



## 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:** 11 *Nocardia* species (7%) were identified. 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.

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## 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*

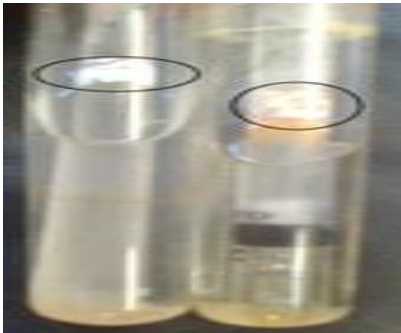
1 gram of soil was added to 10 mL sterile distilled water and shaken for 10 min. After precipitation, 1 ml of the supernatant was added to a tube containing 7 ml of carbon-free broth. Paraffin rods were added to each tube and were incubated for 4 weeks at 35°C (17). After isolation and purification of *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, 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.



**Figure1.** White colonies suspected of *Nocardia* on the paraffin rods



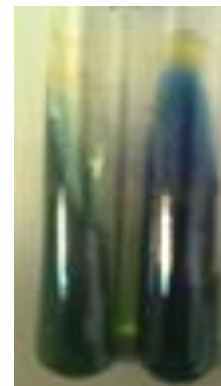
**Figure2.** Colonies suspected *Nocardia* were cultured from the paraffin rods on the Sabouraud dextrose agar with cycloheximide (See mixed colonies) in order to isolation



**Figure3.** Pure culture of *Nocardia* on nutrient agar



**Figure4.** 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.

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## Conflict of interest

None declared conflicts of interest.

**Table 1.** The results of biochemical tests of isolates of *Nocardia*

Bacterial isolates	1	2	3	4	5	6	7	8	9	10	11
<b>Biochemical tests</b>											
Lysozyme	+	+	+	+	+	+	+	+	+	+	+
Color of colonies	White	White	White to Brown	Orange	White	White	White	White to Brown	White	White	White
Casein	-	-	-	-	-	-	-	-	-	-	-
Tyrosine	-	-	-	-	-	-	-	-	-	-	-
Hypoxanthine	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+
Growth at 45C <sup>o</sup>	-	-	-	-	-	-	-	-	-	-	-
Hydrolysis of Gelatin	-	-	-	-	-	-	-	-	-	-	-
Citrate	+	+	-	+	-	+	+	+	+	+	+
urease	+	+	+	+	+	-	-	+	+	+	+
Acid production of a-L-Rhamnose	-	-	-	-	-	-	-	-	-	-	-
D-Sorbitol	-	-	-	-	-	-	-	-	-	-	-
D-Xylose	-	-	-	-	-	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	-	-
Sucrose	+	-	-	-	-	-	-	-	-	-	-
D-Lactose	-	-	-	-	-	-	-	-	-	-	-
L-Arabinose	-	-	-	-	-	-	-	-	-	-	-
Glucose	-	-	-	-	-	-	-	-	-	-	-
Maltose	-	-	-	-	-	-	-	-	-	-	-
D-Galactose	-	-	-	-	-	-	-	-	-	-	-
Salicin	-	-	-	-	-	-	-	-	-	-	-
Xanthine	-	-	-	-	-	-	-	-	-	-	-

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