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Research Article

**EFFECTS OF CUSHING SYNDROME IN PULMONARY
NOCARDIOSIS**¹Anum Saleem, ²Dr. Bushra Rafique, ³Dr. Ali Saeed¹Indus Hospital Sabzazar Lahore²WMO, BHU 147/148 NB, Sillanwali, Sargodha³District Headquarter Hospital, Kasur**Abstract:**

Objective: *Nocardia species is comprised of strict aerobic bacilli which forms the branched hyphae in culture and tissues. Nocardia infection is not well-known but its importance cannot be underestimated due to the mortality and morbidity in organ transplant cases and immunocompromised cases. As Nocardia species is varying in the various regions, this research is targeted on the study an isolate Nocardia bacteria from immune-compromised cases which were referred to the specialists.*

Setting and Duration: *The research was carried out in Nishtar Hospital Multan with 142 participants observed with advanced symptomatic pulmonary disease. Research was completed in the time span of 7 months.*

Method: *In the total research sample 102 cases were assessed for sputum and Broncho alveolar lavage. In the remaining cases the collection of samples was not possible because of the accessibility issue. We prepared three thin smears to observe the samples through microscopic. Culturing of the samples was made in paraffin agar and sabouraud dextrose agar. To analyze the data, we also documented history of the patients through questionnaire. Asteroids of the Nocardia were isolated from one case who was suffering from the syndrome of Cushing having bronchogenic carcinoma. Further in-vitro studies for isolate differentiation were carried out in order to confirm the growth of organism on primary media which was Nocardia steroids complex. **Conclusion:** *It is revealed through NaOH normal concentration 4%, which has been used for the Mycobacteria species identification, can stop the Nocardia growth. Therefore, collected samples were processed through decontamination procedure to isolate Nocardia with the help of NaOH (1%).**

Key Words: *Pulmonary Nocardiosis, Cushing's syndrome, Nocardia asteroids complex and immunocompromised host.*

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INTRODUCTION:

Nocardia, is a gram positive acid-fast aerobic variable bacterium is an opportunistic pathogen in immune-compromised hosts. Nocardia species are available in nature like soil and water borne environments. Nocardia species in various varieties have been identified. In these diseases there are many potential pathogenic issues to humans including Nocardia steroids, *N. otitidiscaviarum*, *N. Brasiliense's* and *N. transvalensis*. Current taxonomic Nocardia steroids taxon research studies have categorized this species with the *N. nova* and *N. farcinica* [1].

Pulmonary Nocardiosis impersonates pulmonary TB through clinical investigations and radiological features, it is also treated in a wrong way through anti TB medications. A systematic immuno-suppression, specifically an immunity mediated through cells disorders is counted as the vital Nocardial attack disposal factor which is harmful for the lungs occurring repeatedly in kidneys, liver, heart and lung graft recipients through the transplantation of the bone marrow [2]. In the patients of non-immunocompromised nature, the incidence of disease is also observed. It is demonstrated through all the previous research studies about the nocardiosis in different disorders which include systemic lupus erythematosus, chronic myelogenous leukemia combined with lung carcinoma, HIV and common pulmonary infection caused by the Nocardia steroids complex in the bronchiectasis patients [3].

Our research was aimed at the Nocardia bacterium isolation from pulmonary infectious diseases patients, for the characterization of the isolated Nocardia on the level of the species; furthermore, it was also aimed at the isolation of bacteria from BAL samples and sputum. Various NaOH concentrations effects on the isolation ability of the organism was also under investigation.

PATIENTS AND METHODS:

Clinical samples: Our research included 142 pulmonary infectious diseases patients in the time span of Jan, 2016 – Aug, 2017 in a hospital of Nishtar Hospital Multan. The selected patients were suffering from the indexes of bronchoscope such as radiological anomalies, progressive pneumonia, atelectasis, pleural effusion and hemoptysis. Every patient was asked to fill a questionnaire before the start of the research. Research questionnaire included personal specifications, disease and patient symptoms and medical history.

Two samples of BAL and sputum were collected from every patient. The instructions were passed to every patient that the sample of sputum is to be taken from the deep part of the trachea. Since the procedures of decontamination may pose certain inhibitory effects Nocardia growth, no chemical treatment was given to the samples of the patients. To treat the BAL samples, we required centrifuge for lavage concentration. In order to achieve this ten minutes' centrifuge process was carried out at the rate of 1500 revolution per minute. We discarded supernatants and assessment was carried out in the deposited material.

Direct Microscopy and Culture: We prepared 3 smears (thin) for every collected sample to carry out Kinyoun and gram staining. Processing of staining was carried out as described. Culturing of the samples was completed on paraffin agar and Sabouraud dextrose agar. Injected media were incubated at the temperature of 37°C & 45°C. The recovery of the Nocardia species can be achieved at 45°C. We also cultured the suspected colonies through blood agar for the assessment and observation of the typical colonies.

Laboratory Diagnosis: After micro & macroscopic assessment we observed that Nocardia existence was differential tests; substances hydrolysis such as Adenine, Esculin, Casein, Gelatin, Tyrosine, Hypoxanthine and Xanthine agar, along with ability tests of organisms for the utilization of the Arabinose, Inositol, Glucose, Rhamnose and Mannitol as the sources carbon.

We also suspended standard Nocardia asteroides organism in mixed and saline for a period of one minute with vortex mixer. Organism's clumps were adjusted to turbidity that was equal to the standard McFarland (0.5). We also treated every bacterial suspension for a time of fifteen minutes with (0.5, 1, 2, 4, & 6 percent) NaOH concentrations containing red phenol. After the fifteen minutes suspension the neutralization process was carried out with HC – 1 and (0.1 ml) of each dilution that was subculture through Sabouraud dextrose agar plates and incubation of forty-eight hours on a temperature of 37°C was also carried out.

RESULTS:

We studied 142 cases in the time of 7 months and patients distribution is reflected in Table – I in age wise order. Both sputum and BAL samples and testing was carried out in 102 patients, remaining forty patients were not accessible for the collection of

sputum sample. High risk disorders of immunocompromised have been shown in Table – II.

Table – I:

High risk patients	Frequency Percentage	
Chronic Pulmonary Infection	5	4
Diabetics	22	18
Cancer-Leukemia	5	4
Other Infectious Diseases	9	7.2
Combined CPI & D	3	2.4
Unclassified	71	58
Corticosteroids treated	5	4
Organ transplantation	3	2.4
TOTAL	123	100

Table – II:

Age Grouping	Frequency	Percentage
20 - 20	5	3.5
21 - 30	9	6.3
31 - 40	15	10.6
41 - 50	33	23.2
51 - 60	40	28.2
61 - 70	21	14.8
> 70	19	13.4
Total	142	100

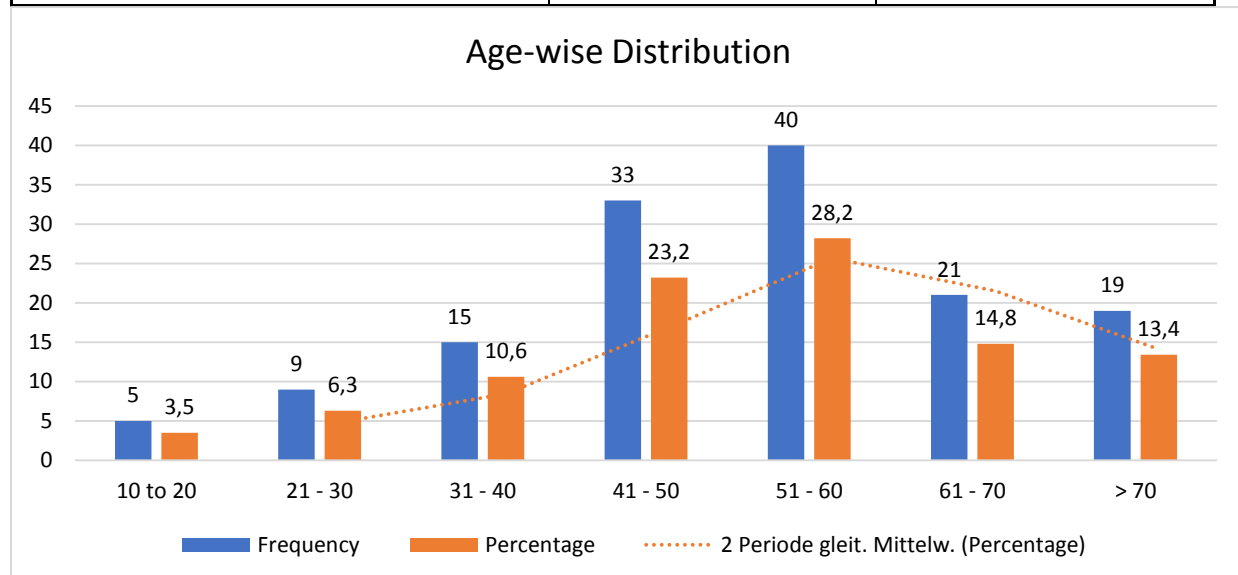


Table – III:

Character	Isolated	Nocardia	Nocardia	Nocardia	Nocardia	Nocardia	Nocardia
	Nocardia	asteroids	farcinica	nova	brasiliensis	caviae	transvalensis
Hydrolysis of: Adenine	-	-	-	-	-	-	v
Casein	-	-	-	-	+	-	-
Esculin	-	-	-	-	+	-	-
Gelatin	-	-	-	-	+	+	+
Hypoxanthine	-	-	-	-	+	-	-
Tyrosine	-	-	-	-	+	±	±
Xanthine	-	-	-	-	-	v	v
Utilization of: Arabinose	-	-	-	-	-	-	-
Citrate	+	+	-	-	+	-	+
Glucose	+	+	+	+	+	+	+
Inositol	-	-	-	-	+	+	+
Mannitol	-	-	-	-	+	v	v
Rhamnose	-	-	+	-	-	-	-
Growth at: 45°C	-	±	+	-	-	±	-

Diagnosis of microbial was made after the isolation of Nocardia in broncho-alveolar lavage (BAL) and in the samples of sputum. Single species isolation was possible through Nocardia in only single case who was affected by Cushing's syndrome having bronchogenic carcinoma (ACTH, ectopic syndrome). The isolate was a gram positive that was also partially fast acid having rod elements which were finely branched. Through differential tests on Casein, Adenine, Esculin, Hypoxanthine, Gelatin, Xanthine and Tyrosine agar, the organism's ability for the Arabinose, Inositol, Glucose, Mannitol, Rhamnose as an alone source of carbon having observations of the microscope which were confirmed through the growth of organism through the Nocardia asteroides as primary media as shown in Table – III.

Decontamination procedure with adverse effects having various NaOH concentrations is also studies in our research. It is controversial to use various substances of chemical as NaOH, L-Cysteine, N-acetyl and benzyl konium chloride in tri-sodium phosphate (Zephiran – TSP) to decontaminate the specimen except Nocardia. For the same purpose we also carried out NaOH serial dilution. The obtained results also revealed that Nocardia growth is inhibited through the NaOH concentrations, which makes NaOH an unreliable substance for the clinical specimen decontamination purpose.

Nocardia growth in NaOH concentrations above (1%) were inhibited as we observed in this research. The dyspnea patient was at the age of forty-three years before being hospitalized felt weakness and fatigue. The patient also shared that severe weakness is observed by him from last one year, he also an incidence of DM. To observe the presence or absence of Cushing's syndrome we carried out Endocrine work. High and low dose dexamethasone suppression helped in the revelation of the plasma high levels with not suppressed cortisol when the dexamethasone dose was high.

Through radiographic observations we observed that entire left lung was involved when used nodular infiltration. Due to the normal levels of ACTH, there was a suspected hypercortisolism from ectopic ACTH tumor production. Through Pathological reports it was evident that patients were facing bronchogenic carcinoma. A deeper investigation also revealed the Ectopic ACTH syndrome presence which was also confirm.

DISCUSSION:

An important identification of the acid fast in organisms can be made through Ziehl – Neelsen (ZN) staining specially in the primary Mycobacterium tuberculosis. However, weaker acid decolorization for the concentration of acid (such as sulfuric acid 1%), can produce variety of the organisms appearing in the acid fast [4]. The fastness of the acid is the

partial feature of Nocardioform family on mycolic acid which is presented in the bacteria cell wall. In case there is an invasion of the Nocardia bacterium in body a number of molecules of the carbon in the walls of the cell increases the protection against the immune system [5]. In this state the mycelium of partial acid-fast stained Nocardia will appear in the shape of filamentous or pink bacilli through light microscope. We have also considered the same feature in this research [6]. Which is also a differential feature that differentiates Nocardia from same natured morphologically organism like Actinomyces and Streptomyces species. After few Nocardia sub-culturing, the partial acid fastness features may disappear and its identification is also difficult through smear for the identification of the mycelia or bacterial element [7].

We undertook the present research for the isolation of the Nocardia bacterium from pulmonary nocardial patients having historical chest symptoms. All negative sputum cases for ZN on direct smear assessment for consecutive probe of the Nocardia through sputum assessment and in bronchoalveolar lavage liquid which is obtained through bronchoscopy [8]. The smears processing was carried out through Kinyoun staining method (Modified ZN). We observed a case of the positive nocardial infection also suffering from the Cushing's syndrome having bronchogenic carcinoma [9].

A similar research was carried out in Japan on the pulmonary nocardiosis patients that was hospitalized to be assessed through multiple nodules; these nodules were disclosed through chest roentgenogram [10]. After that it was identified as the adrenocorticotropic hormone (ACTH) which was also depending on the Cushing's syndrome. Bronchial secretion samples obtained through fiberoptic bronchoscopy contained numerous bacteria's of Nocardia asteroides [11].

It is believed about the therapy of corticosteroids it is a pre-condition for the opportunistic Nocardia. We can conceive on the grounds of this idea that patient with the nocardiosis development in prolonged endogenous hypercortisolism, as in the absence of predisposing factors. Number of lymphocytes and eosinophils can be decreased through the high cortisol concentration in the blood [12]. The occurrence of this phenomenon can be obtained in very less time and worst shape can be obtained through the lengthy processing. Furthermore, in the large dose administration of cortisol results in the shape of severe atrophy in all body lymphoid organs, resultantly the outcome of the antibodies and T cells

production decreased. It is therefore the humoral immunity cannot be that much strong that can fight and defend the agents like Nocardia bacterium [13].

We observed in this research that with the help of differential tests and microscopic observation through various substances, it was confirmed that all the growing organism primarily on the media that was Nocardia asteroides complex. Differentiation of the bacteria can be made through Nocardia nova and Nocardia farcinica with the Rhamnose pattern, Citrate and growth at the temperature of 42°C as shown in Table – III [14].

The research demonstrates Nocardia bacterium identification in bronchoalveolar lavage (BAL), which was successful. An expert was performed all the collections of the samples; whereas, the specimen of the sputum may be free of Nocardia because of the pulmonary infection which is also localized. We can also say that infection is not transferred through pulmonary bronchiole [15]. Bacteria trapping is not possible. In the collection of the samples of the sputum with BAL from every patient that will increase the isolating chances of the Nocardia, it is to be confirmed that sputum is sample is to be collected from depth of the chest through strong coughing. Although Nocardia identification also requires laboratory assessment to observe the partial acid fast in the helpful stained smears [16].

CONCLUSION:

It is revealed through NaOH normal concentration 4%, which has been used for the Mycobacteria species identification, can stop the Nocardia growth. Therefore, collected samples were processed through decontamination procedure to isolate Nocardia with the help of NaOH (1%).

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