

# *Nocardia asteroides* Complex in Patient with Symptomatic Pulmonary Nocardiosis

\*S Eshraghi, M Amin

Dept. of Microbiology, School of Public Health, Tehran University of Medical Sciences, P.O. Box: 6446-14155, Tehran, Iran.

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## ABSTRACT

Pulmonary nocardiosis is an acute or suppurative chronic disease, caused by aerobic actinomycetes which usually originates from soil and compost. Since *Nocardia* species have been isolated from the soil of different regions of Iran, as well as different cases of nocardiosis, this study was undertaken to evaluate the role and frequency of *Nocardia* in pulmonary infections. The present investigation was planned to isolate *Nocardia* bacteria from immunocompromised patients who had been referred to Maseeh-e-Daneshvari University Hospital (Tehran). 142 patients with advanced symptomatic pulmonary disease were studied during a period of seven months. Of all the patients surveyed, 102 were tested both for their BAL (bronchoalveolar lavage) and sputum. For the rest of the patient's sputum samples were not accessible. From each sample, three thin smears were prepared for microscopic observations. The samples were cultured in Sabouraud dextrose agar and paraffin agar. Medical history of patients were also recorded in the certain questionnaire for further data analysis. *Nocardia asteroides* was isolated from only a patient suffering from Cushing's syndrome with bronchogenic carcinoma [Ectopic adrenocorticotrophic hormone (ACTH) syndrome]. Further *in-vitro* investigation for differentiation of the isolate was performed and confirmed that the organism that grew on primary media was *Nocardia asteroides* complex. Our results revealed that the normal concentration of NaOH (4%), which is routinely being used for identification of *Mycobacteria* species, could inhibit the growth of *Nocardia*. Therefore, decontamination procedure of the samples collected for isolation of *Nocardia* was preformed using 1% NaOH in this study.

## INTRODUCTION

*Nocardia*, a gram positive variably acid-fast aerobic bacterium is an opportunistic pathogen in immunocompromised hosts (10,14,17). All species of *Nocardia* are found in nature in the soil and aquatic environments (2,4). A variety of *Nocardia* species have so far been identified (13). Of these, four species have pathogenic potential to human, including *Nocardia asteroides*, *N. brasiliensis*, *N. otitidiscaviarum*, and *N. transvalensis* (11,32,33). Recent taxonomic studies of *Nocardia asteroides* taxon, have classified these species together with *N. farcinica* and *N. nova* as *Nocardia asteroides* complex (1,17,26,29).

Pulmonary nocardiosis mimics pulmonary tuberculosis in clinical symptoms and radiological characteristics, and it is often wrongly treated with anti-tuberculosis drugs (9, 22). Systemic immunosuppression, especially cell mediated immunity dysfunction is an important predisposing factor in Nocardial attack to the lung which mostly occurs in kidney, heart, liver and lung graft recipients (20, 23, 31), and bone marrow transplantation (30). In non-immunocompromised patients the disease has also been recorded (3,6,24). Previous studies have demonstrated nocardiosis in variety of disorders including systemic lupus erythematosus (2,16), HIV (5,15,25), chronic myelogenous leukemia with lung carcinoma (21), and repeated pulmonary infection by *Nocardia asteroides* complex in a patient with bronchiectasis (6).

The purpose of this study was to isolate *Nocardia* bacterium from patients suffering pulmonary infectious diseases, to characterize the isolated *Nocardia* at the species level, and to compare the isolated bacteria from sputum and BAL samples. The effect of various concentrations of NaOH on the ability to isolate the organism was also investigated.

## MATERIALS AND METHODS

### Clinical Samples

142 patients who suffered from pulmonary infectious diseases (between January 1998 and August 1999), were selected at Maseeh-e-Daneshvay Hospital in Tehran. This group of patients had bronchoscopic indexes like, radiological abnormalities, atelectasis, progressive pneumonia, hemoptysis, and pleural effusion. Certain questionnaires were filled out for every patient at the beginning of the investigations. Personal specifications, medical history and symptoms of the patients were included in these questionnaires. Two sputum and BAL samples were collected from the patients. The patients were fully instructed to submit their sputum deeply from the interior parts of their trachea. Since decontamination procedures may have inhibitory effects on the growth of *Nocardia*, the samples were not treated with any chemical substances. For BAL samples, centrifugation was required in order to concentrate the lavage. In this regard, BAL samples were centrifuged for 10 minutes (1500 rpm). Supernatants were discarded and examinations were carried out on the deposited materials.

### Direct Microscopy and Culture

Three thin smears were prepared from each sample for Gram and Kinyoun staining. Staining procedures were done as described (14,18). Sample cultures were done on Sabouraud dextrose agar and paraffin agar. Inoculated media were incubated at 37°C and 45°C. It is believed that 45°C temperature enhances the chance of recovering *Nocardia* species (18, 33). Suspected colonies were then cultured on blood agar in order to observe the typical colonies.

### Laboratory Diagnosis

After the microscopic and macroscopic observations confirmed the existence of *Nocardia* differential tests; hydrolysis of

\*Corresponding author, Tel: +98-21- 6402751; Fax: + 98-21- 6422647; E-mail: eshraghi@spttums.com

substances e.g. adenine, casein, esculin, gelatin, hypoxanthine, tyrosine and xanthine agar, along with the tests for ability of organisms to utilize arabinose, galactose, inositol, mannitol, rhamnose as sole carbon sources were performed. Standard *Nocardia asteroides* organisms were suspended in saline and mixed for 1 minute with vortex mixer. Clumps of organisms were adjusted to a turbidity equivalent to Mc Farland 0.5 standard. Each bacterial suspension was treated for 15 min. with 0.5, 1, 2, 4, and 6% concentrations of NaOH containing phenol red. After 15 min. suspensions were neutralized with HCl and 0.1 ml of each dilution was sub-cultured onto Sabouraud dextrose agar plates, and was incubated in 37°C for 48 hours.

**RESULTS**

One hundred and forty two patients were studied during a period of seven months. Out of the patients, 102 of them were tested both for their BAL and sputum. For the rest of the patients (40 cases) sputum samples were not accessible. The microbial diagnosis was established after isolating *Nocardia* in (BAL) and sputum samples. A single species of *Nocardia* was isolated from only one patient who suffered from Cushing's syndrome with bronchogenic carcinoma (ectopic syndrome ACTH). The isolate was Gram positive, partially acid fast with fine branched rod-shaped elements. The differential tests on adenine, casein, esculin, gelatin, hypoxanthine, tyrosine,

xanthine agar, and the ability of organisms to utilize arabinose, galactose, inositol, mannitol, rhamnose as sole carbon sources along with microscopic observation confirmed that the organism grew on primary media (Table 1) was *Nocardia asteroides* complex. Fig. 1 shows the age group distribution of patients and Fig. 2 shows distribution of the patients by high-risk and immunocompromised disorders. The adverse effects of decontamination procedure with different concentrations of NaOH were tested in this study. There is controversy over using chemical substances like NaOH, N-acetyl-L-Cysteine and benzylammonium chloride in tri-sodium phosphate (Zephiran – TSP ) for decontaminating clinical specimens from the organisms other than *Nocardia* (19). In this regard, an experiment was performed with serial dilution of NaOH. The results obtained from this study revealed that low concentrations of NaOH could inhibit the growth of *Nocardia*, so that NaOH could not be a reliable substance for decontamination purposes of clinical specimens. Growth of *Nocardia* in concentrations above 1% NaOH was inhibited in this study.

Fig. 1. Patient's age group distribution

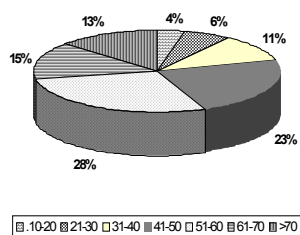


Fig. 2. Relative distribution of 142 High-risk and immunocompromised patients

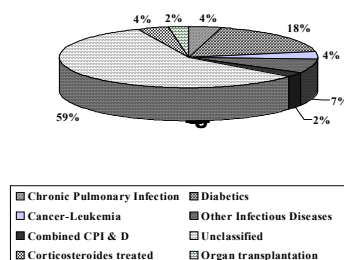


Table 1. The differential tests on isolated *Nocardia* strain and standard bacteria

Character	Isolated <i>Nocardia</i>	<i>Nocardia asteroides</i>	<i>Nocardia nova</i>	<i>Nocardia brasiliensis</i>	<i>Nocardia caviae</i>	<i>Nocardia caviae</i>	<i>Nocardia transvalensis</i>
<b>Hydrolysis of:</b>							
Adenine	-	-	-	-	-	-	V
Casein	-	-	-	-	+	-	-
Esculin	-	-	-	-	+	-	-
Gelatin	-	-	-	-	+	+	+
Hypoxanthine	-	-	-	-	+	-	-
Tyrosine	-	-	-	-	+	±	±
Xanthine	-	-	-	-	-	V	V
<b>Utilization of:</b>							
Arabinose	-	-	-	-	-	-	-
Citrate	+	+	-	-	+	-	+
Galactose	+	+	+	+	+	+	+
Inositol	-	-	-	-	+	+	+
Mannitol	-	-	-	-	+	V	V
Rhamnose	-	-	+	-	-	-	-
<b>Growth at:</b>							
45°C	-7	±	+	-	-	±	-

The patient was a 43-year-old man presented with dyspnea one month before his admission in our Hospital with fatigue and weakness. He claimed that he has had sever weakening on his feet for a year. Diabetes was also included in his medical history. Endocrine work up was carried out and the results suggested the presence of Cushing's syndrome. Low and high dose dexamethasone suppression revealed that a high level of plasma cortisol was not suppressed with high dose of dexamethasone. Radiographic findings showed a well defined nodular infiltrates involving the entire left lung. Because of normal ACTH levels, hypercortisolism from ectopic ACTH-production tumor was suspected. Pathological reports came up the idea that the patient was bearing bronchogenic carcinoma. Further investigations confirmed the presence of Ectopic ACTH syndrome.

## DISCUSSION

The Ziehl-Neelsen (ZN) stain is important in identifying organisms that are acid fast, in principal *Mycobacterium tuberculosis*. However, decolorization with a weaker acid concentration (for example 1% sulfuric acid), can result in a wider variety of organisms appearing acid fast (22). The partially acid fastness property of the family of Nocardioform is upon the mycolic acid present in cell wall of the bacteria. When *Nocardia* bacterium invades the body, the number of carbon molecules of the cell wall is increased to protect the bacterium against the immune system. In this condition the mycelium of partial acid-fast stained *Nocardia* would appear as pink bacilli or filamentous under light microscope. This feature which has also been considered in this study, is a helpful differential characteristic differentiating *Nocardia* from morphologically similar organisms such as *Streptomyces* and *Actinomyces* species. After few sub-culturing of *Nocardia*, the partial acid-fastness property would disappear and it is difficult to identify the bacterial element or mycelia in the smear.

The present study was undertaken to isolate *Nocardia* bacterium from pulmonary nocardial cases having chronic chest symptoms. All the patients who had negative sputum for ZN on direct smear examination consecutively were investigated for *Nocardia* by examining the sputum and also in broncho-alveolar lavage liquid obtained by bronchoscopy. The smears were submitted to the Kinyoun method staining (Modified Ziehl-Neelsen). There was only one positive case of nocardial infection who suffered from Cushing's syndrome with bronchogenic carcinoma (Ectopic ACTH syndrome). In a similar study in Japan, a case of pulmonary nocardiosis was admitted for further investigation of multiple nodules which was disclosed by a chest roentgenogram. It was then diagnosed as adrenocorticotrophic hormone (ACTH)-dependent Cushing's syndrome. Bronchial secretion samples obtained by fiberoptic bronchoscopy contained numerous *Nocardia asteroides* bacteria (7).

It is believed that corticosteroid therapy is a precondition for opportunistic *Nocardia* (8, 28). On the basis of this idea it is conceivable that the patient developed nocardiosis in the presence of prolonged endogenous hypercortisolism, since there were no other predisposing factors. High concentration of cortisol decreases the number of eosinophiles and lymphocytes in the blood, such phenomenon would occur within few minutes and would be worsening after a couple of hours (12). Further, the administration of large doses of cortisol causes sever atrophy in all the lymphoid organs of the body, which in turn decreases the output of both T cells and antibodies production (12). Therefore, humoral immunity could not

be strong enough to defend against opportunistic agents like *Nocardia* bacterium.

In the present investigation the microscopic observation and differential tests on different substances, confirmed that the organism which grew on primary media was *Nocardia asteroides* complex. The bacteria can be differentiated from *Nocardia farcinica* and *Nocardia nova* through the pattern of rhamnose, citrate and the growth at 42°C (Table1).

We have demonstrated that identification of *Nocardia* bacterium in the BAL was successful. Although sample collection was performed by an expert, but the sputum specimens may be free of *Nocardia* due to localized pulmonary infection. In other words, if the infection is not penetrated through pulmonary bronchiole, there is no chance for the bacteria to be trapped. Collecting the sputum samples together with BAL from each patient would increase the chance of isolating *Nocardia*, but the sputum itself must be collected from deep chest by strong coughing. Although identification of *Nocardia* needs a series of laboratory investigations, observing the partially acid fast stained smears is very helpful.

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