ISMB

TUMS

Molecular Typing of Nocardia Species

Seyyed Saeed Eshraghi *

Section Microbiology, Department Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran

ARTICLE INFO

Article type:
Review Article

Article history:
Received: 07 Aug 2012
Revised: 20 Aug 2012
Accepted: 5 Sep 2012

Keywords:
Nocardia

Polymorphism, Restriction Fragment Length

RNA, Ribosomal, 16S

ABSTRACT

Identification of clinically significant Nocardia species is essential for the definitive diagnosis, predict antimicrobial susceptibility, epidemiological purposes, and for an effective treatment. Conventional identification of Nocardia species in routine medical laboratories which is based on phenotypic (cellular morphology, colonial characteristics), biochemical and enzymatic profiles, and chemotaxonomic characteristics is often laborious, and time-consuming. The procedure requires expertise, and newer species can be difficult to differentiate with accuracy from other related species. Alternative methods of identification, such as high performance liquid chromatography (HPLC) and molecular biology techniques allow a better characterization of species. The taxonomy of the genus Nocardia has been dramatically been revised during the last decade and more than 30 valid human clinical significance species of Nocardia have been reported. The use of molecular approaches, including 16S rRNA gene sequencing, restriction fragment length polymorphism (RFLP) or PCR restriction endonuclease analysis has been the focus of recent investigations to distinguish the isolates of Nocardia from other actinomycetes genera. The methods have revolutionized the characterization of the Nocardiae by providing rapid, sensitive, and accurate identification procedures. The present review describes the currently known medically important pathogenic species of Nocardia.

- ***Please cite this paper as:*** Eshraghi SS. Molecular Typing of Nocardia Species**.** *J Med Bacteriol*. 2012; **1** (1, 2): pp. 38-45.

* *Corresponding Author:* Seyyed Saeed Eshraghi, PhD., Section Microbiology, Department Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran. *Tel:* +98 912 6363134, *E-mail:* eshragh@tums.ac.ir

Introduction

Nocardiae are aerobic, Gram-positive, non-motile, weakly acid-fast bacteria that often form branched hyphae in both tissues and culture, and are closely related to the rapidly growing mycobacteria. The hyphae are easily disrupted into rods and cocci elements. Nocardia species are found extensively around the world as saprophytic component of the normal soil microflora, fresh and marine water and may also be associated with decomposing plant materials, dust, and air (1). Nocardia was first isolated by French veterinarian Edmond Nocard in 1888 after isolation from a case of lymphadenitis "bovine farcy" (2). A year after, an Italian scholar Trevisan characterized the organism and named it *Nocardia farcinica* (3). In 1954, *N. farcinica* was made the type species of genus Nocardia, and this strain was designated the type strain of the species *N. farcinica* (4). Because of the uncertain taxonomic status of the *N. farcinica* type strain and because *N. asteroides* had become the most common designation of isolates of this genus, Gordon and Mihm petitioned the International Judicial Commission to group these two species together; the name *N. asteroides* was chosen to replace *N. farcinica* as the type species of the genus Nocardia. At this time, a new type strain, *N. asteroides* ATCC 19247, was also selected (5).

Nocardia, like members of the genus *Mycobacterium*, contain tuberculostearic (mycolic) acids, but in contrast to *mycobacteria*, they possess short-chain mycolic acids (40-60 carbon). Nocardiae also have a type IV cell wall, characterized by a peptidoglycan made up of *meso*-diaminopimelic acid, arabinose, and galactose (6). Nocardiae are found extensively in nature but they are not present as normal flora in the respiratory tract. Nocardia species are facultative intracellular bacteria that can infect both immunocompromised and immunocompetent human hosts. They may invade the body from the environment via skin and respiratory tract, and causes a variety of suppurative and granulomatous infections in humans, ranging from localized cutaneous mycetomas to disseminated systemic diseases (7). Nocardial infection shows a broad and variable geographic distribution. The clinical forms include pulmonary involvement, skin or soft tissue infection, and disseminated forms with brain and pulmonary lesions. Primary Nocardial infection includes pulmonary or cutaneous and/or subcutaneous lesions. Disseminated disease is defined by the identification of Nocardial infection in two or more organs. The lung is the most commonly involved organ, followed by the skin and the brain. The bacteria are considered an unusual opportunistic pathogen among HIV-infected patients (8). From the epidemiological point of view, there is no age, ethnic group, or geographic variation in nocardiosis caused by the different Nocardia species, and the male to female ration for infection is 3 to 1. The identification of Nocardiae is moving steadily away from traditional phenotypic tests to methods based upon genomic analysis (9). In addition, the characterization of many pathogenic determinants and antibiotic resistance genes are also progressing from bioassays to

nucleic acid-based techniques. For such methods, rapid DNA isolation and storage are of crucial importance for a number of procedures, particularly where quality control tests are required (10).

Taxonomy

The improved classification provides a sound framework for the recognition of additional species. Interest in the taxonomy of the Nocardiae has risen as some described species of Nocardia cause a variety of suppurative diseases in human and animals, notably actinomycete mycetoma and nocardiosis (7, 11). Many of the Nocardia species isolates based on a major taxonomic and nomenclature-related issues for Nocardia that have been reported in the literature as "*Nocardia* asteroides" were clearly misidentified by today's standards (12). *Nocardia asteroides* were chosen by Ruth Gordon as the type species of Nocardia, with ATCC 19247 as the type strain of the type species. Recent application of modern taxonomic procedures, including of more extensive phenotypic evaluation, molecular characterization, and numerical taxonomic methods, have expanded our knowledge of their phylogenetic relatedness and taxonomic status (6, 13). Identification of Nocardiae to the species level does predict susceptibility for several species of Nocardia. Generally, only small numbers of some of the species within the complexes have been studied, and thus as new strains are identified, the currently accepted phenotypic descriptions, including antimicrobial susceptibility patterns, may not be valid. This is well illustrated within the *N. nova* complex. Currently this complex is composed of *N. nova, N. africana, N. veterana, N. kruczakiae,* and possible other, yet-unnamed species. With the exception of *N. nova*, only a few isolates of each of these species have been studied in detail, including antimicrobial susceptibility testing. Therefore, until susceptibility testing of large numbers of each of the species to allow for generalization of susceptibility patterns is reported, antimicrobial susceptibility testing of all isolates of Nocardia is warranted (12).

Wallace and colleagues demonstrated six drug pattern types out or 12 antimicrobial agents, among a study of 78 consecutive clinical isolates previously identified as "*Nocardia* asteroides" (14). This report was the largest study of antimicrobial susceptibilities of *N. asteroides* to show the variability of drug susceptibility patterns and the first to show grouping of specific susceptibility patterns. The issue of the type strain was first evident in this study, as none of the six drug pattern groups matched the pattern of the type strain ATCC 19247. This study provided the impetus for investigators to perform studies of drug resistance in *N. asteroides* (subsequently referred to as the *N. asteroides complex*) and for the study of the taxonomic significance of the groups of Nocardia exhibiting the six drug susceptibility patterns. These later studies were performed using more modern molecular methods (*Table 1*).

Table 1. Current issues pertaining to the Nocardia species associated with human pathogens

Commonly associated disease			Formerly species (Type drug pattern)	Currently accepted species
Systemic	Pulmonary	Cutaneous		
+	+		*N. asteroids (I)*	*N. abscessus*
+	+		*N. paucivorans Complex (II)*	*N. paucivorans*
+	+		*N. asteroids (III)*	*N. nova*
	+		*N. asteroids (III)*	*N. africana*
	+		*N. asteroids (III)*	*N. veterana*
+	+		*N. asteroids (IV)*	*N. transvalensis*
+	+		*N. asteroids (V)*	*N. farcinica*
+	+	+	*N. asteroids (VI)*	*N. asteroides*
		+	Discomyces brasiliensis Streptotrix brasiliensis Oospora brasiliensis Actinomyces brasiliensis (NA)	*N. brasiliensis*
	+		(NA)	*N. asiatica*
+	+	+	(NA)	*N. beijingensis*
+	+		*N. cyriacigeorgici (NA)*	*N. cyriacigeorgica*
+	+		(NA)	*N. higoensis*
		+	(NA)	*N. niigatensis*
+	+	+	*N. caviae (NA)*	*N.otitidiscaviarum*
+	+		(NA)	*N. pseudo-brasiliensis*

Data retrieved from (12, 15)
NA = not validated

Recently, more than 50 species of the genus Nocardia have been characterized by phenotypic and molecular analysis. Many of these can cause clinical diseases in humans and animals, including pulmonary, central nervous system, and cutaneous infections that are all diagnosed by culture and specific identification of these organisms (*Table 1*) (12).

Molecular Typing Methods

Molecular methods were developed in the 1990s, which, through advancement of the last decade, have had an enormous impact on the classification of Nocardia species. These methods comparing with conventional taxonomy represent a simple and rapid means to finalize identification to the species level. As a result, the identification of Nocardia species by conventional taxonomy was badly underestimated and misunderstood (16). Knowledge of the genetic complement of Nocardiae enables their specific detection, typing and recognition of key aspect of genotypic variation by the application of specific gene probes (hybridization) and by the use of targeted DNA amplification technologies. DNA Hybridization assays require one nucleic acid strand as probe (originate from an organism of known identity) and another strand as target (originate from an unknown organism to be identified). The use of two constructed DNA probes to identify *Nocardia asteroides* in substantially shorter time has been examined. The probes hybridized with 31% of the *N. asteroides* strains in a reference collection without cross-hybridization with related Actinomycetales (17). However, the application of DNA probe technique for the identification of microorganisms requires extraction of large amounts of DNA for probe production and hybridization studies. Because of the presence of a complex cell wall structure similar to that of mycobacteria, Nocardia species are resistant to procedures routinely used to extract DNA from most bacteria. Prolonged exposure of sensitized organisms to high concentrations of lysozyme is required (18). The modification procedure

substantially increased the yield of chromosomal DNA from harvested cells and may be applicable to the bacterial-DNA extraction procedures (19). The application of the in situ hybridization technique to detect Nocardial 16S rRNA using a Nocardia specific digoxigenin-labeled probe in specimens from the cerebral cortices of monkeys experimentally infected with *N. asteroides* have been described. Results from these studies suggested a possible association between the presence of Nocardia and neurodegenerative disorders. In 1996 Laurent used ribotyping (rRNA gene restriction patterns) as a taxonomic tool for studying of the *Nocardia asteroides* complex and related strains. The probe used was obtained by PCR from DNA extracted from the *N. asteroides* ATCC 19247 type strain. The technique involves extraction of the genomic DNA and subsequent digestion with a restriction endonuclease, electrophoresis of the digest, followed by Southern blot using a labeled DNA probe, resulting in a banding pattern specific for individual species. However, ribotyping has been used to differentiate a limited number of Nocardia species. Various banding patterns for a number of isolates identified as belonging to the *N. asteroides* complex. Interpretation of hybridization after EcoRI restriction of total DNA isolated from 21 strains of the *N. asteroides* complex allowed for the identification of only four different ribotypes: two related to *N. asteroides* sensu stricto, one related to N. farcinica and one related to N. nova (20). Additionally, Clinical isolate identified as N. farcinica by phenotypic methods showed a ribotype identical to that obtained with the type strain of N. farcinica but was different from those obtained with the type strains of N. nova and N. asteroids (21).

PCR and PCR-RFLP molecular analysis

The PCR, alone and in combined (combination) with other molecular analyses methods such as PRA (PCR restriction enzyme pattern analysis), was one of the first molecular techniques to be used for the characterization and identification of Nocardia species. These techniques involve PCR amplification of the 65-kDa heat shock proteins of the hsp65 gene or the 16S rRNA gene and subsequent digestion with specific restriction endonucleases for each gene. The restriction digest is then electrophoresed on an agarose gel, resulting in a restriction fragment length with special polymorphism pattern, which may be species specific (22). PRA techniques have provided reliable information to characterize biotypes and to better understand the epidemiology of the infections caused by Nocardia genus. These features obtained by PCR-RFLP had been reported in a nosocomial outbreak and a pseudo-epidemic strain associated with Nocardia. PRA of a 441-bp fragment of the hsp65 gene proteins was developed to identify individual Nocardia species. A PRA procedure technique using a 999-bp region of the 16S rRNA gene, which allowed differentiation of most of the species of Nocardia commonly isolated from clinical specimens, was also developed to detect the species of Nocardia (22).

DNA-based typing methods, including pulsed-field gel electrophoresis and randomly amplified

polymorphic-DNA analyses, have been used to characterize clinically related *N. asteroides* strains (2324).The PCR targeting portions of the *hsp* gene and the 16S rRNA gene coupled with restriction endonuclease digestion of PCR products have also been developed and used for Nocardia-specific primers to distinguish isolates from the other actinomycetes genera (22). Comparison of identifications obtained using PRA of both the *hsp65* kDa and 16S rRNA genes showed that some isolates exhibited identical groupings with both genes. However, in some cases, PRA results obtained with the two genes did not correlate with each other; subsequent 16S rRNA gene sequencing of these isolates indicated these to be members of un-described species75. Nevertheless, an important interspecies polymorphism has been observed in *N. nova, N. otitidiscaviarum* and *N. transvalensis* by PCR-RFLP technique. This method clearly differentiates these groups from the *N. asteroides* complex. The use of a PCR-RAPD fingerprinting technique has been applied for its ability to distinguish among various species of Nocardia. This technique involves PCR of extracted DNA with a set of arbitrary primers and gel electrophoresis of the resulting fragments (24).

Sequencing and Enzymatic Digestion of Nucleic Acids

Sequencing is the determination of the order of bases in a piece of DNA. Analysis of gene sequences has increased understanding the phylogenetic relationships of Nocardia and has resulted in the recognition of numerous new species (25). Moreover, sequencing methods are important identification tools, and those based on 16S rRNA gene polymorphism have been studied and applied to Nocardia species (26, 27). The Micro sequencing 500 16S rRNA gene kit (PE Applied Biosystems) and the RIDOM database and BIBI database relied on this methodology have recently been applied to the species identification of Mycobacterium and Nocardia isolates (26, 27). This method as important components in the identification of Nocardia isolates is the quality of the databases used for sequence comparisons. These approaches proved to be as efficient as conventional methods (biochemical tests, high-pressure liquid chromatography, and molecular probes) for many but not all Nocardia species (26). Recently, the technique of pyrosequencing has been evaluated for use in the identification of various clinically relevant Nocardia species. Pyrosequencing involves preliminary amplification of a specific gene region and subsequent short-read sequencing of a section of that region which includes variable bases. Touhy et al. utilized pyrosequencing of the hypervariable region of the 16S rRNA gene and identified isolates by comparison of resulting sequences contained in GenBank and in an in-house database. Thirty-eight of 45 identifications obtained by pyrosequencing were identical to identifications obtained for those isolates by PRA of the hsp65 gene (12).

References

1. Das M, Royer TV, Leff LG. Diversity of Fungi, Bacteria, and Actinomycetes on Leaves Decomposing in a Stream. *Applied*

and Environmental Microbiology 2007; 7**3** (7): 756-67.

2. Nocard ME. Note sur la maladie des boeufs de la Guadeloupe connue sous le nom de farcin. *Ann Inst Pasteur* 1888; **2**: 293-302.
3. Trevisan V. *Zanaboni and Gabuzz, Milan, Italyi*. 1889
4. Lechevalier HA. Nocardioforms. *Bergey's manual of systematic bacteriology* 1986; **2**: 1458-9.
5. Gordon RE, Mihm Jm. The type species of the genus Nocardia. *J Gen Microbiol* 1962; **27**: 1-10.
6. Saubolle MA, Sussland D. Nocardiosis: review of clinical and laboratory experience. *J Clin Microbiol* 2003; **41** (10): 4497-4501.
7. Moghaddami M, Kordbachae P. Report of thirteen cases of mycetoma. *Medical Journal of the Islamic Republic of Iran* 1989; **3** (3): 183-6.
8. Jinno S, Jirakulaporn T, Bankowski Mj, *et al.* Rare case of *Nocardia asteroides* pericarditis in a human immunodeficiency virus-infected patient. *J Clin Microbiol* 2007; **45** (7): 2330-3.
9. Filice G. Nocardiosis in persons with human immunodeficiency virus infection, transplant recipients, and large, geographically defined populations. *The Journal of laboratory and clinical medicine* 2005; **145** (3): 156-162.
10. Conville PS, Witebsky FG. Organisms designated as *Nocardia asteroides* drug pattern type VI are members of the species *Nocardia cyriacigeorgica*. *J Clin Microbiol* 2007; **45** (7): 2257-9.
11. Iona E, Giannoni F, Brunori L, *et al.* Isolation of *Nocardia asiatica* from Cutaneous Ulcers of a Human Immunodeficiency Virus-Infected Patient in Italy. *Journal of Clinical Microbiology* 2007; **45** (6):2088-9.
12. Brown-Elliott BA, Brown JM, Conville PS, *et al.* Clinical and laboratory features of the Nocardia spp. based on current molecular taxonomy. *Clin Microbiol Rev* 2006; **19** (2):259-82.
13. Kiska DL, Hicks K, Pettit DJ. Identification of medically relevant Nocardia species with an abbreviated battery of tests. *J Clin Microbiol* 2002; **40** (4): 1346-51.
14. Wallace RJ, Jr, Steele LC, Sumter G, *et al.* Antimicrobial susceptibility patterns of *Nocardia asteroides*. *Antimicrob Agents Chemother* 1988; **32** (12): 1776-9.
15. Leegaard TM, Caugant DA, Froholm LO, *et al*. Apparent differences in antimicrobial susceptibility as a consequence of national guidelines. *Clinical Microbiology & Infection* 2000; **6** (6): 290-3.
16. Goodfellow M. Numerical taxonomy of some nocardioform bacteria. *J Gen Microbiol* 1971; **69** (1): 33-80.
17. Brownell GH, Belcher KE. DNA probes for the identification of *Nocardia asteroides*. *Journal of Clinical Microbiology* 1990; **28** (9): 2082-6.
18. Mordarski M, Szyba K, Pulverer G, *et al*. Deoxyribonucleic acid reassociation in the classification of the 'rhodochrous' complex and allied taxa. *J Gen Microbiol* 1976; **94** (2): 235-45.
19. Loeffelholz MJ, Scholl DR. Method for improved extraction of DNA from *Nocardia asteroides*. *J Clin Microbiol* 1989; **27** (8): 1880-1.
20. Laurent F, Carlotti A, Boiron P, *et al*. Ribotyping: a tool for taxonomy and identification of the *Nocardia asteroides*

complex species. *J Clin Microbiol* 1996; **34** (5): 1079-82.

21. McNeil MM, Ray S, Kozarsky PE, *et al*. *Nocardia farcinica* pneumonia in a previously healthy woman: species characterization with use of a digoxigenin-labeled cDNA probe. *Clin Infect Dis* 1997; **25** (4): 933-4.
22. Conville PS, Fischer SH, Cartwright CP, *et al*. Identification of Nocardia species by restriction endonuclease analysis of an amplified portion of the 16S rRNA gene. *J Clin Microbiol* 2000; **38** (1): 158-164.
23. Isik K, Goodfellow M. Differentiation of Nocardia species by PCR-randomly amplified polymorphic DNA fingerprinting. *Syst Appl Microbiol* 2002; **25** (1): 60-7.
24. Kalpoe JS, Templeton Ke, Horrevorts Am, *et al*. Molecular Typing of a Suspected Cluster of *Nocardia farcinica* Infections by Use of Randomly Amplified Polymorphic DNA, Pulsed-Field Gel Electrophoresis, and Amplified Fragment Length Polymorphism Analyses. *J Clin Microbiol* 2007; **45** (12): 4048-50.
25. Conville PS, Witebsky FG. Multiple copies of the 16S rRNA gene in *Nocardia nova* isolates and implications for sequence-based identification procedures. *J Clin Microbiol* 2005; **43** (6): 2881-5.
26. Patel JB, Wallace RJ, Jr, Brown-Elliott Ba, *et al*. Sequence-based identification of aerobic actinomycetes. *J Clin Microbiol* 2004; **42**: 2530-40.
27. Mellmann A, Cloud JL, Andrees S, *et al*. Evaluation of RIDOM, MicroSeq, and Genbank services in the molecular identification of Nocardia species. *Int J Med Microbiol* 2003; **293** (5): 359-70.