PEARLS

Pathogenic Nocardia: A diverse genus of emerging pathogens or just poorly recognized?

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Introduction to pathogenic Nocardia, a clinically relevant non-ESKAPE pathogen

The clear and present danger posed by the ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa, and Enterobacter species) and their ability to evade antimicrobials is generally well appreciated [1]. There are, however, a multitude of pathogens, some more rare than others, that can be clinically challenging to diagnose and treat. Nocardia, a genus of aerobic actinomycetes found ubiquitously in soil and water, harbors one such group of pathogens with unique attributes and often complicated properties.

Opportunistic infections caused by Nocardia often afflict immunocompromised individuals like cancer patients receiving chemotherapy, individuals with AIDS, and organ transplant recipients. Although commonly considered opportunistic, among 1,000 cases of Nocardia infection published between 1950 and 1991, about one-third occurred in patients with no identifiable underlying predisposing conditions [2], implying that immune status is not the only factor affecting infectivity. Skin and lung are the primary infection sites for these rod shaped, gram-positive bacteria. Nocardia also have the ability to survive as facultative intracellular parasites within macrophages [3,4] and escape killing by human neutrophils and monocytes [5]. Infections can sometimes be largely asymptomatic, which, when coupled with the slow growth rate of Nocardia, makes them difficult to identify in clinical specimens [6]. Dissemination, particularly to the central nervous system (CNS), is relatively common and can be life threatening, with mortality rates as high as 85% in immunocompromised individuals [6–8]. To date, 119 species of Nocardia have been documented (http://www.bacterio.net/nocardia.html), with more than 40 of them being considered clinically relevant (https://www.cdc.gov/nocardiosis/health-care-workers/index.html).

Nocardia infections: Difficult to identify and difficult to treat

The ability of this organism to cause infection was first recognized in 1888 [9]. However, to date, Nocardia has received relatively little attention as a human pathogen. The Centers for Disease Control and Prevention (CDC) estimates approximately 500 to 1000 new cases of nocardiosis infections occur every year in the United States (https://www.cdc.gov/nocardiosis/infection/index.html). This statistic is based on a study conducted in 1976 [9] but is likely to be a significant underestimate given the paucity of molecular diagnostics in that era. Nocardia isolates can take up to two weeks to grow on routine culture media used in clinical labs, and mixed bacterial infections can further obscure identification [3,10]. Concomitant infections in immunocompromised individuals are common and can include Nocardia co-infecting with...
other bacterial, fungal, and viral pathogens [11,12]. Tuberculosis (TB) caused by another actinomycte, *Mycobacterium tuberculosis*, is associated with chronic lung disease, and owing to the similarity in diagnosis and clinical manifestation of nocardiosis and TB, accurate identification of these two acid-fast bacilli can be difficult [13–15]. In some cases, concomitant nocardial and TB infections have also been observed and are more likely to occur in HIV-infected individuals [16]. Treatment of choice for tuberculosis is ineffective for nocardiosis, underscoring the importance of accurate diagnosis for effective treatment [13,14,17].

Diagnostic difficulty is compounded by the ability of *Nocardia* to disseminate to a variety of sites after primary infection. This makes the site and type of infection difficult to identify, often requiring invasive biopsies [18]. Time taken from identifying symptoms to making a diagnosis can vary from 3 days to as much as 30 days [19–21]. The severity of an infection sometimes leads to administration of antimicrobial treatment prior to accurate diagnosis [22], causing some infections to go undiagnosed.

Virulence in *Nocardia* has been attributed to its ability to survive and grow in a variety of human cells and evade the host immune response by production of catalase and superoxide dismutase (SOD), inhibition of phagosome-lysosome fusion, reduction of intracellular acid phosphatase levels in macrophages, and secretion of toxins and (in some cases) hemolysin [2,4,23–25]. Furthermore, a phenotypically distinct form of *Nocardia* called L-phase variants or cell wall deficient variants is known to be induced within lungs and is involved in pathogenesis in in vivo animal models [26]. These forms, however, are not recovered from homogenates of infected lungs, making diagnosis difficult, and have been implicated in contributing to latency of disease [26].

In addition to issues related to immune evasion and diagnosis, strain identification also affects treatment outcomes. Large amounts of heterogeneity exist among different *Nocardia* species with genome sizes ranging from 6 to 10 million base pairs (Mbp) [27,28]. Virulence and antimicrobial susceptibilities of various pathogenic *Nocardia* species vastly differ from one another. The most recent classification distributes the clinically relevant *Nocardia* species into 13 antimicrobial susceptibility patterns [29]. *Nocardia farcinica* tends to be a more virulent species, intrinsically resistant to various antibiotics, including third-generation cephalosporins [18]. In addition, *N. cyriacigeorgica*, *N. nova*, and *N. pseudobrasiliensis* are considered major pathogenic species. The species also differ from each other in biochemical characteristics, such as their ability to utilize different carbon sources and hydrolyze different substrates. All these differences are used as criteria for species identification [18,30]. However, these techniques are laborious and require considerable expertise [31]. As a result, most clinical laboratories rarely identify *Nocardia* infections to the species level [32]. On account of the diverse antimicrobial susceptibilities associated with this genus, identification at species level is crucial for empirical treatment of infection with the appropriate antibiotic in the clinic [29].

Once diagnosed, treatment of nocardiosis is usually prolonged because of the risk of relapse [33,34]. Six to 12 months of antimicrobial therapy for immunocompetent patients and a minimum of 12 months of treatment for immunocompromised patients or those with CNS dissemination is often recommended [8]. In spite of the diverse susceptibility patterns among *Nocardia* species, all 13 patterns show sensitivity to the combination drug trimethoprim–sulfamethoxazole (TMP–SMX) and the more expensive, linezolid [29]. As a result, TMP–SMX is the treatment of choice for nocardial infections [29,35,36]. Imipenem, amikacin, and third-generation cephalosporins are also used, and combination therapy can yield better results [8,20].

**Antimicrobial resistance in *Nocardia*: Is it too late to prevent?**

Resistance to antibiotics is a pressing problem and one of the biggest global threats facing the healthcare industry [37]. *Nocardia* species possess various patterns of intrinsic resistance to
antibiotics, as mentioned above, and TMP–SMX is usually the treatment of choice. In addition to being used as a combination drug for long term treatment, TMP–SMX is also commonly used as a prophylactic agent at low doses to prevent *Nocardia* and *Pneumocystis jirovecii* infections in immunocompromised individuals [34,35,38]. However, this extended antibiotic regimen and low dose exposure provide a greater opportunity for the evolution of resistance.

Unfortunately, resistance to TMP–SMX is already rampant. 42% of 765 isolates of *Nocardia* submitted to the CDC between 1995 and 2004 showed TMP–SMX resistance [39]. Similar levels of TMP–SMX resistance were also seen among 157 isolates from Canada [40]. Similarly, breakthrough infections in immunocompromised individuals receiving TMP–SMX prophylaxis are also being observed [34,35].

TMP–SMX inhibits the folate biosynthesis pathway in bacteria. Because these are two of the earliest antibiotic compounds with broad-spectrum efficacy to be administered to humans, resistance to these drugs is fairly common and well characterized in clinically important bacteria [41]. Resistance is often attributed to mutations or regulatory changes in target enzymes, efflux pumps, and acquired resistance via horizontal gene transfer [41]. The cause of resistance in clinical isolates of *Nocardia*, however, is understudied.

**What is known about the genetic basis of antimicrobial resistance in clinical *Nocardia* isolates?**

A study in 2015 by Valdezate and colleagues showed that out of 76 TMP–SMX-resistant patient isolates of *Nocardia* belonging to 12 species, 75 carried Class 1 and/or Class 3 integrons, which are mobile elements associated with antimicrobial resistance [42,43]. In addition to carrying plasmid-borne variants of dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR) (which are folate biosynthetic pathway enzymes targeted by SMX and TMP, respectively) the strains also carried genes encoding efflux pumps, as well as β-lactamases, aminoglycoside modifying enzymes, RNA methylases, and ribosomal protection proteins that are implicated in resistance to β-lactams, aminoglycosides, macrolides, and tetracyclines, respectively [43]. Although some isolates containing genetic determinants of resistance exhibited susceptible phenotypes, potentially owing to gene silencing or lack of functionality [43], these findings are alarming because they suggest that *Nocardia* isolates are capable of and already have acquired mobile elements carrying resistance conferring alleles that can spread rapidly via horizontal gene transfer. Although not much more is known about the genetic basis of resistance in *Nocardia*, these findings highlight the necessity to use caution in clinical settings during diagnosis and treatment of *Nocardia* infections.

**In vitro evolution of *Nocardia* to TMP–SMX**

In 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 to recapitulate the paths leading to antimicrobial resistance in bacteria [44–46]. Recently, our group conducted in vitro experimental evolution to adapt susceptible clinical isolates of *N. nova* and *N. cyriacigeorgica* to the treatment of choice, TMP–SMX [28]. 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 and DHPS. Some of those mutations were identical to mutations implicated in resistance in other bacterial species like *Escherichia coli* and were involved in substrate or inhibitor binding. In addition 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. This
resistance mechanism of overexpression of folate biosynthesis genes to increase flux through the pathway has also been observed in *Plasmodium falciparum* [47] and *E. coli* [48].

Interestingly, in addition to identifying known mechanisms of TMP–SMX resistance in *Nocardia*, this organism was able to achieve resistance via an as yet uncharacterized process. A homolog (*folP2*) of the gene encoding DHPS (*folP*) exists in most actinomycetes including *Nocardia* and *Mycobacterium*. Although considered to be nonfunctional and unable to serve as a bypass for DHPS in *Mycobacterium* [49], 8 out of 10 *Nocardia* strains evolved to TMP–SMX had acquired mutations in *folP2* [28], suggesting a role for this homolog in resistance. While sequence data of clinically TMP–SMX resistant *Nocardia* strains is lacking, this study provides potential biomarkers for diagnostic purposes. With next generation sequencing technology becoming cheaper and more easily accessible, we are moving closer to sequencing-based diagnostics for identification of infecting agents as well as their antimicrobial susceptibility profiles. Having prior knowledge of potential alleles involved in resistance will facilitate this process.

**Concluding remarks**

The genus *Nocardia* encompasses a diverse group of species. While the incidence of nocardial disease is increasing and so is the number of species being identified [50], there is debate about the use of the phrase “emerging pathogen” for this organism [51]. It may be argued that modern molecular biology tools have improved diagnosis of this elusive pathogen and enabled finer species level identification, giving increased recognition to this well-established, rather than emerging, genus as an agent of infection. Conversely, information obtained from whole genome sequencing of at least one *N. cyriacigeorgica* genome suggests that this organism is on the path of an ongoing adaptation from an environmental bacterium to an emerging pathogen [52]. In either case, it is clear that accurate diagnosis and timely treatment of nocardial infections are crucial because undiagnosed and latent infections have the ability to spread from primary sites to more sensitive regions in the body that can be fatal. Development of PCR based diagnostic assays for early diagnosis of infection and rapid antimicrobial susceptibility testing to identify appropriate treatment options should be considered a priority for this pathogen. As rapid molecular diagnostic technology continues to improve it is likely that other organisms, like *Nocardia*, may soon be joining an extended pantheon of medically important pathogens.

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**References**

1. Rice LB. Federal Funding for the Study of Antimicrobial Resistance in Nosocomial Pathogens: No ESKAPE. J Infect Dis [Internet]. 2008; 197:1079–81. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1086/533452. [cited 2019 July 19] PMID: 18419525
2. Beaman BL, Beaman L. Nocardia Species: Host-Parasite Relationships. Clin Microbiol Rev. 1994; 7 (2):213–64. https://doi.org/10.1128/cmr.7.2.213 PMID: 8055469
3. Lerner PI. Nocardiosis. Clin Infect Dis. 1996; 22:891–905. https://doi.org/10.1093/clinids/22.6.891 PMID: 8783685
4. Davis-Scibienki C, Beaman BL. Interaction of *Nocardia asteroides* with Rabbit Alveolar Macrophages: Association of Virulence, Viability, Ultrastructural Damage, and Phagosome-Lysosome Fusion. Infect Immun. 1980; 28(2):610–9. PMID: 6995313
5. Filice GA, Beaman BL, Kirck JA, Remington JS. Effects of human neutrophils and monocytes on *Nocardia asteroides*: Failure of killing despite occurrence of the oxidative metabolic burst. J Infect Dis. 1980; 142(3):432–8. https://doi.org/10.1093/infdis/142.3.432 PMID: 6777435
6. McNeil MM, Brown JM. The medically important aerobic actinomycetes: Epidemiology and microbiology. Clin Microbiol Rev. 1994; 7(3):357–417. https://doi.org/10.1128/cmrx.7.3.357 PMID: 7923055

7. Cattaneo C, Antoniazzii F, Cairia 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 [Internet]. 2013; 17:e610–4. Available from: [cited 2019 July 19]. https://doi.org/10.1016/j.ijid.2013.01.013 PMID: 23453714

8. Wilson JW. Nocardiosis: updates and clinical overview. Mayo Clin Proc [Internet]. 2012; 87(4):403–7. Available from: https://doi.org/10.1016/j.mayocp.2011.11.016 PMID: 22469352

9. Bearman BL, Burnside J, Edwards B, Causey W. Nocardial Infections in the United States, 1972–1974. J Infect Dis. 1976; 134(3):286–9. https://doi.org/10.1093/infdis/134.3.286 PMID: 789786

10. Baio PVP, Ramos JN, dos Santos LS, Soriano MF, Ladeira EM, Souza MC, et al. Molecular identification of Nocardia isolates from clinical samples and an overview of human nocardiosis in Brazil. PLoS Negl Trop Dis. 2013; 7(12):e2573. https://doi.org/10.1371/journal.pntd.0002573 PMID: 24340116

11. Steinbrink J, Leavens J, Kauffman CA, Mcclellan MH. Manifestations and outcomes of nocardia infections Comparison of immunocompromised and nonimmunocompromised adult patients. Medicine (Baltimore). 2018; 97(40):e12436.

12. Dorman SE, Guide S V., Conville PS, Malech HL, Gallin JI, et al. Nocardia Infection in Chronic Granulomatous Disease. Clin Infect Dis. 2002; 35:390–4. https://doi.org/10.1086/341416 PMID: 12145721

13. Sakyi SA, Danquah KO, Ephraim RD, Enimil A, Frimpong V, Ahenkorah Fondjo L, et al. Evaluating the Contribution of Nocardia spp. and Mycobacterium tuberculosis to Pulmonary Infections among HIV and Non-HIV Patients at the Komfo Anokye Teaching Hospital, Ghana. Can J Infect Dis Med Microbiol. 2018;2018:Article ID 2910198, 7 pages.

14. Hoza AS, Mfinanga SGS, Moser I, König B. Isolation, biochemical and molecular identification of Nocardia species among TB suspects in northeastern, Tanzania; A forgotten or neglected threat? BMC Infect Dis. 2017; 17(1):407. https://doi.org/10.1186/s12879-017-2520-8 PMID: 28595598

15. Olson ES, Simpson AJH, Norton AJ, Das SS. Not everything acid fast is Mycobacterium tuberculosis—A case report. J Clin Pathol. 1998; 51(7):535–6. https://doi.org/10.1136/jcp.51.7.535 PMID: 9797732

16. Ekrami A, Khosravi AD, Samarbaf-Zadeh AR, Hashemzadeh M. Nocardia co-infection in patients with pulmonary tuberculosis. Jundishapur J Microbiol. 2014; 7(12):e12495. https://doi.org/10.5812/jjm.12495 PMID: 25741428

17. Khadka P, Basnet RB, Rijal BP, Sherchand JB. Pulmonary nocardiosis masquerading as tuberculosis in an immunocompetent host: A case report from Nepal. BMC Res Notes [Internet]. 2018; 11(1):488. Available from: [cited 2019 Nov 14]. https://doi.org/10.1186/s13104-018-3604-2 PMID: 30016976

18. Bell M, McNeil MM, Brown JM. Nocardia species (Nocardiosis) [Internet]. Antimicrobe. Available from: http://www.antimicrobe.org/b117.asp. [cited 2019 July 11]. (Accessed on July 11, 2019).

19. Lebeaux D, Freund R, Delden C Van, Guillot H, Marbus SD, Matignon M, et al. Outcome and Treatment of Nocardiosis after Solid Organ Transplantation: New Insights From a European Study. Clin Infect Dis. 2017; 64(10):1396–405. https://doi.org/10.1093/cid/cix124 PMID: 28329348

20. Poonyagairiyagorn HK, Gersham A, Avery R, Minai O, Blazey H, Asamoto K, et al. Challenges in the diagnosis and management of Nocardia infections in lung transplant recipients. Transpl Infect Dis. 2008; 10:403–8. https://doi.org/10.1111/j.1399-3062.2008.00338.x PMID: 18823356

21. Ott SR, Meier N, Kolditz M, Bauer TT, Rohde G, Presterl E, et al. Pulmonary nocardiosis in Western Europe—Clinical evaluation of 43 patients and population-based estimates of hospitalization rates. Int J Infect Dis [Internet]. 2019; 81:140–8. Available from: [cited 2019 July 11]. (Accessed on July 11, 2019). https://doi.org/10.1016/j.ijid.2018.12.010 PMID: 30658169

22. Rouzaud C, Rodriguez-nava V, Catherinot E, Mêchâi F, Bergeron E, Farfour E, et al. Clinical Assessment of a Nocardia PCR-Based Assay for Diagnosis of Nocardiosis. J Clin Microbiol. 2018; 56(6): e00002–18. https://doi.org/10.1128/JCM.00002-18 PMID: 29563199

23. Beaman BL, Black CM, Doughty F, Beaman L. Role of superoxide dismutase and catalase as determinants of pathogenicity of Nocardia asteroides: Importance in resistance to microbial activities of human polymorphonuclear neutrophils. Infect Immun. 1985; 47(1):135–41. PMID: 3880721

24. Black CM, Beaman BL, Donovan RM, Goldstein E. Intracellular acid phosphatase content and ability of different macrophage populations to kill Nocardia asteroides. Infect Immun. 1985; 47(2):375–83. PMID: 3881345

25. Mikami Y, Yu SF, Yazawa K, Fukushima K, Maeda A, Uno J, et al. A toxic substance produced by Nocardia otitidiscaviarum isolated from cutaneous nocardiosis. Mycopathologia. 1990; 112(2):113–8. https://doi.org/10.1007/bf00436506 PMID: 2293032
26. Beaman BL. Induction of L-Phase Variants of *Nocardia caviae* Within Intact Murine Lungs. Infect Immun. 1980; 29(1):244–51. PMID: 7399704

27. Yasuik M, Nishiki I, Iwasaki Y, Nakamura Y, Fujiiwara A, Shimahara Y, et al. Analysis of the complete genome sequence of *Nocardia seriolae* UTF1, the causative agent of fish nocardiosis: The first reference genome sequence of the fish pathogenic *Nocardia* species. PLoS ONE. 2017; 12(3):e0173198. https://doi.org/10.1371/journal.pone.0173198 PMID: 28257489

28. Mehta H, Weng J, Prater A, Elworth RAL, Han X, Shamoo Y. Pathogenic *Nocardia cyriacigeorgica* and *Nocardia nova* evolve to resist trimethoprim-sulfamethoxazole by both expected and unexpected pathways. Antimicrob Agents Chemother. 2018; 62:e00364–18. https://doi.org/10.1128/AAC.00364-18 PMID: 29686152

29. Zhao P, Zhang X, Du P, Li G, Li L, Li Z. Susceptibility profiles of *Nocardia* spp. to antimicrobial and antituberculous agents detected by a microplate Alamar Blue assay. Sci Rep [Internet]. 2017;(7):43660. Available from: http://dx.doi.org/10.1038/srep43660. [cited 2019 July 16].

30. Conville PS, Brown-Elliott BA, Smith T, Zelazny M. The Complexities of Nocardiosis 1997–2003. J Med Microbiol. 2007; 56:545–50. https://doi.org/10.1099/jmm.0.46774-0 PMID: 17374898

31. 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; 142:513–23. https://doi.org/10.1093/ajcp/awu087 PMID: 25239419

32. Saubolte MA, Sussland D. Nocardiosis: Review of Clinical and Laboratory Experience. J Clin Microbiol. 1988; 26:224–37. https://doi.org/10.1128/CMR.19.2.259-282.2006 PMID: 16614249

33. Minero MV, Marín M, Cercenado E, Rabadán PM, Bouza E, Munoz P. Nocardiosis at the turn of the century. Medicine (Baltimore). 2009; 88(4):250–61.

34. Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ. Clinical and Laboratory Features of the *Nocardia* spp. Based on Current Molecular Taxonomy. Clin Microbiol Rev. 2006; 19(2):259–82. https://doi.org/10.1128/CMR.19.2.259-282.2006 PMID: 16614249

35. Centers for Disease Control and Prevention. Antibiotic Resistance: A Global Threat [Internet]. Available from: https://www.cdc.gov/features/antibiotic-resistance-global/index.html. [cited 2019 July 5].

36. Torres HA, Reddy BT, Raad II, Tarrand J, Bodey GP, Hanna HA, et al. Nocardiosis in cancer patients. Medicine (Baltimore). 2002; 81(5):388–97.

37. Huovinen P. Resistance to trimethoprim-sulfamethoxazole. Clin Infect Dis. 2001; 32:1608–14. https://doi.org/10.1086/320532 PMID: 11340533

38. Gillings MR. Integrons: Past, Present, and Future Structure of Integrons. Microbiol Mol Biol Rev. 2014; 78(2):257–77. https://doi.org/10.1128/MMBR.00056-13 PMID: 24847022

39. Valdezate S, Garrido N, Carrasco G, Villalon P, Medina-Pascual MJ, Saez-Nieto JA. Resistance gene pool to co-trimoxazole in non-susceptible *Nocardia* strains. Front Microbiol. 2015; 6:376. https://doi.org/10.3389/fmicb.2015.00376 PMID: 25972856

40. Miller C, Kong-J, Tran TT, Arias CA, Saxer G, Shamoo Y. Adaptation of *Enterococcus faecalis* to daptomycin reveals an ordered progression to resistance. Antimicrob Agents Chemother [Internet]. 2013; 57(11):5373–83. Available from: [cited 2019 July 19]. https://doi.org/10.1128/AAC.01473-13 PMID: 23959318

41. Arias CA, Panesso D, McGrath DM, Qin X, Mojica MF, Miller C, et al. Genetic Basis for In Vivo Daptomycin Resistance in *Enterococcus*. N Engl J Med [Internet]. 2011; 365(10):892–900. Available from: [cited 2019 July 19]. https://doi.org/10.1056/NEJMoa1101138 PMID: 21899450

42. Mehta HH, Prater AG, Shamoo Y. Using experimental evolution to identify druggable targets that could inhibit the evolution of antimicrobial resistance. J Antimicrob (Tokyo). 2018; 71(2):279–86.
47. Heinberg A, Kirkman L. The molecular basis of antifolate resistance in *Plasmodium falciparum*: Looking beyond point mutations. Ann N Y Acad Sci. 2015; 1342(1):10–8.

48. Palmer AC, Toprak E, Baym M, Kim S, Veres A, Bershtein S, et al. Delayed commitment to evolutionary fate in antibiotic resistance fitness landscapes. Nat Commun [Internet]. 2015; 6:7385. Available from: [cited 2019 July 19]. https://doi.org/10.1038/ncomms8385 PMID: 26060115

49. Gengenbacher M, Xu T, Niyomrattanakit P, Spraggon G, Dick T. Biochemical and structural characterization of the putative dihydropteroate synthase ortholog Rv1207 of *Mycobacterium tuberculosis*. FEMS Microbiol Lett. 2008; 287:128–35. https://doi.org/10.1111/j.1574-6968.2008.01302.x PMID: 18680522

50. Larruskain J, Idigoras P, Marimon JM, Perez-Trallero E. Susceptibility of 186 *Nocardia* sp. Isolates to 20 Antimicrobial Agents. Antimicrob Agents Chemother. 2011; 55(6):2995–8. https://doi.org/10.1128/AAC.01279-10 PMID: 21402847

51. Witebsky FG, Conville PS, Wallace RJ, Brown-elliott BA. *Nocardia cyriacigeorgica*—an Established Rather than an Emerging Pathogen. J Clin Microbiol. 2008; 46(7):2469–70. https://doi.org/10.1128/JCM.00510-08 PMID: 18614666

52. Zoropogui A, Pujic P, Normand P, Barbe V, Belli P, Graindorge A, et al. The *Nocardia cyriacigeorgica* GUH-2 genome shows ongoing adaptation of an environmental Actinobacteria to a pathogen's lifestyle. BMC Genomics. 2013; 14:286. https://doi.org/10.1186/1471-2164-14-286 PMID: 23622346