	17.9	50
TMP-SMX	0.012 – >32	0.094	1	92.9	–	7.1
Linezolid	0.25 – 1.5	0.75	0.75	100	–	–
Moxifloxacin	0.094 – >32	0.5	>32	82.1	14.3	3.6
Tigecycline	0.047 – 2	0.125	0.75	100	–	–

Abbreviations: S = susceptible; I = intermediate; R = resistant; TMP-SMX = trimethoprim-sulfamethoxazole

N. cyriacigeorgica and *N. abscessus* isolates were uniformely susceptible to amoxicillin-clavulanic acid, while in contrast all of the *N. otitidiscaviarum* and *N. farcinica* isolates were resistant. Cefotaxime, ceftriaxone and cefepime showed high susceptibility rates against *N. cyriacigeorgica* and *N. abscessus* (100%), whereas *N. brasiliensis* and *N. farcinica* were more frequently nonsusceptible (33.3% and 100%, respectively). In addition, 100% of *N. farcinica,* and *N. cyriacigeorgica,* 50% of *N. abscessus* and 11.1% of *N. brasiliensis* isolates were susceptible to imipenem. Except for *N. farcinica*, gentamicin and tobramicin exhibited excellent in vitro activity against *N. brasiliensis, N. otitidiscaviarum, N. cyriacigeorgica,* and *N. abscessus*. Non susceptibility to tetracyclines, minocycline and doxycycline, was more frequent among *Nocardia cyriacigeorgica*. For fluoroquinolone antibiotics, ciprofloxacin had poor *in vitro* activity against all species except for *N. farcinica*, while moxifloxacin demonstrated good in vitro susceptibility against *N. brasiliensis* (100%), *N. farcinica* (100%), *N. abscessus* (100%) and *N. otitidiscaviarum* (85.7%).

Treatment and outcome

The 26 patients were treated with various antibiotics and in 5 (19.2%) of them medical treatment was combined with surgical debridement or drainage. TMP-SMX was the antibiotic most commonly used (92.3%) either alone (9/24, 37.5%) or in combination (15/24, 62.5%). Details of the antibiotic treatment regimens that were administered are shown in Table 4. The median duration of treatment was 18 weeks (range, 1–52 weeks). Favorable outcome was detected in 24 (92.3%) patients. One patient relapsed after treatment. Death was considered related to nocardiosis in one patient (3.8%) suffering from disseminated infection.

Table 4. Antibiotic treatment of the 26 patients with nocardiosis

Antibiotic regimen	No. (%) of patients
TMP-SMX	9 (34.6)
TMP-SMX + meropenem	4 (15.4)
TMP-SMX + amoxicillin/clavulanic acid	3 (11.5)
TMP-SMX + linezolid	2 (7.7)
TMP-SMX + amikacin	1 (3.8)
TMP-SMX + moxifloxacin	1 (3.8)
TMP-SMX + ceftriaxone	1 (3.8)
TMP-SMX + minocycline	1 (3.8)
TMP-SMX + meropenem + amikacin	1 (3.8)
TMP-SMX + ceftriaxone + amikacin	1 (3.8)
Meropenem + amikacin	1 (3.8)
Linezolid + minocycline	1 (3.8)

Abbreviation: TMP-SMX = trimethoprim-sulfamethoxazole

DISCUSSION

This retrospective review at a Greek tertiary care hospital identified 26 patients with microbiologically proven *Nocardia* infection over 5 years. Compared to our previous study from the same center, nocardiosis appear to have increased in the last years, likely due to the expanding immunocompromised population living in rural areas exposed to environmental sources of the pathogen [15]. Several reports have described increasing incidence over time, while others have reported stable incidence [18–20]. The number of cases in males almost doubles that of females, similar to previously published reports [15, 20]. This may be due to the different exposure caused by the different profession and lifestyle of men vs. women. The mean age of the patients was 62.4 ± 16.6 years with 53.8% being over 65 years. According to several

reports, the age distribution varies widely and may be associated with risk factors and the exposure to the pathogen.

As widely reported, the administration of immunosuppressive therapy, high doses of corticosteroids, solid tumors and haematological malignancies, solid organ and haematopoietic stem cell transplantation (HSCT) and HIV are the most common predisposing factors for nocardiosis [3, 4, 21, 22]. The major risk factor in our study group was immunosuppressive treatment and chronic corticosteroid administration, present in 42.3% of our patients. Only one patient with nocardiosis had HIV infection, reflecting the increasing use of retroviral therapy. Furthermore, comorbidities causing local impairment of the lung defences predisposes to pulmonary nocardiosis. COPD was the most common respiratory disease among patients with pulmonary nocardiosis (27.3%). In patients with COPD, bronchiectasis or cystic fibrosis, bacteria colonizing the lower airways alter ciliary motility and cause epithelial damage, leading to colonization and infection by *Nocardia* [23]. Additionally, diabetes mellitus, chronic kidney disease and autoimmune disease (7.7% each one), were also found to be risk factors in our study. Notably, among the 26 included cases, 11 (42.3%) cases showed no evidence of underlying diseases. Previous studies have shown that approximately one-third of infected patients were immunocompetent [24, 25].

More than 100 *Nocardia* species have been characterized by molecular methods, with almost half of them affecting humans. Species epidemiology varies with geographical area. In the present study, *N. brasiliensis* was the most frequently isolated species, followed by *N. otitidiscaviarum*, *N. farcinica* and *N. cyriacigeorgica*. Likewise, in Taiwan *N. brasiliensis* is the species more frequently identified [26]. Some evidence suggests that *N. brasiliensis* prevails in countries with tropical and subtropical climates. But our country has a temperate climate, so the different distribution of species seems not to be affected by the climatic conditions. In Italy, France and Belgium *N. farcinica* is the commonest species, in Spain *N. cyriacigeorgica*, *N. farcinica* and *N. otitidiscaviarum* are the three dominant isolated species, whilst *Nocardia nova* is the predominant species encountered in the USA and Australia [27–32].

As previously reported, some *Nocardia species* have closely correlated with clinical presentation [1, 33]. The majority of skin and soft tissue infections cases (69.2%) were caused by *N. brasiliensis*, most commonly acquired through direct inoculation after trauma-related injuries such as road accidents, skin abrasion, insect bites and through nosocomial exposure. *N. farcinica* was most commonly isolated in blood cultures, which is in agreement with many previous studies [33, 34].

During the study period, we found that skin and soft tissue was the most common site of infection, followed by pulmonary involvement and disseminated infection. This finding is inconsistent with many studies reporting pulmonary infection as the most common manifestation of nocardiosis [19, 20, 30, 32, 33, 35]. Pleural effusion was developed in 2/11 (18.2%) patients suffering pulmonary nocardiosis. Unlike pulmonary nocardiosis that involved mostly immunocompromised patients (83.3%), skin and soft tissue infection developed most often (81.8%) in immunocompetent hosts.

Accurate identification of *Nocardia* isolates by use of phenotypic and genotypic methods is important to define the spectrum of diseases caused by each species, to determine the epidemiology, and to predict antimicrobial susceptibility, that is essential to guide treatment. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has been recognized as a reliable method for rapid, cost-effective and accurate identification of *Nocardia* species [36]. MALDI-TOF MS cannot be used alone for identification of *Nocardia* species, because the databases of instruments contain a limited number of species. This was the case of our isolate that was misidentified as *N. veterana*. The database of Vitek MS version 3.2.0 we are using contains only 16 *Nocardia* species. It has been shown that if a species is not included in the manufacturer's database, it can be misidentified as a similar species rather than not identified [36]. Hodille et al. comparing the results of *Nocardia* species identification by Vitek MS to 16S rRNA and hsp65 sequencing-based identification found few discrepancies related to phylogenetically close species [37]. Misidentifications could potentially impact therapeutic management, therefore molecular methods such as DNA sequencing, currently considered the gold standard, should be also employed for definitive identification of *Nocardia* species [38].

TMP-SMX is considered the drug of choice for the treatment of nocardiosis, since more than 90% of strains have been reported to be susceptible in most studies [16, 21, 26]. TMP-SMX can be administered either as monotherapy in immunocompetent patients with primary skin infections or combined with other antimicrobials for disseminated and CNS nocardiosis. Alternative antimicrobial agents used in case of allergy, include linezolid, amikacin, minocycline, moxifloxacin, and amoxicillin-clavulanic acid. Linezolid has been demonstrated to have excellent in vitro activity against all *Nocardia* species, is available in both an intravenous and oral form and has the ability to penetrate the CNS. Despite these advantages, prolonged linezolid usage may be associated by serious adverse drug reactions, namely hematological toxicity and neuropathy [39]. The duration of therapy depends on the severity and the localization of the infection and the immune status of the patient. Because the disease has a tendency to recur, prolonged treatment is recommended in immunocompromised patients, those with disseminated or CNS nocardiosis. The median duration of treatment in the current cohort was 18 weeks. The mortality rates associated with nocardiosis range from 26% to 90%, depending on the form of the infection and the immune status of the patient [21, 40]. Mortality is high among immunocompromised patients and those with bacteremia, disseminated and CNS nocardiosis [40]. In the present study, the mortality rate was low (3.8%).

The main limitation of this study is the small number of patients with nocardiosis originating from a single geographical area. This implies that the present findings regarding the epidemiology of nocardiosis could not be applicable to the whole country.

CONCLUSION

Nocardiosis is a rare opportunistic infection difficult to diagnose, requiring long-term antimicrobial treatment. In recent years, its incidence has increased in our region due to the increasing number of immunocompromised individuals living in rural areas exposed to environmental sources. Nocardiosis should be considered in patients with underlying risk factors, especially those on immunosuppressant or long-term corticosteroid therapy, and microbiologists should be notified for prolonged incubation of cultures and proper culture evaluation. Early diagnosis and prompt initiation of appropriate treatment are essential for good prognosis.

Funding: No funds, grants, or other support was received.

Conflict of interest: The authors have no relevant financial or non-financial interests to disclose.

Ethics approval: This study was approved by the Ethical Committee of the University Hospital of Heraklion and met the guidelines of Helsinki declarations.

REFERENCES

1. Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ Jr. Clinical and laboratory features of the *Nocardia* spp. based on current molecular taxonomy. Clin Microbiol Rev 2006; 19: 259–82.
2. Wilson JW. Nocardiosis: updates and clinical overview. Mayo Clin Proc 2012; 87: 403–7.
3. Coussement J, Lebeaux D, van Delden C, Guillot H, Freund R, Marbus S, et al. *Nocardia* infection in solid organ transplant recipients: a multicenter European case-control study. Clin Infect Dis 2016; 63: 338–45.
4. Palomba E, Liparoti A, Tonizzo A, Castelli V, Alagna L, Bozzi G, et al. *Nocardia* infections in the immunocompromised host: a case series and literature review. Microorganisms 2022; 10: 1120.
5. Steinbrink J, Leavens J, Kauffman CA, Miceli MH. Manifestations and outcomes of *Nocardia* infections: comparison of immunocompromised and nonimmunocompromised adult patients. Medicine (Baltimore) 2018; 97: e12436.
6. Marques C, Ribeiro M, Bonccoraglio MT, Braga J, Pereira AF, Ogando A. Disseminated nocardiosis: the complexity of the diagnosis. J Med Cases 2021; 12: 205–8.
7. Margalit I, Muhsen K, Ben Ari Y, Ben-Zvi H, Shostak Y, Krause I, et al. *Nocardia* colonization in contrast to nocardiosis: a comparison of patients' clinical characteristics. Eur J Clin Microbiol Infect Dis 2020; 39: 759–63.
8. Al Akhrass F, Hachem R, Mohamed JA, Tarrand J, Kontoyiannis DP, Chandra J, et al. Central venous catheter-associated *Nocardia* bacteremia in cancer patients. Emerg Infect Dis 2011; 17: 1651–8.
9. Vuotto F, Faure K, Queyre V, Dessein R, Pasquet A, Lambert M, et al. Vascular nosocomial *Nocardia farcinica* infection after arterial stenting in an immunocompetent patient. Can J Infect Dis Med Microbiol 2011; 22: e10–1.
10. Exmelin L, Malbruny B, Vergnaud M, Prosvost F, Boiron P, Morel C. Molecular study of nosocomial nocardiosis outbreak involving heart transplant recipients. J Clin Microbiol 1996; 34: 1014–6.
11. Wenger PN, Brown JM, McNeil MM, Jarvis WR. *Nocardia farcinica* sternotomy site infections in patients following open heart surgery. J Infect Dis 1998; 178: 1539–43.
12. Kachi S, Okazaki M, Takeda H, Igarashi H, Kobayashi O, Watanabe H, et al. Outbreak of *Nocardia farcinica* infection with the same pattern in randomly amplified polymo-rphic DNA analysis. J Hosp Infect 2006; 62: 502–6.
13. Maraki S, Mavromanolaki VE, Detorakis EE, Stafylaki D, Moraitis P, Scoulica E. *Nocardia elegans* primary iliopsoas abscess: a case report and literature review. Acta Microbiol Immunol Hung 2022; 69: 351–7.
14. Mylona E, Papastamopoulos V, Giannopoulou M, Perivolioti E, Psaroudaki Z, Skoutelis A. Lymphocutaneous nocardiosis in a kidney transplant patient successfully treated with tigecycline. Saudi J Kidney Dis Transpl 2018; 29: 1207–10.
15. Maraki S, Scoulica E, Nioti E, Tselentis Y. Nocardial infection in Crete, Greece: review of fifteen cases from 2003 to 2007. Scand J Infect Dis 2009; 41: 122–7.
16. Glupczynski Y, Berhin C, Janssens M, Wauters G. Determination of antimicrobial susceptibility patterns of *Nocardia* spp. from clinical specimens by Etest. Clin Microbiol Infect 2006; 12: 905–12.
17. Clinical and Laboratory Standards Institute. Performance standards for susceptibility testing of mycobacteria, *Nocardia* spp. and other aerobic actinomycetes- 2 nd ed.:; approved standard M24S. 2023. Wayne, PA: CLSI. Wayne, PA: CLSI.
18. Woodworth MH, Saullo JL, Lantos PM, Cox GM, Stout JE. Increasing *Nocardia* incidence associated with bronchiectasis at a tertiary care center. Ann Am Thorac Soc 2017; 14: 347–54.
19. Yang J, Ren HT, Wang J, Dong AY, Chen YL, Hu DX, et al. Clinical characteristics, susceptibility profiles, and treatment of nocardiosis: a multicenter retrospective study in 2015–2021. Int J Infect Dis 2023; 130: 136–43.
20. Pintado V, Gomez-Mampaso E, Fortun J, Meseguer MA, Cobo J, Navas E, et al. Infection with *Nocardia* species: clinical spectrum of disease and species distribution in Madrid, Spain, 1978–2001. Infection 2002; 30: 338–40.
21. Torres HA, Reddy BT, Raad II, Tarrand J, Bodey GP, Hanna HA, et al. Nocardiosis in cancer patients. Medicine (Baltimore) 2002; 81: 388–97.
22. Cattaneo C, Antoniazzi F, Caira M, Castagnola C, Delia M, Tumbarello M, et al. *Nocardia* spp infections among hematological patients: results of a retrospective multicenter study. Int J Infect Dis 2013; 17: e610–4.
23. Martínez Tomás R, Menéndez Villanueva R, Reyes Calzada S, Santos Durantez M, Vallés Tarazona JM, Modesto Alapont M, et al. Pulmonary nocardiosis: risk factors and outcomes. Respirology 2007; 12: 394–400.
24. Colaneri M, Lombardi A, Morea A, Monzillo V, Mariani B, Marone M, et al. An eight-year experience of *Nocardia* infection in Italy: does immunosuppression matter? New Microbiol 2021; 44: 111–6.

25. Fujita T, Ikari J, Watanabe A, Tatsumi K. Clinical characteristics of pulmonary nocardiosis in immunocompetent patients. J Infect Chemother 2016; 22: 738–43.
26. Yang CH, Kuo SF, Chen FJ, Lee CH. Clinical manifestations and outcome of nocardiosis and antimicrobial susceptibility of *Nocardia* species in southern Taiwan, 2011–2021. J Microbiol Immunol Infect 2023; 56: 382–91.
27. Cordioli G, Di Pietra G, Asaad S, Meneghello S, Del Vecchio C, De Canale E, et al. *Nocardi*a infections in Italy: from a local to a national view. New Microbiol 2023; 46: 68–74.
28. Lebeaux D, Bergeron E, Berthet J, Djadi-Prat J, Mouniée D, Boiron P, et al. Antibiotic susceptibility testing and species identification of *Nocardia* isolates: a retrospective analysis of data from a French expert laboratory, 2010–2015. Clin Microbiol Infect 2019; 25: 489–95.
29. Averbuch D, De Greef J, Duréault A, Wendel L, Tridello G, Lebeaux D, et al. *Nocardia* infections in hematopoietic cell transplant recipients: a multicenter international retrospective study of the infectious diseases working party of the European Society for Blood and Marrow Transplantation. Clin Infect Dis 2022; 75: 88–97.
30. Galar A, Martín-Rabadán P, Marín M, Cercenado E, Sánchez-Carrillo C, Valerio M, et al. Revisiting nocardiosis at a tertiary care institution: any change in recent years? Int J Infect Dis 2021; 102: 446–54.
31. Hamdi AM, Fida M, Deml SM, Abu Saleh OM, Wengenack NL. Retrospective analysis of antimicrobial susceptibility profiles of *Nocardia* species from a tertiary hospital and reference laboratory, 2011 to 2017. Antimicrob Agents Chemother 2020; 64: e01868–19.
32. Sundac L, Dang L, Ho J, Hanson J, Smith S, Choong K, et al. A multi-centre retrospective study of *Nocardia* speciation and antimicrobial susceptibility in Queensland, Australia. Eur J Clin Microbiol Infect Dis 2023; 42: 339–45.
33. Wang H, Zhu Y, Cui Q, Wu W, Li G, Chen D, et al. Epidemiology and antimicrobial resistance profiles of the *Nocardia* species in China, 2009 to 2021. Microbiol Spectr 2022; 10: e0156021.
34. Williams E, Jenney AW, Spelman DW. *Nocardia* bacteremia: a single-center retrospective review and a systematic review of the literature. Int J Infect Dis 2020; 92: 197–207.
35. Yang M, Xu M, Wei W, Gao H, Zhang X, Zhao H, et al. Clinical findings of 40 patients with nocardiosis: a retrospective analysis in a tertiary hospital. Exp Ther Med 2014; 8: 25–30.
36. Khot PD, Bird BA, Durrant RJ, Fisher MA. Identification of *Nocardia* species by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2015; 53: 3366–9.
37. Hodille E, Prudhomme C, Dumitrescu O, Benito Y, Dauwalder O, Lina G. Rapid, easy, and reliable identification of *Nocardia* sp. by MALDI-TOF Mass Spectrometry, VITEK®-MS IVD V3.2 database, using direct deposit. Int J Mol Sci 2023; 24: 5469.
38. Durand T, Vautrin F, Bergeron E, Girard V, Polsinelli S, Monnin V, et al. Assessment of VITEK® MS IVD database V3.0 for identification of *Nocardia* spp. using two culture media and comparing direct smear and protein extraction procedures. Eur J Clin Microbiol Infect Dis 2020; 39: 559–67.
39. Kishor K, Dhasmana N, Kamble SS, Sahu RK. Linezolid induced adverse drug reactions - an update. Curr Drug Metab 2015; 16: 553–9.
40. Traxler RM, Bell ME, Lasker B, Headd B, Shieh WJ, McQuiston JR. Updated review on *Nocardia* species: 2006–2021. Clin Microbiol Rev 2022; 35: e0002721.