Razavi Int J Med. 2019 March; 7(1): e78763 doi: 10.30483/RIJM.2019.48

Published online 2019 March 30 **Letter to editor**

The Role of Molecular Methods in Identification of Nocardiosis

Masoud Keikha[1-3,*]

[1] Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Department of Microbiology and Virology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran

*** *Corresponding author:*** Masoud Keikha, Department of Microbiology, Antimicrobial Resistance Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran. Tel: +989386836425; Email: Masoud.keykha90@gmail.com

Received 2018 June 06**; Accepted** 2018 June 12.

Keywords: Mollecular idetification, Nocardia spp, Nocardiosis, 16S rRNA

Dear Editor,

The genus *Nocardia*is one of the important pathogenic group of aerobic *actinomycetes*, which includes high rate of GC, presence of mycolic acid (60-40 carbons), and the type IV of peptidoglycan (consisting ofmeso-diaminopimelic acid, arabinose and galactose) in the cell wall structure (1-2). This group of bacteria was introduced by Edmond Nocard in 1888 from bovine farcy; the term "*Nocardia*" was established by Trevisan in 1889, and the first report of human infection of nocardiosis was described by Epinger in 1890 (1-3).

Nocardia spp. are gram positive, branching, relatively slow-growing and partially acid-fast bacteria, which can enter into the human body using inhalation of aerosol or traumatic inclusion causing various infections asnocardiosis that can be the localized cutaneous infection to pulmonary, brain abscess, keratitis, as well as the disseminated and catheter-related infection in immune-compromised patients and healthy individuals. According to the literatures, the infection rate of nocardiosis in Iran is estimated to be 1.88% (2-4). The selection of appropriate *Nocardia* isolation method is one of the important steps for diagnosing and the treatment of nocardiosis. Isolation of *Nocardia* species from clinical specimens are difficult, as *Nocardia* spp. have slow growth (between 7-30 days), are sensitive to decontaminating materials, and misidentified as fungi and contamination in medical laboratories. The paraffin baiting technique is the reliable and suitable method for the diagnosis of nocardiosis infections, which is popular and frequently used by various researchers (1-2,5-6). This method (the paraffin baiting method) was introduced by Mishra et al., which includesthe carbon free media, such as McClung carbon-free broth and a paraffin coated rod glass. Unlike the other bacteria, genus *Nocardia* produced enzymes, which metabolized the paraffin, and the colonies appear between 1-4 weeks (7).

Nocardia species are identified and classified using conventional methods, such as the colony morphology, growth rate, Kinyoun staining, aerial hyphae, growth to lysozyme broth, growth in different temperatures, pigment production, hydrolysis of tyrosine, xanthine, hypoxanthine, casein, esculin, urease production, consumption of citrate, gelatinase activity, nitrate reductase and utilization of various carbohydrates as the sole source of carbon. These methods are time-consuming, labor-intensive, expensive, need skilled microbiologists, and are difficult to be standardized, and confusing. Moreover, the presence of complexes such as *Nocardiaasteroides* complex (*N. asteroides*, *Nocardiacyriacigeorgica*, *N. farcinica*), *Nocardia nova* complex (*Nocardia nova*, *Nocardiavetera*, *Nocardia-africana*, *Nocardiakruczakiae*), *Nocardiatransvalensis* complex (*Nocardiatransvalensis*, *Nocardiablacklockiae*, *Nocardiawallacei*) in the genus *Nocardia* providedextensive difficulties. However, molecular tests are inexpensive, accurate, reliable, fast in emergency cases, being appropriate identifying methods, which include sequencing, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and DNA hybridization technique using housekeeping genes such as 16S rRNA, ITS (16S-23S rRNA internal transcribed spacer), *rpoB*, *hsp65*, *secA*, *gyrB* and *sodA* genes (1-2). Among the mentioned methods, 16S rRNA direct-sequencing is the gold standard method for identification and differentiation of nocardial species, especially in emerging *Nocardia* spp. 16S rRNA has made from hyper-variable and conserved regions that according to Laurent et al.,*Nocardia spp.* can easily be identified using a 596-bp region in 16S rRNA gene, which can be amplified by NG1 (5′-ACCGACCACAAGGGG-3′ and NG2 (5′-GGTTGTAACCTCTTCGA-3') paired primers. However, some reports have proved otherwise; e.g. the presence of two different copies of 16S rRNA in *N. nova*, *Nocardiaconcava*, *Nocardiaignorata* or *Nocardiayamanashiensis*, and similarities between closely related species of *N. nova* complex and diversity in the 16S rRNA gene sequence of *N. cyriacigeorgica*can be observed (1-2,8). Thus,it is better to survey and study the two or more housekeeping genes using MLSA (Multi-locus sequence analysis) technique (9). In addition, the

REA (Restriction Endonuclease Analysis) method was also an inexpensive and a rapid method for identification of *Nocardia* spp., achieved via restriction of the enzymes that cleavage the unique palindrom sequences;e.g. hsp65 sequence via *BstE*II, *Hin*fI, *Hae*III, *BsaH*I and *Msp*I or 16S rRNA using *BstE*II, *Dpn*II, *Hind*III, *Hha*I, *Nde*II, *Sph*I and *Hin*PII restricted the enzymes and subsequently compared with the available reported REA patterns (1-2,10).

Nowadays, the prevalence of nocardiosis infection is increasing, and this increase can be due to enhancing the number of patients affected to immune system disorders and also improving the diagnosing methods, especially the development of molecular methods (11-12).

The genus *Nocardia* has six drug-susceptibility patterns consisting of*N. abscessus* as type I, *Nocardiabrevicatena-paucivorans* complex as type II, *N. nova* complex as type III, *N.transvalensis* complex as type IV, *N. farcinica*as type V and *N. cyriacigeorgica*as type VI; but due to spreading of antibiotic-resistant gene elements among community of bacteria and emerging of novel *Nocardia* spp., the drug susceptibility patterns did not cover all the species (1-2).

In brief, the identification and differentiation of *Nocardia* species are essential for the final diagnosis, patient management, appropriate treatment, and epidemiological studies. Moreover, this group of bacteria can be efficient in the biodegradation process and production of secondary metabolites such as antibiotic. Molecular methods are the best options for rapid and accurate identification and differentiation of *Nocardia* species.

Conflicts of interest

None to declare.

References

1. 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. doi: 10.1128/CMR.19.2.259-282.2006. [PubMed: 16614249].
2. Fatahi-Bafghi M. Nocardiosis from 1888 to 2017. *Microb Pathog*. 2017;**114**:369-84. doi: 10.1016/j.micpath.2017.11.012. [PubMed: 29146497].
3. Rahdar HA, Azadi D, Shojaei H, Daei-Naser A. Molecular analysis and species diversity of Nocardia in the hospital environment in a developing country, a potential health hazard. *J Med Microbiol*. 2017;**66**(3):334-41. doi: 10.1099/jmm.0.000436. [PubMed: 28100300].
4. Boiron P, Provost F, Chevrier G, Dupont B. Review of nocardial infections in France 1987 to 1990. *Eur J Clin Microbiol Infect Dis*. 1992;**11**(8):709-14. [PubMed: 1425729].
5. Bafghi MF, Heidarieh P, Soori T, Saber S, Meysamie A, Gheitoli K, et al. Nocardia isolation from clinical samples with the paraffin baiting technique. *Germs*. 2015;**5**(1):12-6. doi: 10.11599/germs.2015.1066. [PubMed: 25763363].
6. Shawar RM, Moore DG, LaRocco MT. Cultivation of *Nocardia* spp. on chemically defined media for selective recovery of isolates from clinical specimens. *J Clin Microbiol*. 1990;**28**(3):508-12. [PubMed: 2182669].
7. Mishra S, Randhawa H. Application of paraffin bait technique to the isolation of Nocardia asteroides from clinical specimens. *Appl Microbiol*. 1969;**18**(4):686-7. [PubMed: 4905040].
8. Kong F, Chen SC, Chen X, Sintchenko V, Halliday C, Cai L, et al. Assignment of reference 5'-end 16S rDNA sequences and species-specific sequence polymorphisms improves species identification of Nocardia. *Open Microbiol J*. 2009;**3**:97-105. doi: 10.2174/1874285800903010097. [PubMed: 19639036].
9. McTaggart LR, Richardson SE, Witkowska M, Zhang SX. Phylogeny and identification of Nocardia species on the basis of multilocus sequence analysis. *J Clin Microbiol*. 2010;**48**(12):4525-33. doi: 10.1128/JCM.00883-10. [PubMed: 20844218].
10. Fatahi-Bafghi M, Heidarieh P, Rasouli-Nasab M, Habibnia S, Hashemi-Shahraki A, Eshraghi SS. Comparison of restriction enzyme pattern analysis and full gene sequencing of 16S rRNA gene for Nocardia species identification, the first report of Nocardia transvalensis isolated of sputum from Iran, and review of the literature. *Antonie Van Leeuwenhoek*. 2016;**109**(10):1285-98. doi: 10.1007/s10482-016-0746-x. [PubMed: 27613736].
11. Molina A, Winston DJ, Pan D, Schiller GJ. Increased incidence of nocardial infections in an era of atovaquone prophylaxis in allogeneic hematopoietic stem cell transplant recipients. *Biol Blood Marrow Transplant*. 2018;**24**(8):1715-20. doi: 10.1016/j.bbmt.2018.03.010. [PubMed: 29555315].
12. Keikha M. Necessity of studying the methods for isolation of *Nocardia* from environmental and clinical samples. *Zahedan J Res Med Sci*. 2017;**19**(11):e58134. doi: 10.5812/zjrms.58134.