TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2018) 48: 366-371
© TÜBİTAK
doi:10.3906/sag-1801-138

Microbiological and clinical data analysis of 32 patients with *Nocardia* infections in Yantai

Hong-Xia YU[1], Ming LIU[2], Zeng-Hui PU[1], Yan LIU[1], Mao-Mao ZHAO[1,*]

[1]Department of Infectious Disease, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, P.R. China
[2]Department of Interventional MRI, Shandong Medical Imaging Research Institute affiliated to Shandong University, Shandong Key Laboratory of Advanced Medical Imaging Technologies and Applications, Jinan, P.R. China

Received: 17.01.2018 • **Accepted/Published Online:** 31.03.2018 • **Final Version:** 30.04.2018

Background/aim: *Nocardia* is an opportunistic pathogen that mostly affects hosts with immune deficiencies. Recently, the widespread use of immunosuppressive agents and antitumor drugs has led to an increasing number of *Nocardia* infections being reported. However, it is difficult to confirm this diagnosis owing to the slow growth of the bacterium and its complex resultant clinical manifestations, potentially delaying treatment and increasing mortality. Thus, further knowledge on the clinical characteristics of *Nocardia* infection is required. Hence, this study aimed to review the demographics, comorbidities, clinical presentation, microbiology, treatment, and outcomes of *Nocardia* infections in Yantai.

Materials and methods: This is a retrospective study including 32 patients identified to have *Nocardia* infection from the Yantai Yuhuangding Hospital. The relevant patient samples were collected by two researchers, while the other researchers analyzed the relevant data.

Results: The male to female ratio among the 32 patients was 3:5, and 23 patients (71.9%) were immunocompromised. Pulmonary sites of infection were the most common (65.6% of patients). *N. brasiliensis* infections were present in 25.0% and *N. asteroides* infections were present in 21.9% of patients. Because of limited biotechnological resources, *Nocardia* spp. in 50.0% of cases were not classified. The TMP-SMX resistance rate among isolates was 9.4%. All isolates were susceptible to amikacin, ceftriaxone, and imipenem.

Conclusion: In Yantai, immunocompromised patients predominate among cases of *Nocardia* infection. The rate of occurrence was higher in females than in males. Because of potential TMP-SMX resistance, treatment for *Nocardia* infection should be based on drug susceptibility or should include combination therapy.

Key words: *Nocardia* infections, data collection, diagnosis, therapeutics

1. Introduction

Nocardia is a common opportunistic pathogen; over 90 *Nocardia* spp. have been identified to date, of which more than 30 are human pathogens (1–4). *Nocardia* infections are commonly observed in individuals who undergo bone marrow or solid organ transplantation, patients on long-term steroid therapy, and patients with malignancies (5).

Nocardiosis usually manifests in the pulmonary system, the central nervous system (CNS), or the subcutaneous tissue (6–8). Pulmonary nocardiosis is the most common clinical manifestation and is mainly acquired through inhalation of the organism from environment. Especially in an immunocompromised host, the infection may then spread to involve the brain, eye, bone, joints, heart, kidneys, skin, or other organs and tissues (9). A quick diagnosis has usually been hampered owing to the biological characteristics of *Nocardia brasiliensis* and the lack of specificity in clinical manifestations. *Nocardia* infections are often misdiagnosed as actinomycosis or *Mycobacterium* or *Cryptococcus* infections, delaying diagnosis and leading to the institution of inappropriate therapy, which may influence the patient outcome.

In recent years, many researchers have focused on *Nocardia* infections in immunocompromised hosts as this organism is reemerging as an important opportunistic pathogen (10). Only a few cases of *Nocardia* infection in hosts with normal immune function have been reported, indicating a lack of systematic research on *Nocardia* infections in the Yantai area. Thus, we conducted a 7-year retrospective systematic retrospective study, and we hope that our findings can spread awareness about *Nocardia* infections.

* Correspondence: docmaomaozhao@163.com

2. Materials and methods

2.1. Design

This is a retrospective study of patients with nocardiosis managed at our hospital between January 2010 and December 2016. Ethical approval was provided by the Human Research Ethics Committee of our hospital.

2.2. Patients

All patients with clinically significant symptoms and culture-positive *Nocardia* infection was identified, and 32 patients diagnosed between January 2010 and December 2016 were eligible. Potential cases were identified through the microbiology isolate database and hospital discharge summary coding data. All cases were reviewed by at least 2 experienced investigators.

2.3. Data collection

Data on demographics, comorbidities, clinical manifestations, immunosuppressive agent and glucocorticoid administration, treatments, and outcomes were obtained from medical records. Microbiological data (specimen type, *Nocardia* species, and antimicrobial susceptibility) were obtained from the laboratory database.

2.4. *Nocardia* identification

Samples were inoculated on a blood agar plate. Dry colonies of white, light yellow, or orange color embedded in the plate and having an earthy smell as observed after 24–72 h of culture were highly suggestive of *Nocardia*. Colonies were subjected to Gram stain and acid-fast stain. The presence of *Nocardia* was confirmed on microscopic observation of small gram-positive branched bacilli with weakly positive acid-fast stain observed under microscope. *Nocardia* spp. were identified following the novel method described in 2016 (11). Antimicrobial susceptibility testing was performed using the Clinical and Laboratory Standards Institute (CLSI) standardized broth microdilution method (12).

3. Results

3.1. Patient demographics

Between January 2010 and December 2016, 32 patients were diagnosed with *Nocardia* infection and their demographic characteristics are described in Table 1. The median patient age was 48 years (range: 18–84), and 12 patients (37.5%) were male and 20 patients (62.5%) were female; furthermore, 9 patients (28.1%) were immunocompetent. The most frequently identified predisposing factor to *Nocardia* infection was chronic lung disease (present in 5 patients [15.6%]), primarily related to chronic obstructive pulmonary disease. The most common immunocompromising factors identified were immunosuppressive drug therapy, especially corticosteroid-based therapy.

Table 1. Demographic features of 32 *Nocardia* infections.

Patient characteristics	Number (n/%)
Age	
18–65	27 (84.37)
≥65	5 (15.63)
Sex	
Male	12 (37.50)
Female	20 (62.50)
Immunocompetent	9 (28.13)
Immunocompromising factor	
Chronic lung diseases	5 (15.62)
Diabetes mellitus	1 (3.12)
Immunosuppressive mediation [a]	
High-dose corticosteroids-based	4 (12.50)
Therapy in preceding 3 months [b]	
Active malignancy	
Other immunosuppressive therapy [c]	6 (18.75)
Hematological diseases	3 (9.38)
Hepatitis	2 (6.25)

[a] Immunosuppressive medications included corticosteroids, mycophenolate mofetil, azathioprine, and methotrexate.
[b] Defined as ≥20 mg/day prednisolone or >2 mg/day dexamethasone for 1 month or >2 pulses of 1 g of intravenous methylprednisolone with or without other immunosuppressive agents.
[c] Patients receiving low-dose prednisolone (<20 mg/day) and at least 1 other immunosuppressive agent.

3.2. Clinical characteristics

Infection sites for all patients are shown in Figure 1. Twenty-one patients (65.6%) had primary pulmonary infections, 4 patients (12.5%) had primary cutaneous infections, and 4 patients (12.5%) had blood infections and disseminated diseases. Three patients (9.4%) showed evidence of CNS disease. Early presentations of pulmonary infection were noted as multiple patchy shadows with a hole-forming tendency (Figure 2). Cerebral presentation was noted as a polycystic cavity abscess (Figure 3).

The clinical disease pattern was associated with the patients' immune status (Figure 4). Of 4 patients with primary cutaneous disease, 3 were immunocompromised (history of active malignancy and autoimmune hepatitis on prednisolone and azathioprine). Of the 21 patients

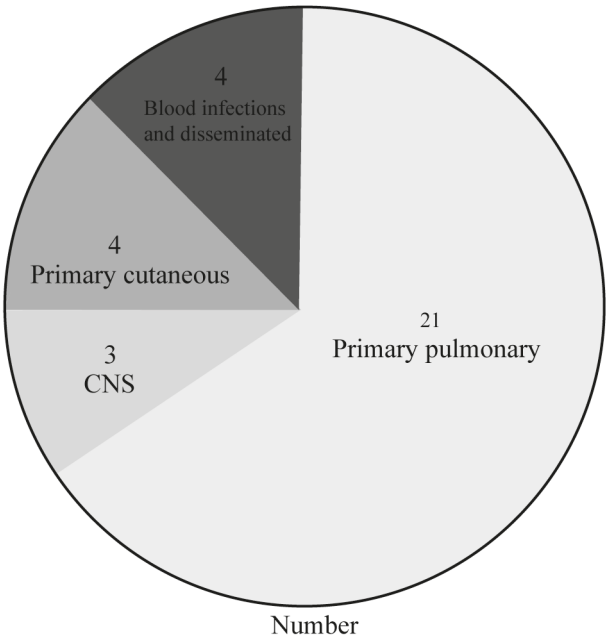

Figure 1. Sites of infection for 32 patients with *Nocardia*.

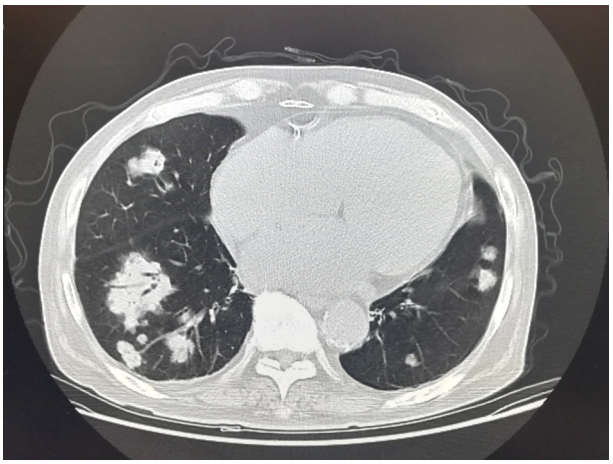

Figure 2. Pulmonary imaging of *Nocardia* infection.

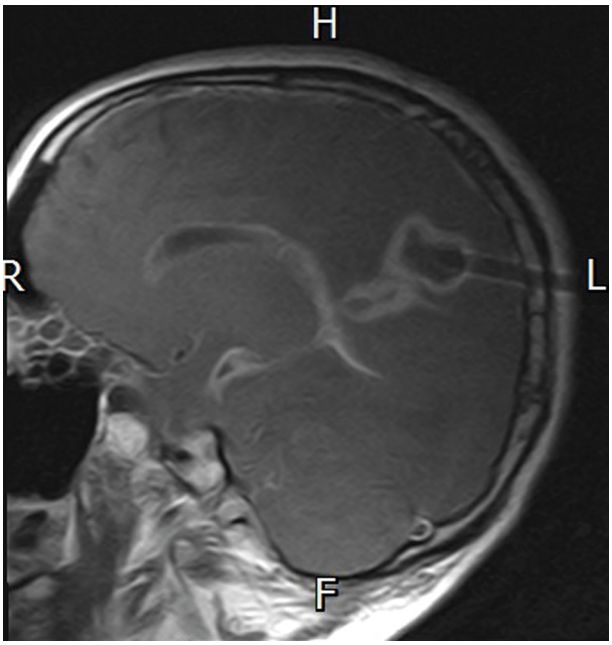

Figure 3. The brain abscess of *Nocardia* infection.

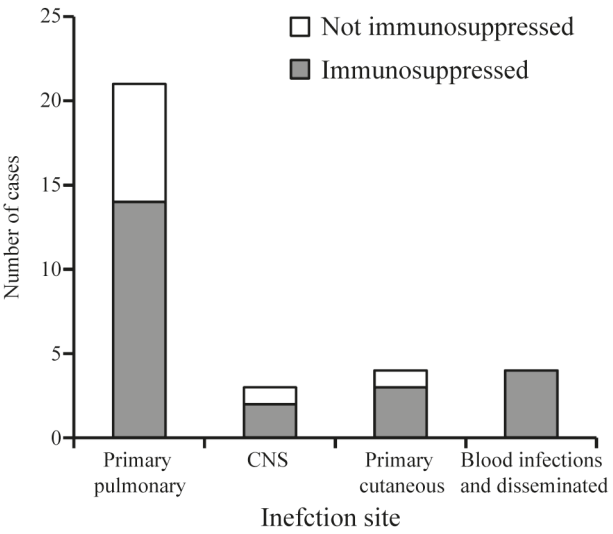

Figure 4. Patients' immune status and disease sites. Immunosuppressive factors included immunosuppressive medications and active malignancy.

with pulmonary disease, 14 were immunocompromised. All 4 patients with blood infections and disseminated diseases and 3 of 4 patients with CNS infections were immunocompromised.

3.3. Microbiology and antimicrobial susceptibility profiles

Samples collected from 32 patients included sputum, bronchoalveolar lavage fluid, abscess puncture fluid, blood, and skin, brain, and lung tissue samples (Table 2).

Table 2. *Nocardia* classification in different samples.

Sample type	Species (n)			
	Nocardia brasiliensis	*Nocardia asteroids*	*Nocardia farcinica*	Unclassified
Sputum	2	3	0	9
BALF	2	1	0	0
Puncture fluid	0	0	1	0
Blood	0	0	0	4
Skin biopsy	4	0	0	0
Brain biopsy	0	0	0	1
Lung biopsy	0	3	0	2

BALF: Bronchoalveolar lavage fluid.

N. brasiliensis was isolated from 8 patients (25.0%), *N. asteroides* from 7 patients (21.9%), and *N. farcinica* from 1 patient (3.1%); *Nocardia* samples isolated from the remaining 16 patients (50.0%) were unclassified owing to the lack of a typical biochemical reaction or poor emulsification. Drug sensitivity tests indicated that isolates from only 3 patients were resistant to TMP-SMX, of which 1 was an isolate of *N. brasiliensis* and 2 were unclassified *Nocardia* spp.; these isolates were all sensitive to amikacin, ceftriaxone, and imipenem.

3.4. Treatment and outcome

Among the 32 patients with confirmed nocardiosis, 12 were treated with ceftriaxone or amoxicillin clavulanate potassium combined with amikacin (3 were drug-resistant, and 9 could not tolerate TMP-SMX). The other 20 patients were treated with TMP-SMX-based therapy for 6–12 months (average duration: 6.3 months). The prognosis of patients with nocardiosis was generally good. There were 9 deaths (28.1%) in total: 1 case of old age combined with diabetes mellitus, 3 cases of bloodstream infection disseminated to the entire body with long-term application of high-dose glucocorticoids, and 5 cases of severe pulmonary infection combined with destruction of the lung structure.

4. Discussion

As *Nocardia* is an opportunistic pathogen, most patients with *Nocardia* infections are immunocompromised owing to an active malignancy or owing to hormone and immunosuppressant use. The overall proportion of immunocompromised hosts in our study (71.9%) was higher than that in a previous review of *Nocardia* infections (13). Patients with systemic disseminated infection accounted for 12.5% of our cases, which was lower than the proportion reported in highly immunosuppressed populations (20%–21%) such as solid-organ transplant patients (14), possibly owing to our smaller sample size.

In this study, patients aged between 18 and 65 years accounted for 84.4% (27/32) of all cases, of whom 33.3% (9/27) were immunocompetent. All patients aged >65 years were free from immunosuppressant therapy and did not have underlying diseases, likely owing to an age-related reduction in CD4 cell count and function (15). In addition, our results suggest higher morbidity in women than in men, contrary to the findings of previous studies (16), possibly owing to 45.0% of our female patients having immunological diseases.

Human *Nocardia* infection can be caused by inhalation (pulmonary nocardiosis: pneumonia, lung abscess, and cavitary lesions) or bacterial contact through a cut or abraded skin (cutaneous nocardiosis: cellulitis and ulcers), and the infection can then disseminate to the brain, kidneys, joints, heart, eyes, and bones (17–20). In our study, pulmonary infection was most common (65.6% of all cases [21/32]), followed by skin infection and bloodstream infection with multiorgan systemic dissemination. Pulmonary infection with *Nocardia* spp. presented clinical symptoms similar to pulmonary tuberculosis (fever, cough, chest pain, night sweats, weight loss, and pneumonia) (21). In addition, pulmonary nocardiosis has various imaging manifestations, including pulmonary consolidation, presence of nodules and masses, pleural effusion, and extension of lung infection towards the chest wall resulting in an abscess (22), which makes clinical diagnosis challenging, delays treatment, and increases the mortality rate. Therefore, laboratory tests including biopsy samples are highly necessary.

Nocardia spp. are associated with human infections and include the *N. asteroides* complex (more than 50% human cases), *N. brasiliensis*, *N. abscessus*, *N. cyriacigeorgica*, *N.*

farcinica, *N. nova*, the *N. transvalensis* complex, the *N. nova* complex, *N. pseudobrasiliensis*, and the recently reported *N. veteran* and *N. cerradoensis* (23,24). Pulmonary infection is usually caused by *N. asteroides*, while skin infection and subcutaneous abscesses are mostly caused by *N. brasiliensis*. *Nocardia* identification is more rapid and precise when using polymerase chain reaction and 16S RNA sequencing than when using conventional phenotypic methods that study microscopic, cultural, and biochemical properties (25,26). However, owing to limited laboratory resources, 16S RNA sequencing was not utilized in this study. Therefore, *Nocardia* spp. was not classified in 50.00% of cases, which is a limitation of this study.

Previously, TMP-SMX was considered the primary therapeutic option for nocardiosis; however, antimicrobial susceptibility studies of *Nocardia* isolates performed in the USA, Canada, Spain, and Taiwan have revealed TMP-SMX resistance among 2% to 43% of cases (27–31). In our study, the TMP-SMX resistance rate was 9.4%, and all isolates were susceptible to amikacin, ceftriaxone, and imipenem.

Clinically, an increase has been observed in *Nocardia* infection owing to the application of immunosuppressant and antineoplastic drugs. However, owing to a lack of specific clinical manifestations and limited laboratory resources, the diagnosis of *Nocardia* infection is often difficult or delayed, greatly affecting patient prognosis. Therefore, a clear understanding of epidemiological and clinical data of *Nocardia* infections in a local region can greatly contribute to the rapid diagnosis of *Nocardia* infection.

References

1. Ribeiro MG, Salerno T, Mattos-Guaraldi AL, Camello TC, Langoni H, Siqueira AK, Paes AC, Fernandes MC, Lara GH. Nocardiosis: an overview and additional report of 28 cases in cattle and dogs. Rev Inst Med Trop Sao Paulo 2008; 50: 177-185.
2. Shahapur PR, Peerapur BV, Shahapur RP, Honnutagi RM, Biradar MS. Lymphocutaneous nocardiosis caused by *Nocardia otitidiscaviarum*: a case report and review of literature. J Nat Sci Biol Med 2014; 5: 197-201.
3. Condas LA, Ribeiro MG, Gonoi T, Matsuzawa T, Yazawa K, Motta RG, Franco MM, Listoni FJ. Molecular identification and thermoresistance to boiling of *Nocardia farcinica* and *Nocardia cyriacigeorgica* from bovine bulk tank milk. Braz J Microbiol 2012; 43: 1038-1041.
4. Parte AC. LPSN--list of prokaryotic names with standing in nomenclature. Nucleic Acids Res 2014; 42: D613-D616.
5. Ishihara M, Takada D, Sugimoto K, Oguro H, Gonoi T, Akiyama Y, Yamaguchi S. Primary brain abscess caused by *Nocardia otitidiscaviarum*. Intern Med 2014; 53: 2007-2012.
6. Wang HL, Seo YH, LaSala PR, Tarrand JJ, Han XY. Nocardiosis in 132 patients with cancer: microbiological and clinical analyses. Am J Clin Pathol 2014; 142: 513-523.
7. Han T, Liu YM, Yang T, Dai HP, Zhang XL. A novel case of pulmonary nocardiosis with secondary hemophagocytic lymphohistiocytosis. Chin Med J (Engl) 2017; 130: 2128-2129.
8. Igbaseimokumo U, El Shafie S, Al Khal AL. First human infection of *Nocardia Crassostreae* in an immunocompetent patient. Chin Med J (Engl) 2016; 129: 114-115.
9. Cattaneo C, Antoniazzi F, Caira M, Castagnola C, Delia M, Tumbarello M, Rossi G, Pagano L. *Nocardia* spp infections among hematological patients: Results of a retrospective multicenter study. Int J Infect Dis 2013; 17: e610-e614.
10. Ramamoorthi K, Pruthvi BC, Rao NR, Belle J, Chawla K. Pulmonary nocardiosis due to *Nocardia otitidiscaviarum* in an immunocompetent host- a rare case report. Asian Pac J Trop Med 2011; 4: 414-416.
11. Takiguchi Y, Ishizaki S, Kobayashi T, Sato S, Hashimoto Y, Suruga Y, Akiba Y. Pulmonary nocardiosis: a clinical analysis of 30 cases. Intern Med 2017; 56: 1485-1490.
12. CLSI. Susceptibility Testing of Mycobacteria, Nocardiae and Other Aerobic Actinomycetes; Approved Standard-Second Edition. CLSI Document M24-A2. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2011.
13. Girard V, Mailler S, Welker M, Arsac M, Cellière B, Cotte-Pattat PJ, Chatellier S, Durand G, Béni AM, Schrenzel J et al. Identification of *Mycobacterium* spp. and nocardia spp. from solid and liquid cultures by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Diagn Microbiol Infect Dis 2016; 86: 277-283.
14. Santos M, Gil-Brusola A, Morales P. Infection by *Nocardia* in solid organ transplantation: thirty years of experience. Transplant Proc 2011; 43: 2141-2144.
15. Maly K, Schirmer M. The story of CD4+CD28– T cells revisited: solved or still ongoing? J Immunol Res 2015; 2015: 348746.
16. Lebeaux D, Morelon E, Suarez F, Lanternier F, Scemla A, Frange P, Mainardi JL, Lecuit M, Lortholary O. Nocardiosis in transplant recipients. Eur J Clin Microbiol Infect Dis 2014; 33: 689-702.
17. Piau C, Kerjouan M, Le Mouel M, Patrat-Delon S, Henaux PL, Brun V, Morin MP, Gautier P, Rodriguez-Nava V, Kayal S. First case of disseminated infection with *Nocardia cerradoensis* in a human. J Clin Microbiol 2015; 53: 1034-1037.
18. Ambrosioni J, Lew D, Garbino J. Nocardiosis updated clinical review and experience at a tertiary center. Infection 2010; 38: 89-97.

19. Flateau C, Jurado V, Lemaître N, Loïez C, Wallet F, Saiz-Jimenez C, Decoene C, Bergeron E, Boiron P, Faure K et al. First case of cerebral abscess due to a novel *Nocardia* species in an immunocompromised patient. J Clin Microbiol 2013; 51: 696-700.

20. Haussaire D, Fournier PE, Djiguiba K, Moal V, Legris T, Purgus R, Bismuth J, Elharrar X, Reynaud-Gaubert M, Vacher-Coponat H. Nocardiosis in the south of France over a 10-years period, 2004-2014. Int J Infect Dis 2017; 57: 13-20.

21. Eschapasse E, Hussenet C, Bergeron A, Lebeaux D. Respiratory infections caused by slow-growing bacteria: *Nocardia*, *Actinomyces*, *Rhodococcus*. Rev Mal Respir 2017; 34: 661-671.

22. Kanne JP, Yandow DR, Mohammed TL, Meyer CA. CT findings of pulmonary nocardiosis. AJR Am J Roentgenol 2011; 197: W266-W272.

23. Centers for Disease Control and Prevention. Nocardiosis. Atlanta, GA, USA: CDC; 2013.

24. Conville PS, Brown JM, Steigerwalt AG, Lee JW, Byrer DE, Anderson VL, Dorman SE, Holland SM, Cahill B, Carroll KC et al. *Nocardia veterana* as a pathogen in North American patients. J Clin Microbiol 2003; 41: 2560-2568.

25. Woo PC, Lau SK, Teng JL, Tse H, Yuen KY. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin Microbiol Infect 2008; 14: 908-934.

26. Wei M, Wang P, Qu J, Li R, Liu Y, Gu L, Yang C. Identification and antimicrobial susceptibility of clinical *Nocardia* species in a tertiary hospital in China. J Glob Antimicrob Resist 2017; 11: 183-187.

27. Schlaberg R, Fisher MA, Hanson KE. Susceptibility profiles of *Nocardia* isolates based on current taxonomy. Antimicrob Agents Chemother 2014; 58: 795-800.

28. Larruskain J, Idigoras P, Marimón JM, Pérez-Trallero E. Susceptibility of 186 *Nocardia* sp. isolates to 20 antimicrobial agents. Antimicrob Agents Chemother 2011; 55: 2995-2998.

29. Lai CC, Liu WL, Ko WC, Chen YH, Tang HJ, Huang YT, Hsueh PR. Antimicrobial-resistant *Nocardia* isolates, Taiwan, 1998-2009. Clin Infect Dis 2011; 52: 833-835.

30. Brown-Elliott BA, Biehle J, Conville PS, Cohen S, Saubolle M, Sussland D, Wengenack N, Kriel K, Bridge L, McNulty S et al. Sulfonamide resistance in isolates of *Nocardia* spp. from a US multicenter survey. J Clin Microbiol 2012; 50: 670-672.

31. Uhde KB, Pathak S, McCullum I, Jannat-Khah DP, Shadomy SV, Dykewicz CA, Clark TA, Smith TL, Brown JM. Antimicrobial-resistant *Nocardia* isolates, United States, 1995-2004. Clin Infect Dis 2010; 51: 1445-1448.