

Detection of Pulmonary Nocardiosis Mimicking Tuberculosis: Role of Sputum Microscopy

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ABSTRACT

Background: Nocardiosis is an opportunistic infection and most commonly presents as pulmonary disease. Unless investigations like Gram's stain and modified acid-fast stain are specially done, pulmonary infection may be mistaken for tuberculosis. They are useful for early diagnosis of Nocardia infection. The present study was conducted for the early detection of pulmonary nocardiosis mimicking tuberculosis by sputum microscopy with Gram staining, modified Ziehl-Neelsen (ZN) staining. **Material and methods:** The current study was conducted on 2,466 sputum samples over a period of 4 years from July 2011 to June 2015 for ZN staining for evaluation of pulmonary tuberculosis. Those sputum samples which were negative with 20% H₂SO₄ and showed weakly stained acid-fast bacilli (AFB) were confirmed for Nocardia by modified ZN staining with 1% H₂SO₄, Gram's staining and culture. **Result:** Out of 2,466 sputum samples, 433 (17.55%) were found positive for AFB by microscopic examination. Ten (2.30%) out of 433 cases were positive for Nocardia. Amongst the Nocardia positive samples, 7 (70%) were of male and 3 (30%) were of female. **Conclusion:** Gram's staining and modified acid-fast stain are useful for early diagnosis and appropriate treatment of pulmonary nocardiosis.

Keywords: Pulmonary nocardiosis, sputum microscopy, modified Ziehl-Neelsen staining

Nocardiosis is a rare disorder caused by Gram-positive, weakly acid-fast, filamentous aerobic actinomycetes, which tends to affect the lung, brain and skin. The genus *Nocardia* belongs specifically to the family Mycobacteriaceae and contains tuberculostearic acid but differ from the mycobacteria by possession of shorter-chained (40- to 60-carbon) mycolic acids.¹

Pulmonary nocardiosis is the most common clinical presentation and has several features similar to tuberculosis. It is a major cause of morbidity and mortality in immunocompromised patients.² Lack of suspicion, nonspecific clinicoradiological presentation, diagnostic intricacies and lack of systematic reporting are the probable reasons that have hindered the true

estimation of its incidence, worldwide.³ In tuberculosis endemic countries like India, nocardiosis should always be excluded among patients not responding to antitubercular treatment. Early recognition and appropriate individualized treatment is the key to a successful outcome.² New methodologies were developed for the identification of *Nocardia*, but evaluation of appropriate specimens by smear and culture remains the principal method of diagnosis.^{4,5} Unless investigations like Gram's stain and modified acid-fast stain are specially done, pulmonary infection may be mistaken for tuberculosis and are useful for early diagnosis of *Nocardia* infection.⁶ Hence, the present study was conducted for the early detection of pulmonary nocardiosis mimicking tuberculosis by sputum microscopy with Gram staining, modified Ziehl-Neelsen (ZN) staining.

AIM

Early detection of pulmonary nocardiosis mimicking *Mycobacterium tuberculosis* by sputum microscopy.

MATERIAL AND METHODS

The current study was conducted on 2,466 sputum samples which were received in the Microbiology Department of a tertiary care hospital in North India,

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over a period of 4 years from July 2011 to June 2015 for acid-fast bacilli (AFB) by ZN staining for evaluation of pulmonary tuberculosis.

All the samples were processed in laboratory without delay in order to avoid contaminants to grow. Homogenization of sputum samples were done by Petroff's method.⁷ Next, concentrated specimens were used for smear preparation and culture. Few slides on ZN staining showed weakly stained AFB with some filamentary structure. *Nocardia* spp. are acid-fast and can survive the decontamination of clinical specimens with sodium hydroxide method.⁸

These patient's sputum samples were repeated with another two fresh sputum samples to exclude *Nocardia* by doing Gram's staining and modified ZN staining⁹ using 1% H₂SO₄. On microscopic examination, Gram-stained smear showed thin, delicate, weakly to strongly Gram-positive, irregularly stained or beaded branching filament and Modified ZN staining revealed filamentous acid-fast organism suggestive of *Nocardia*.

Further 250 µL of each concentrated sputum specimen suspected to be *Nocardia* was inoculated onto Sabouraud's dextrose agar (SDA) media and blood agar. All cultures were incubated at 37°C with 5% CO₂ and humidity. Cultures were examined daily for the growth of *Nocardia* species for up to 3 weeks. On SDA, colonies were white to brown in appearance and on blood agar, filamentous colonies having chalky-white or cotton-ball appearance were seen.⁴ Colonies were examined with Gram staining and modified ZN staining methods to confirm the growth of *Nocardia* (Fig. 1).

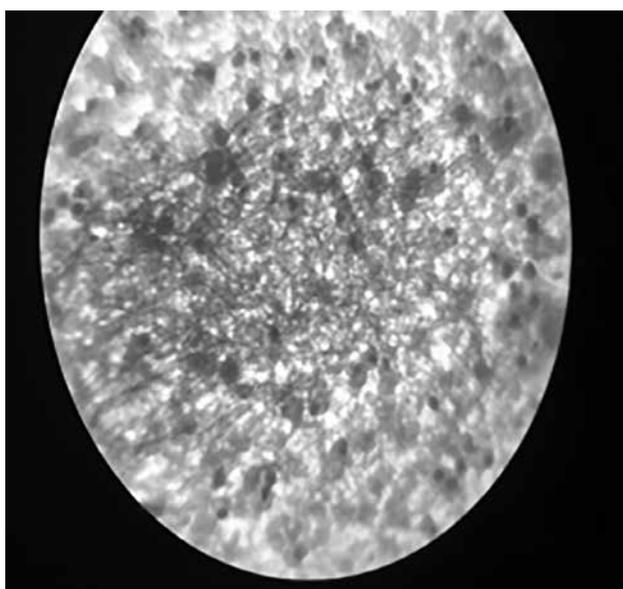


Figure 1. Microscopic picture of *Nocardia* (Gram's staining).

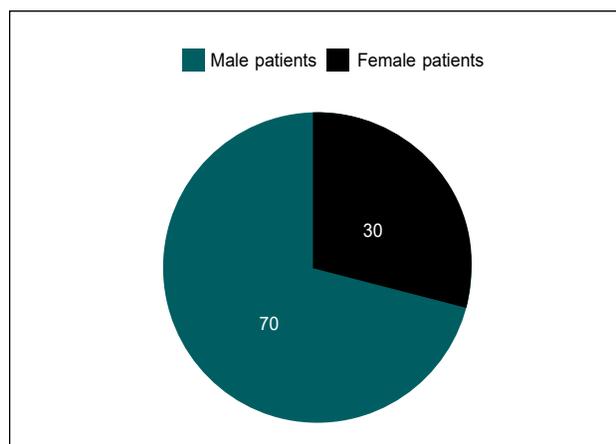


Figure 2. The percentage of *Nocardia* positivity in patients.

RESULTS

Two thousand four hundred sixty-six samples from patients of all ages and both sexes were studied during a period of 4 years (July 2011 to June 2015). Out of the total, 73.52% were males and 26.48% were females. Of the total cases, 433 (17.55%) were found positive for AFB by microscopic examination. Ten (2.30%) out of 433 cases were positive for *Nocardia*, which were confirmed by Gram's staining, modified ZN staining and culture. Out of these 10 cases, 70% were males and 30% were female cases (Fig. 2).

DISCUSSION

Pulmonary nocardiosis is the most common clinical presentation of nocardial infection because inhalation is the primary route of bacterial exposure.¹⁰ It can be fatal if untreated. Untreated pulmonary nocardiosis is similar to tuberculosis and *Nocardia asteroides* is the most frequent cause of pulmonary infection in humans (85%).¹¹ In the present study, we included 2,466 sputum samples, out of which 73.52% were from males and 26.48% were from female patients, whereas a similar study done by other workers included 44.8% sputum samples of male and 55.2% sputum samples of females.¹² Lungs are the most common site of involvement for tuberculosis and *Nocardia*.¹³ In our study, 17.55% sputum samples were found positive for AFB, whereas other workers reported 7.6% positivity by microscopy examination.¹⁴ Pulmonary nocardiosis is the most common clinical presentation of infection and can occur in persons of all ages, even neonates.¹⁰

In our study, out of 433 AFB positive sputum samples, 10 (2.30%) were positive for *Nocardia*, whereas other workers reported it to be 3.57%,¹⁵ which was confirmed

by Gram's staining, modified ZN staining (1% H₂SO₄) and by culture. Nocardia incidence in pulmonary disease has been reported to be 1.4%, 2.7% and 4% by different workers in different areas.¹⁶⁻¹⁸ McNeil and Brown highlighted the importance of direct microscopic examination because, despite the new methodologies developed, there is no test replacing it.⁴ Amongst the Nocardia positive samples, 70% samples were of male patients and 30% were of female patients almost similar results were reported by other workers also (75% and 25%, respectively).¹⁹

The exact reason for gender difference is not known, though hormonal effect may be attributed to virulence or growth of Nocardia species.¹⁵ In India, the prevalence of Nocardia as reported in 1973 was 4.6% among patients who were suspected to have tuberculosis.²⁰ The clinical diagnosis of nocardiosis is difficult. Signs, symptoms and radiologic studies may suggest the diagnosis but are not pathognomonic. Serologic diagnosis is unreliable, and serologic tests are not available commercially,⁵ so isolation and identification of the organism from the clinical specimens form the backbone for diagnosis of pulmonary nocardiosis.

CONCLUSION

This study highlights the importance of nocardiosis in differential diagnosis of pulmonary disease patients. The initial diagnosis of pulmonary nocardiosis requires fast and accurate methodology for early recognition and appropriate individualized treatment due to the clinical aspects and bacteriologic similarity to the genus Mycobacterium. The direct microscopic examination of sputum specially, with Gram's stain and modified acid-fast stain with 1% sulfuric acid highlighted the importance in early diagnosis despite of the development of new methodologies. Hence, a microbiologist should process the respiratory tract specimens not only for Mycobacteria but should also be alert to the fact that organisms such as Nocardia may cause pulmonary infection.

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