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RESEARCH ARTICLE

Epidemiology of Nocardiosis -A six years study from Northern India

Reetika Dawar[1], Ruchi Girotra[1], Seema Quadri[1], Leena Mendiratta[1], Hena Rani[1], Firdaus Imdadi1, Avdesh Bansal[2], Raman Sardana[1]

[1] Department of Microbiology & [2] Department of Respiratory, Critical care &Sleep Medicine,

Indraprastha Apollo Hospitals, New Delhi, India

ABSTRACT

Objective: To isolate and speciate *Nocardia species* from clinical samples and to study their antimicrobial susceptibility pattern to different antimicrobials. Various risk factors associated with nocardiosis were also studied.

Methods: 32 clinical specimens with clinical history of pneumonia, abscesses, or disseminated infections were collected over a period of 6 years (2009-2014) from Inpatient and Outpatient departments and processed for *Nocardia* cultures and sensitivity.

Results: Twelve cases of nocardiosis were reported out of 32 clinically suspected cases. The mean age of presentation in our study was 57.9 years. Pneumonia was the most common clinical presentation followed by primary cutaneous disease and one case of disseminated disease. 8/ 10 patients with nocardiosis were immunocompromised with history of organ transplantation, use of immunosuppressive agents or steroids. Based on biochemical reactions 5 of the isolates were identified as *N. asteroides*, 3 *N. brasiliensis*, 2 N. *farcinica* and 1 each were *N. transvalensis*, & *N. nova*. All were sensitive to linezolid followed by cotrimoxazole (91.6%)

Conclusions: With increasing number of immunocompromised patients and an increased incidence of nocardiosis, diagnosis of *Nocardia* infections should always be kept in mind as it can present with nonspecific symptoms and can mimic confused with other diseases. Linezolid, Cotrimoxazole, imipenem and minocycline were found to be very effective, in vitro, against most *Nocardia* species. *J Microbiol Infect Dis 2016;6(2): 60-64*

Key words: Nocardiosis, pulmonary, Incidence, India, resistance, immunocompromised, bacterial infections

Nokardiyoz Epidemiyolojisi – Kuzey Hindistan'dan Altı Yıllık Bir Çalışma

ÖZET

Amaç: Klinik örneklerden yeni *Nocardia* türlerini izole etmek ve bunların farklı antibiyotiklere olan duyarlılık paternlerini çalışmak. Nokardiyoza eşlik eden değişik risk faktörleri de çalışıldı.

Metotlar: Yatan hasta ve polikliniklerden altı ayı aşkın bir sürede toplanan ve klinik hikayesinde pnömoni, apse veya yaygın enfeksiyon olan 32 klinik örnek işleme alındı ve Nocardia kültürü yapılarak duyarlılıkları çalışıldı.

Bulgular: Şüpheli 32 örnekten 12'sinde nokardiyoz rapor edildi. Başvuran hastaların yaş ortalaması 57,9 idi. Pnömoni en sık karşılaşılan klinik durumdu, onu primer kütanöz hastalık ve yaygın enfeksiyon tablosu takip ediyordu. Nokarddiyozlu on hastanın sekizi; organ transplantasyon hikayesi ve immünsüpresif ilaç veya steroid kullanımı nedeni ile immün yetmezlikli idiler. Biyokimyasal testlerle izole edilen suşların beşi *N. asteroides*, üçü *N. brasiliensis*, ikisi *N. farcinica* ve diğerleri birer tane olarak *N. transvalensis* ve *N. nova* olarak adlandırıldılar. Tamamı linezolide duyarlıydı (% 100) ve bunu cotrimoksazol (% 91,6) takip ediyordu.

Sonuç: İmmünyetmezlikli hastaların sayısındaki artış ve nokardiyoz insidansındaki artış ile birlikte özgün olmayan semptomlarla başvuran ve diğer hastalıkları taklit ederek karışıklık oluşturan *Nocardia* enfeksiyonlarının tanısı her zaman akılda tutulmalıdır. Linozolid, kotrimoksazol, imipenem ve minosiklin *Nocardia* türlerine en iyi etki eden ilaçlar olarak görülmektedir.

Anahtar kelimeler: Nokardiyoz, pulmoner, insidans, Hindistan, direnç, immünyetmezlikli, bakteriyel enfektiyonlar

Correspondence: Reetika Dawar, Senior Consultant-Microbiology Department of Microbiology, Indraprastha Apollo Hospitals, Sarita Vihar, Delhi-Mathura Road, New Delhi, India-110076 Email: reetika_d@apollohospitals.com
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INTRODUCTION

Nocardia species strictly aerobic actinomycetes which are gram positive and weakly acid fast. They form filamentous branched cells which fragment into pleomorphic rods shaped or coccoid elements [1,2]. They are ubiquitous soil saprophytes and enter body via inhalation or via wounds contaminated with dust and soil. *Nocardia* are opportunistic pathogens causing exogenous infection, usually primary pulmonary infections which disseminates. Almost every organ of the body can be infected manifesting as empyema, bacteremia, brain abscess, pericarditis, peritonitis, corneal ulcers and soft tissue and skeletal system infections [3].

Risk factors are deficient cell mediated immunity as with lymphoma, transplantation, glucocorticoid therapy, treatment with monoclonal antibodies, AIDS, pulmonary alveolar proteinosis and tuberculosis [4]. As a group, aerobic actinomycetes may take several days to grow and are not easily amenable to identification with commercially available panel to test carbohydrate utilization [5].

Gram staining of direct samples is an important diagnostic aid as compared to modified acid-fast staining [6]. As *Nocardia* is known to produce significant morbidity especially in the immunocompromised individuals and as the treatment is for an extended period (6-12 months) clinicians are particularly interested in antimicrobial susceptibility pattern [4]. Speciation of *Nocardia* also helps in determining susceptibility pattern. Likewise antimicrobial susceptibility pattern can help in speciation of *Nocardia* [7].

Even though sulfonamides and co- trimoxazole (TMP-SMX) have been the drugs of choice in the past, optimal antimicrobial therapy for patients with nocardiosis remains to be established. Hence it is important to identify alternative antimicrobials and perform in vitro susceptibility testing with these agents to determine their efficacy against isolates of *Nocardia*.

The first recommendations for antimicrobial susceptibility testing of aerobic actinomycetes including *Nocardia* was published in 2003 by "The Clinical and Laboratory Standards Institute" (CLSI) wherein susceptibility testing of aerobic actinomycetes by broth microdilution dilution is the approved method [8].

Studies showed that performance of E test for susceptibility of *Nocardia* showed 90% concordance with disc diffusion, broth micro dilution and agar dilution methods for in-vitro susceptibility of *N. asteriodes* to a panel of drugs [7,9].

The objective of our study was isolation and speciation of *Nocardia* species from clinical samples and to study their antimicrobial susceptibility pattern to different antimicrobials. Various risk factors associated with nocardiosis were also studied.

METHODS

Thirty two clinical specimens with clinical history of pneumonia, abscesses, or disseminated infections from Inpatient and Outpatient department of Indraprastha Apollo Hospitals were collected over a period of six years (2009-2014). These included 27 respiratory samples (13 bronchoalveolar aspirates, 9 sputum, 3 endotracheal secretions, 1 transtracheal secretion and 1 pleural fluid), 3 pus samples from cutaneous abscesses and 1 blood culture from patient with history of fever. Age, gender and presence of underlying immunocompromised conditions like HIV status, intake of steroids, history of organ transplantation, cancers, smoking, diabetes mellitus, alcohol use, chronic obstructive pulmonary disease (COPD) were noted. Type of specimens positive for *Nocardia* were documented. Specimens were processed immediately. If delay in processing of any specimen was anticipated for more than 2 hours, it was stored in refrigerator at 2-8 ºC and subsequently processed. Blood was always immediately processed without delay by loading in BACTEC 9120 (Becton Dickinson and Company).

Specimens were stained with Gram's and modified Ziehl Neelsen's technique using 1% H2SO4 as decoloriser).

Culture

The specimens were then inoculated on routine media like Columbia blood agar, brain heart infusion (BHI)agar and chocolate agar, incubated at 35 °C under aerobic conditions, in carbon dioxide incubator and anaerobic conditions for up to 3 weeks and observed every third day for growth for up to 21 days. Löwenstein–Jensen media was also inoculated and incubated at 35 °C aerobically. Dry and chalky colonies were further subjected to gram staining and Modified Ziehl-Neelsen's staining for morphology.

Identification

Further biochemical tests were put up after identification of *Nocardia* like organisms on staining. *No-*

cardia can be identified by the traditional method to the species level by using a battery of biochemical tests. We tested for hydrolysis of casein, tyrosine, xanthine, hypoxanthine, starch as well as utilization of glucose, inositol and rhamnose [7,9]. Plates of glucose agar, inositol and rhamnose agar medium were spot inoculated with *Nocardia* and were incubated at 37 °C for upto three weeks.

Starch hydrolysis test was put up and the test was read after 2-3 days. Xanthine, hypoxanthine, tyrosine and casein plates were put up for hydrolysis for upto 2-3 weeks [10]. Three day and 14 day arylsufatase test was also put up .Gelatin liquefaction was tested by spot inoculating plates and incubating at 35 ºC for up to 2 weeks and then alternatively refrigerating plate at 4 °C for 30 min. Further tests for nitrate reduction, citrate utilization, urease hydrolysis in tubes were put up.

Antimicrobial susceptibility testing; Inoculum was prepared by taking freshly subcultured isolates on sheep blood agar in Mueller-Hinton (MH) broth .Vortex this suspension along with glass beads (3 mm in diameter) so as to get a uniform suspension with no clumps. The final inoculum should had OD equivalent to 0.5 McFarland standard with a densitometer (Densimat, Biomeriux, France). Inoculum was swabbed on to MH agar to give a confluent growth & E- strips (AB-Biodisk) applied. Plates were incubated at 35 °C at ambient temperature for 48 hours to 72 hours (if growth was insufficient at 48 hours).

Susceptibility was done for ceftriaxone (CRO) (MIC-0.016-256 µg/ml), ciprofloxacin (CIP) (0.002-32 µg/ml), clarithromycin (CLR) (0.016-256 µg/ml), imipenem (IPM) (0.002-32 µg/ml), linezolid (LZD) (0.016-256 µg/ml), minocycline(MIN) (0.016-256 µg/ml), trimethoprim + sulfamethoxazole (SXT) (0.002-32 µg/ml) and tobramycin (TM) (0.064-1024µg/ml). The minimum inhibitory concentrations (MIC) was read in µg/ml .It was determined in accordance with guidelines provided by manufacturer and extrapolation of CLSI breakpoints [9].

RESULTS

A total of 32 clinical specimens were studied for recovery of *Nocardia* over this period. *Nocardia* was detected in 12 out of 32 specimens by culture, smears or both (37.5%). A total of eight respiratory specimens, three pus specimens and one blood specimen were positive for *Nocardia.*

Ten specimens were positive for acid fast filamentous branching in smear stained by modified Ziehl Neelsen technique. Twelve specimens grew *Nocardia* in culture on brain heart infusion agar which was reconfirmed by Gram's and Modified Ziehl Neelsen staining. Mean age of presentation of nocardiosis was 57.9 years.

Eleven (91.6%) patients were in the age group of 40-80 years and only one in was between of 20-40 years. Higher incidence was seen in the older age group 60-80 years.

With respect to gender, 10 men had Nocardiosis (total; 20 while only 2 women were positive out of a total of 12 women who were studied.

Eight patients who were positive for *Nocardia*, out of a total of 32 had pneumonia. Three patients had primary cutaneous disease and one had disseminated disease) (Table 1). When taking risk factors into consideration a total of 8 patients out of 12 patients with nocardiosis were immunocompromised. Eight patients had H/O organ transplantation with immunosuppressives, three had history of COPD and smoking and one had malignancy three had a history of chronic use of steroids (for Connective Tissue Disorder). One of the patients was a farmer with history of chronic smoking. None were HIV positive.

Among the *Nocardia species* isolated, based on biochemical reaction, 5 were *Nocardia asteroides*, 3 were identified as *Nocardia brasiliensis*, 2 *Nocardia farcinica* and 1 each were *Nocardia transvalensis* and *Nocardia nova*. A detailed clinical follow up in terms of radiological features and relapses could not be conducted. The results of antimicrobial susceptibility were also interpreted. Figure 1 displays the results of sensitivities of all antimicrobials that were put up for various *Nocardia species*. The five *Nocardia asteroides* isolated were resistant to clarithromycin, 3 out of five strains being resistant to ciprofloxacin but were sensitive to all the other drugs tested. All the Nocardia strains were sensitive to linezolid (100%) followed by cotrimoxazole (91.6%).90% of the strains were sensitive to minocycline followed by Tobramycin (83.3%), Imipenem (75%) and ceftriaxone (50%). Low % sensitivity was observed towards Ciprofloxacin (41.6 0%). Least (16.6%) sensitivity was seen in Clarithromycin. Early diagnosis and treatment with proper drug regimen resulted in complete resolution of infection as evident by radiological and clinical evaluation.

Table 1. Prevalence of *Nocardia* species in patients positive for Nocardiosis with respect to underlying diseases

	Number of *Nocardia* isolates	*Nocardia asteroides*	*Nocardia brasiliensis*	*Nocardia farcinica*	*Nocardia nova*	*Nocardia transvalensis*
Primary Cutaneousdisease	3	0	3	0	0	0
Pulmonary Infections	8	4	0	2	1	1
Disseminated disease	1	1	0	0	0	0

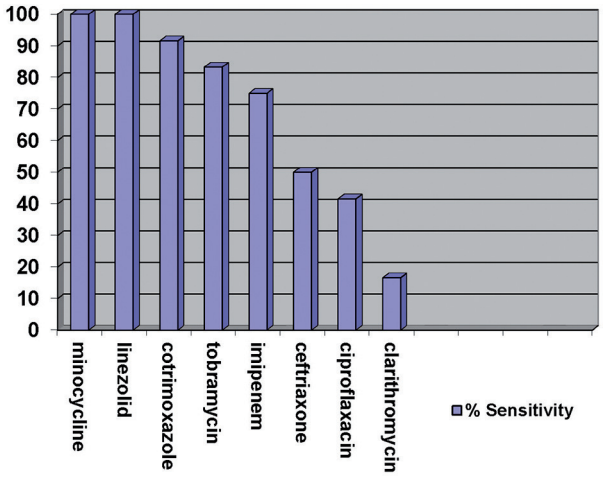

Figure 1. Antibiotic susceptibility pattern of isolates of *Nocardia* species.

DISCUSSION

Nocardial infections occur worldwide and are more prevalent in tropical and subtropical environments [11]. There has been a recent increase in the reporting of nocardiosis in India. The largest series from an Indian hospital was of 860 patients with tuberculosis among which 32 had Nocardiosis and had been treated previously with antitubercular drugs. [12]. A study on nocardiosis in a tertiary care hospital reported 12 consecutive cases over a span of 26 months [13]. It is likely that most of the cases remained undetected in the past. An increase in use of corticosteroids, immunosuppressive agents and immunosuppression due to newer diseases like HIV may also be the cause.

In the present study maximum incidence of nocardiosis was seen in the older age group of 40-80 years (91.6%) indicating that nocardiosis affects mainly the ageing population. A study conducted in Japan from 1992-2001) reports maximum incidence in elderly [14]. Mean age of presentation was 57.90 years in our study. In contrast in a study done in tertiary care hospital Chandigarh mean age of presentation was 38.4 years [13].

Maximum incidence was observed in males indicating that males were prone to infection. In a study on renal transplant recipients 77% of cases of pulmonary nocardiosis were males (M) while 23% were females; thus M/F ratio was 3.4 [15]. Eighty percent t of patient positive for the *Nocardia* spp were male in a study in Thailand in between 1996-2001 [16]. The predominance of men remaining oudoors and certain hormonal effects on bacterial growth and virulence may be responsible for higher male predisposition. [15].

Incidence of pulmonary nocardiosis as one of the opportunistic infection in immuno-suppressed patients is increasing [17]. Immuno-suppression was found to be an important association with nocardiosis in this study with 66.6% patients being immunosuppressed. In our study the most common species of Nocardia isolated from pulmonary infections was *N. asteroides*. In all primary cutaneous cases *N. brasiliensis* was isolated. Earlier reports have documented that the most common clinical presentation was pleuropulmonary infection (44.3%).Skin and soft tissue infection were 22.8% and multiorgan dissemination was seen in 11.4% cases with 20% mortality rate16 and 40% incidence of pulmonary co-infections [14].

N. brasiliensis has been reported to be the commonest causal organism of primary cutaneous nocardiosis [18]. The study on susceptibility of various antimicrobials was found to be in concordance with other studies on antimicrobial agents such as cotrimoxazole, linezolid and minocycline which were found to be very effective on the basis of susceptibility testing results against most *Nocardia* species except *N. brasilensis* displaying resistance to imipenem [19]. Clarithromycin didn't seem to be an effective agent with only 2 of strains being sensitive.

Sulfonamides remain the 1st line agents in management of nocardiosis, though resistance is most commonly seen among *N. farcinica* isolates [20]. However a single isolate of *N. farcinica* in our study was sensitive to cotrimoxazole. Carbapenems should be used as an alternative for treatment

of severely ill patients. 91.6% of the isolates were sensitive to cotrimoxazole in our present study. Lower sensitivity has been reported to cotrimoxazole (76.5%) from Pakistan 21. In renal transplant patients 76% sensitivity towards Cotrimoxazole has been reported. Cotrimoxazole is given as monotherapy in 71% of cases [15].

Linezolid offers an additional option against *Nocardia* as it has clinical activity against all species of *Nocardia*. In addition it can be given orally. Other choices -ceftriaxone and imipenem are administered intravenously. Minocycline has the best in vitro activity and added benefit that it can be used for patients who are allergic to sulfonamides [8].

The limitations of our study was that the sample size was small and molecular tests for identification could not be performed.

CONCLUSION

With increasing incidence of nocardiosis, diagnosis of nocardia should always be kept in mind as it can be easily confused with other diseases. Speciation and susceptibility pattern of nocardia should also be done keeping in view, the varied antimicrobial profile of different nocardia species and the vulnerability of patient population.

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