

Original article:

Pulmonary Nocardiosis Among Suspected Cases of Pulmonary Tuberculosis in Pune, India”.

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Abstract

Introduction: Pulmonary nocardiosis (PN) is a severe infection, infecting both immunocompromised and immunocompetent patients. It mimicks tuberculosis of lung or can occur along with tuberculosis as co-infection non-responding to anti tuberculosis treatment. Therefore, diagnosis at the early stage of the disease could be lifesaving.

Objectives: The goal of this study was to assess the frequency of pulmonary nocardiosis in the area where tuberculosis is endemic along with its associated risk factors

Patients and Methods: A total of 926 patients, who were suspected of having pulmonary tuberculosis infection based on their symptoms and radiological findings, attending tertiary care hospital during the period from July 2012 to June 2013 were included in this study. Their respiratory samples were processed for both Nocardia and tuberculosis examined by Gram and Ziehl-Neelsen staining, culture methods and antimicrobial susceptibility testing.

Results: Out of 926 patients, Nocardia was isolated from nine patients (1%). The most common predisposing factor was HIV infection (33.3%). Nocardia asteroides complex was isolated from seven patients (77.7%) and Nocardia nova from two patients (22.2%). All the nine isolates were sensitive to Gentamicin, Amikacin and Trimethoprim/Sulfamethoxazole

Conclusions: Correct early diagnosis and treatment with appropriate drug regimen in seven patients (77.8%) resulted in complete resolution of infection. So high index of suspicion for nocardiosis is must in cases with risk factors non-responding to anti tubercular treatment can be helpful in reducing morbidity and mortality.

Keyword:- Pulmonary nocardiosis, predisposing factors, laboratory diagnosis, Nocardia asteroid complex

Introduction:

Nocardia are aerobic Gram-positive and weakly acid fast filamentous bacteria of the order Actinomycetales that are ubiquitous in nature.^[1] Infections from nocardia are generally acquired from the environment and transmission from person to person has not been reported.^[2] They usually cause an opportunistic infection in immunocompromised hosts especially those who are deficient in cell-mediated

immunity but cases of nocardiosis have also been reported in some immunocompetent individuals.^[3] Manifestations of the disease range from cutaneous infection caused by traumatic inoculation of the organism in a normal host to severe pulmonary or central nervous system (CNS) infections especially in an immunocompromised host.^[4] Among these, pulmonary and cutaneous nocardiosis are the most common presentations. In humans, Nocardia

asteroides complex is the predominant pathogen, but several other species such as *N. brasiliensis* and *N. otitidiscaviarum* have also been reported.^[5] Pulmonary infection is usually produced by *N. asteroides* (85%), whereas *N. brasiliensis* causes cutaneous and subcutaneous abscesses.^[6]

Pulmonary nocardiosis (PN) is an infrequent but severe infection that commonly presents as a subacute or chronic suppurative disease, mimicking pulmonary tuberculosis, lung carcinoma or abscess and is often misdiagnosed as tuberculosis clinically and radiologically unless investigations like gram stain or modified ZN stain are specially performed.^[6] As a result, the diagnosis of nocardiosis can be missed or delayed especially in tuberculosis endemic regions.^[7] To achieve early detection and treatment of nocardia infections, it is important to understanding the burden, risk factors and clinical outcomes of nocardia infections among suspected TB cases. Therefore, the present study was undertaken to assess the frequency of pulmonary nocardiosis among suspected TB cases in western India and to determine the risk factors for this infection.

Material and methods:

Study design and Population. A prospective review of microbiology records was performed at Byramjee-Jeejeebhoy Government Medical College & Sassoon General Hospitals (BJGMC-SGH), Pune, Maharashtra. BJGMC-SGH is a public, teaching tertiary-care facility that serves over seven million populations in western Maharashtra. We extracted data of demographic and clinical characteristics when available from accessible mycobacteriology laboratory records of patients suspected to have TB between July 2012 and June 2013. The BJMC institutional ethics committee approval was obtained.

All patients had been referred from BJMC chest clinic or hospital setting if they were suspected to have TB according to the revised national tuberculosis control programme (RNTCP) guidelines for TB. 926 patients suspected to have TB were referred to the laboratory for investigations over a one year period.

Sputum, gastric aspirate, broncho alveolar lavage (BAL) from suspected TB cases that were received in the mycobacteriology laboratory were processed for both tuberculosis and *Nocardia*. Standard isolation and identification techniques as described by the American Society of Microbiology (ASM) were used for the diagnosis of nocardial infections.^[8] All respiratory samples were microscopically examined for *Nocardia* by Gram's staining and modified Kinyoun's staining techniques (fig 1).^[9] Lowenstein-Jensen (LJ) slopes and Sabouraud's Dextrose agar (SDA) were used for isolation and incubated at 37°C for up-to six weeks and examined every alternate day for any *Nocardial* growth (fig 2).^[10] Identification of nocardia was performed on the basis of microscopic morphology, colonial morphology and biochemical tests (casein, tyrosine, xanthine and urea hydrolysis).^[11] Antimicrobial sensitivity was performed using the Modified Kirby-Bauer's disk diffusion method using discs of Trimethoprim /Sulfamethoxazole (1.25 + 23.75 µg), Gentamicin (10 µg), Tobramycin (10µg), Amikacin (30µg) and Erythromycin (10µg) as per CLSI guidelines (fig 3).^[12] Further *Nocardia* speciation was carried out using Arylsulfatase, growth at 45° C and opacification of Middle brook 7H11 agar (fig 4 & 5)^[11]

Results

Of the 926 patients screened for suspected TB, *Nocardia* was isolated from nine patients, demonstrating a prevalence rate of 1%. Of these, the

prevalence was 1.29% (7/540) in the males as against 0.5% (2/386) in the females. Predisposing risk factors or underlying diseases were noted in six cases (66.6%) while in three cases (33.3%) none were found.

Of these six cases in which predisposing conditions were found, three patients were seropositive for HIV (33.3%), one patient had underlying COPD (11.1%) and two pediatric patients were malnourished (22.2%).

All cultures grew within 8-12 days. Out of nine isolates of Nocardia, seven isolates belonged to Nocardia asteroides complex (77.7%) and two isolates were identified as Nocardia nova (22.2%). The age, sex, clinical diagnosis of patients, type of species isolated and associated risk factors of these nine patients are given in Table No. 1.

All the nine isolates were sensitive to Gentamicin, Amikacin and Trimethoprim /Sulfamethoxazole (TMP-SXT) (Table no.2).

Out of nine cases, six cases were already started on Anti tubercular treatment (ATT) based on signs and symptoms. Two of these patients had completed ATT Cat I but were non responders and two cases which were HIV infected cases (22.2%) died, due to delay in appropriate diagnosis and receiving of correct treatment.

Correct early diagnosis and treatment with proper drug regimen in seven patients (77.8%) resulted in complete resolution of infection.

Discussion: Nocardiosis, once considered to be a rare disease, is often underdiagnosed by clinicians. As clinical manifestation of pulmonary tuberculosis and nocardiosis are similar, specific laboratory tests are required in order to distinguish nocardiosis from tuberculosis. In USA one incidence of nocardiosis is estimated to be 500-1000 of new cases annually.^[13]

However, in India due to under reporting and lack of awareness exact figures are not known.^[14]—In the present study the prevalence of pulmonary nocardiosis was 1% in suspected cases of tuberculosis which was comparable to studies by Bareja et al (1%), Singh M et al (1.4%) and Malik A et al (1.5%).^[10] Another study from Shimla by Gupta ML et al, (1991) reported slightly higher rates (2.9%) of pulmonary nocardiosis from patients of pulmonary tuberculosis.^[15] A prospective study conducted by Koffi N recruiting 120 patients admitted to the chest department in a French hospital has shown the prevalence of pulmonary nocardiosis to be 4.2%.^[16] Isolation of Nocardia was higher in males (1.29%) than in females (0.5%) in the present study. Many different studies have given similar findings.^[17-19] This predominance in the male sex cannot be explained however researchers believe that this could be due to hormonal effects on the virulence of organisms.^[20]

Pulmonary nocardiosis can occur in both immunocompetent and immunocompromised patients but is usually more common in immunocompromised patients with lymphoreticular malignancies, Cushings disease, organ transplant recipient, steroid therapy, chronic alcoholism, diabetes mellitus and AIDS.^[21]In this study predisposing factors were found in 6 patients (66.6%) and three patients (33.3%) had no risk factors. Among patients with risk factors HIV infection (33.3%) was most common, followed by malnutrition (22.2%) and chronic obstructive pulmonary disease (COPD) (11.1%). In one observational study on Pulmonary nocardiosis over a 13 year period, specific risk factors were seen in 94% of cases and the most common being prolonged steroid therapy (64.5%) followed by immune suppression in transplant patients(29%), COPD

(23%), HIV infection (19%) and alcoholism (6.5%).^[22] A review of Nocardiosis patients in a large hospital in South Florida over 6 years has described 25 cases of which 76% of them were infected with HIV.^[23] So in cases where pneumonia is not responding to treatment and there is presence of any of the above mentioned risk factors pulmonary Nocardiasis should be considered.

In the present study *N. asteroides* complex was isolated in 77.7% of cases. In the study by Bareja R et al (10), *N. asteroides* was the only species that was isolated. Some other workers also have also reported *N. asteroides* as the only isolate in their patients.^[24,25-26]

In the present study *Nocardia nova* was isolated from two cases. Other *Nocardia* species isolates reported from Nocardiosis are *N. brasiliensis*, *N. farcinica* and *N. pneumoniae*.^[27,28]

The treatment of choice for this infection includes sulphonamides and, more recently, TMP-SMX(13).

In the present study all isolates were sensitive to TMP-SMX. However, resistance with TMP-SMX has been reported in a study by Bareja R et al in two out of seven cases (29%). In their study three patients treated with TMP-SMX that showed in vitro susceptibility developed a local spreading or disseminated infection, with a fatal outcome in two cases. In the present study seven patients responded well to appropriate therapy and two cases who were HIV positive succumbed due to delay in starting appropriate therapy.

Conclusion: To conclude, pulmonary nocardiosis resembles tuberculosis. The first line anti-tuberculous drugs are not effective in treating Nocardiosis. Therefore, it is important to establish early diagnosis to start correct treatment especially in non responding patients with risk factor, for reducing morbidity and mortality in them. It would also help in reducing the unnecessary use of empirical anti tubercular therapy, which could be ineffective and potentially harmful.

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Table.no 1. Distribution of pulmonary nocardiosis according to age, sex, clinical diagnosis, risk factor, species isolated and outcome

Sr. no	Lab. no	Age/ Sex	Species	Sample	Clinical Diagnosis and treatment	Risk factors	Outcome
1	144	25yrs/M	N. asteroides VI	Sputum	Pulmonary tuberculosis on anti tubercular therapy	ICS	Expired
2	240	17yrs/M	N. nova	Sputum	Pulmonary tuberculosis Cat I antitubercular therapy completed-non responder	-	Survived
3	256	2yrs/M	N. asteroides VI	Gastric aspirate	Pulmonary tuberculosis Cat I antitubercular therapy completed non responder	-	Survived
4	326	1.5yrs/M	N. asteroides VI	Gastric aspirate	Lower Respiratory Tract Infection	Malnutrition	Survived
5	501	27yrs/F	N. asteroides I	Sputum	?MDR Pulmonary tuberculosis on anti tubercular therapy since 6 months	-	Survived
6	581	2 yrs/F	N. asteroides I	Gastric aspirate	cough, fever since 1mnth, Lower Respiratory Tract Infection antibiotics	Malnutrition	Survived
7	629	50 yrs/M	N. asteroides VI	BAL	COPD with c/o chronic cough with expectoration, Antibiotics	COPD	Survived
8	1104	3 yrs/M	N. asteroides IV	Gastric aspirate	Pulmonary tuberculosis on anti tubercular therapy	ICS	Survived
9	1234	34 yrs/M	N. nova	Sputum	Pulmonary tuberculosis on anti tubercular therapy	ICS	Expired

Table no. 2. Biochemical Reactions of N asteroids complex and N nova

Species	Isolates	Aryl sulphatase	Growth at 45°C	Opacification of 7H11A	SXT-TMP	Gentamicin	Tobramycin	Amikacin	Erythromycin
N. asteroids IV	4	-	+	-	S	S	S	S	R
N. asteroides I	2	-	-	-	S	S	R	S	R
N. asteroides VI	1	-	-	-	S	S	S	S	R
N. nova	2	+	-	-	S	S	S	S	S

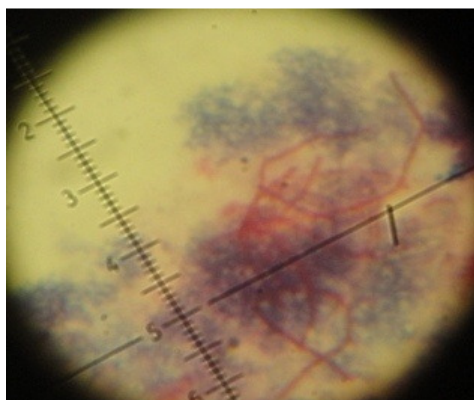


Figure 1. Modified Kinyoun's staining showing Acid fast branching filamentous bacteria



Figure 2. Growth LJ and SDA slope



Figure 3 Antibiotic susceptibility test



Figure 4. Arylsulfatase test



Figure 5. Opacity testing on Middlebrook 7H11 agar