Isolation and Identification of *Nocardia* spp. Using Phenotypic Methods from Soil Samples of North Khorasan Province

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**ABSTRACT**

**Introduction:** *Nocardia* is saprophytic soil bacterium of the actinomycetes families. *Nocardia* has high diversity of species and due to species diversity of *Nocardia*, phenotypic tests are essential for early identification of *Nocardia* species.  
**Methods:** 155 soil samples from different regions of North Khorasan province in Iran were collected, including: urban and rural areas. Isolation of *Nocardia* was performed by paraffin baiting Technique. Identification of *Nocardia* species was performed by phenotypic methods that are including: hydrolysis of the amino acids, acid production of carbohydrates and, growth at 35°C and 45°C.  
**Results:** From 155 soil samples, 8 isolates (5.2%) were identified as the genus *Nocardia*. These bacteria were related to the gardens, the sands soils, and soil of town square. No growth was observed at 45°C. As the result, our isolates were identified as *Nocardia asteroides* complex.  
**Conclusion:** Isolation and identification of *Nocardia* spp. from soil of different regions in North Khorasan province in Iran can help to enhance our understanding of epidemiological and ecological of the pathogenic *Nocardia* species.

Introduction

Aerobic actinomycetes are including pathogens commonly which they are found in the soil. They are lead to the opportunistic infections in humans and animals. Nocardia is the opportunistic infrequent bacterium but it can cause severe disease with various clinical forms such as skin, pulmonary infections, disseminated infection in the central nervous system, and, rarely other organs such as the kidney. The agent of this disease is the genus Nocardia which belongs to the aerobic actinomycetes. Nocardia is Gram-positive, partially acid-fast, branching hyphae and saprophytic of the actinomycetes families that causes nocardiosis. This bacterium is a soil saprophyte that transfers through the inhalation, leading to disease. Nocardia infections are an infection that occurs in the immunocompromised patients, including sarcoidosis, chronic granulomatous, diabetes and AIDS, and trauma disease (1-7). Various factors such as pH, soil type, climate, temperature, humidity and other factors can effect on the abundance of Nocardia species isolated from soil of different regions (8, 9). The weather has an effect on the geographical distribution of various Nocardia species. For example, Nocardia brasiliensis associated with skin infections such as mycetoma which is endemic in tropical and subtropical areas and it is common in the southwestern and southeastern of the United States (10-12). In addition of the epidemiological and ecological importance, the isolation and identification of Nocardia from soil can also help diagnose and treat the nocardiosis (13). Some of Nocardia species can be identified by using phenotypic and carbohydrate tests and drug susceptibility pattern (8, 14). One of the best methods for Nocardia isolation from soil is paraffin baiting technique (15, 16). The purpose of this study is the identification of Nocardia spp. using phenotypic methods from soil samples of North Khorasan province.

Materials and Methods

155 soil samples of the various regions of North Khorasan province were collected from rural areas including gardens and agricultural fields, silt, animal home range, rodent nests, and urban areas (parks, along the roads and streets and squares). The samples were sent to the laboratory within 48 hours.

Preparing the suspension of soil

1 gram of soil was added to 10 mL sterile distilled water and shaken for 10 min. After precipitation, 1 ml of the supernatant was added to a tube containing 7 ml of carbon-free broth. Paraffin rods were added to each tube and were incubated for 4 weeks at 35°C (17). After isolation and purification of Nocardia from paraffin rod, cultured in nutrient agar (Figure 1-3). After 4-7 days, tree smears were prepared of Gram-stain, kinyoun acid-fast and partially acid-fast. Morphology of colonies was observed by stereomicroscope and growth of bacteria was performed in lysozyme broth for confirmation the genus Nocardia (14).

Phenotypic identification

The phenotypic tests including: hydrolysis of the amino acids such as xanthine, hypoxanthine, tyrosine, casein, liquefaction of gelatin, decomposition of urea, citrate utilization, production of nitrate reduction, growth at 45°C, and acid production from carbohydrates such as maltose, sorbitol, glucose, lactose, galactose, arabinose, rhamnose and xylose were performed to Nocardia identification in level species(14, 18).

Results

From 155 soil samples, 11(7%) isolates belong to the genus Nocardia. Four (36%) isolates from a sample of sands soils, 7 (64%) isolates from clay soils have been identified. Gram-positive,
Andalibi, F et al. Isolation and Identification of Nocardia spp. Partially acid-fast, negative kinyoun acid-fast and resistant to lysozyme broth were identified as the genus Nocardia. Biochemical tests results have been showed in the table1. All isolates were identified as Nocardia asteroides complex due to hydrolysis of casein, tyrosine, hypoxanthine, xanthine, gelatin were negative and also no growth at 45 °C.

**Figure 1.** White colonies suspected of Nocardia on the paraffin rods

**Figure 2.** Colonies suspected Nocardia were cultured from the paraffin rods on the Sabouraud dextrose agar with cycloheximide (See mixed colonies) in order to isolation

**Figure 3.** Pure culture of Nocardia on nutrient agar

**Figure 4.** Left to Right
Urease- negative
Urease- positive

**Figure 5.** Left to Right
Citrate- negative
Citrate- positive
Discussion

In current study Nocardia identification was performed of soil samples from North Khorasan province. The results are show that the frequency of Nocardia from soil samples of North Khorasan province is 7%. Nocardia species are soil saprophytes. They are able to causing the diseases with a wide range in healthy individuals and especially in patients with immune system disorder (1-6). The isolation of the genus Nocardia is a difficult, because is bacterium with growth of slowly. One of the best suitable methods for Nocardia isolation from poly-microbial samples is paraffin baiting technique (15). Gordon and Hagan were offered this method in 1936 and developed by McClung in 1960 (19). Based on the results of previous studies, prevalence of Nocardia species from the soil in various locations are different which the range of 5-50% has been reported (20-23). In India, the frequency of Nocardia from soil has been estimated approximately 8% (22). Van Gelderen de et al. identified Nocardia from soil in Argentina. The most abundant of the known species were Nocardia brasiliensis, Nocardia asteroides and Nocardia caviae, with a frequency of 85%, 9%, 6% respectively. These various results in literatures are depending on the geographical location, regional climate and soil types (8, 9).

Conclusion

Due to pathogenicity and species diversity of Nocardia, using phenotypic tests are essential for identification of Nocardia species. Isolation and identification of Nocardia spp. from soil of different regions can help to enhance our understanding of epidemiological and ecological of the pathogenic Nocardia species.

Acknowledgments

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Conflict of interest

None declared conflicts of interest.
**Table 1.** The results of biochemical tests of isolates of *Nocardia*

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<td>Color of colonies</td>
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<td>Hypoxanthine</td>
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