

Prevalence of *Nocardia* Species Isolated from Patients with Respiratory Tract Infections at Siriraj Hospital, Thailand

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ABSTRACT

Infections caused by *Nocardia* spp. are rare in humans. In our study, *Nocardia* spp. isolated from patients admitted at Siriraj Hospital, Bangkok, Thailand during the 6-year period from January 1998 to December 2003 were examined. The results showed that there were fifteen patients infected with *Nocardia* spp. Of these patients, 53.3 percent were male and 46.7 percent were female. Our study also showed the number of patients infected with *Nocardia* spp. based on different age groups. The infections were caused by *N. asteroides* (66.7%) and *N. farcinica* (33.3%). (*J Infect Dis Antimicrob Agents* 2007;24:1-6.)

INTRODUCTION

Nocardia spp., strictly aerobic actinomycetes, have been implicated as the cause of cutaneous, ocular, pulmonary, and disseminated disease in both immunocompetent and immunocompromised human hosts.¹ The incubation period is unknown. No racial predilection exists. They are Gram-positive, weakly acid-fast, dichotomous branching bacilli. *Nocardia* spp. are essentially soil saprophytes worldwide, and involve in the decomposition of plant material. They are not normal flora in humans or animals.

Regarding pathogenesis, *Nocardia* spp. can escape phagocytosis by inhibiting phagosome-lysosome fusion. Natural infections occur in domestic animals. Human infections usually result from the inhalation of airborne bacilli or the traumatic inoculation of the organisms into the skin. Human-to-human transmission has not been reported. Natural resistance, mediated by intact mucous membrane as well as alveolar and tissue phagocytes, is quite strong. Thus, disseminated and fulminant diseases mainly occur in immunocompromised hosts (among persons with deficient cell-mediated

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Received for publication: October 25, 2006.

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Keywords: *Nocardia*, respiratory tract infection

immune response, and chronic granulomatous disease). The pulmonary infection results in a lesion that most often manifest as an abscess, and rarely, granuloma with occasionally hematogenous metastasis to the central nervous system (CNS).²⁻⁴

Identification of *Nocardia* isolates to the species level is important to define the spectrum of diseases caused by each species, to predict their antimicrobial susceptibility patterns, and to determine the geographic distribution of these organisms.^{2,5} Twelve species within the genus *Nocardia* have been identified, using advanced laboratory techniques. Of these species, *N. asteroides sensu stricto* (means in a strict sense) is the best known. According to the current classification of *Nocardia* spp., this group excludes *N. nova*, *N. farcinica*, and *N. transvalensis*, which in older studies, were not recognized as species and were included in what was called "*N. asteroides*" or "*N. asteroides* complex". *N. farcinica*, *N. nova*, *N. brasiliensis*, *N. otitidiscaviarum*, and *N. transvalensis* complex are other species frequently associated with infections in humans.⁶ Members of the *N. asteroides* complex are responsible for 80 percent of non-cutaneous invasive diseases and for most systemic and CNS diseases. *N. farcinica* is most often associated with antimicrobial resistance, and more than 50 percent of cases involve disseminated infections.⁶ Less frequently, other *Nocardia* spp., including *N. brasiliensis*, *N. transvalensis*, and *N. otitidiscaviarum* have also been reported.² Recently, a new pathogenic species, *N. africana*, was isolated from sputum samples of a patient with chronic pulmonary infection in Africa.⁷

The severity of nocardial infections and the high morbidity in disseminated or CNS infections requires additional tests for full identification of the clinical isolates. Antimicrobial susceptibility is useful in the classification and identification of actinomycetes.⁸ The identification of *Nocardia* spp. at the species level and

antimicrobial susceptibility is difficult in the laboratory. This may explain why species distribution in clinical isolates has been poorly documented up to now, and many laboratories still report the "*N. asteroides* complex" as the most frequently isolated *Nocardia* spp. in humans.^{9,10} The purpose of this study was to analyze the data of *Nocardia* spp. to identify the species most commonly encountered in clinical isolates.

MATERIALS AND METHODS

Fifteen *Nocardia* isolates were collected from different patients admitted at Siriraj Hospital, Bangkok, Thailand during the 6-year period from January 1998 to December 2003. Only one isolate from each patient was analyzed. The clinical specimen was sputum. Only the sputum with < 25 squamous epithelial cells per low-power field was acceptable for cultures on blood, chocolate, and MacConkey agars. Bacterial pathogens were isolated and identified by the standard microbiological methods.^{11,12} *Nocardia* spp. grew on blood agar after 3 to 5 days of incubation at 35°C in an ambient incubator. They formed dry, powdery colonies which were very adherent to agar, with white-to-light-coral pigmentation. A typical Gram-stained morphology of *Nocardia* spp., branching Gram-positive bacilli with beaded staining, was noted and the bacteria were positive for modified Ziehl-Neelsen acid-fast stain (Figure 1). *Nocardia* cultures were further identified, using various physiological characteristics including a) growth on Sabouraud dextrose agar at 45°C; b) hydrolysis of adenine, casein, hypoxanthine, tyrosine, xanthine and urea; c) acid production from adonitol, arabinose, erythritol, galactose, glucose, inositol, maltose, mannose, rhamnose, and sorbitol; and d) susceptibility to 5-fluorouracil, imipenem, tobramycin, and kanamycin.

RESULTS

Fifteen *Nocardia* isolates were obtained from 18,750 (0.08%) patients who were admitted at Siriraj Hospital from January 1998 to December 2003. Of these patients, 53.3 percent were men and 46.7 percent were women. The ratio of male and female was patients 1.14:1. The clinical data was available in six (42%) patients. There were two patients with human immunodeficiency virus infection and one patient each with breast cancer, chronic kidney disease, pulmonary tuberculosis, and urinary tract infection. Table 1 shows the number of patients infected with *Nocardia*, based on different age groups, ranging from 22 to 81 years

(mean of 46 years). All isolates were from adult patients. Most patients were (33.3%) in the age group of 31-50 years. The infections were caused by *N. asteroides* (66.7%), and *N. farcinica* (33.3%).

DISCUSSION

Nocardiosis is usually an opportunistic infection, and most commonly presents as pulmonary disease. There are very few studies of *Nocardia* spp. in Thailand in general and specifically in small hospitals. The ratio of male to female nocardiosis patients was reported to be approximately 2:1 or 3:1.¹³ In our study, the ratio was lower (1.14:1).



Figure 1. Gram-positive branching filaments of *Nocardia* species in a direct smear of sputum.

Table 1. Number of patients infected with *Nocardia* spp. in each age group.

Age groups (year)	Number of patients	Percent
1-10	0	0
11-30	4	26.7
31-50	5	33.3
51-70	4	26.7
>70	2	13.3
Total	15	100

No age predilection exists in the patients because nocardiosis may occur from infancy to old age.¹³ In our study, all patients were adults (range from 22 to 81 years). A previous study in Mexico found the age range of the patients was from 15 to 60 years.¹⁴

In our study, fifteen isolates from different patients were obtained. Of these species, the most common species was *N. asteroides* sensu stricto, followed by *N. farcinica*. This result supports a previous study which demonstrated that *N. asteroides* sensu stricto type VI was distributed evenly throughout the USA.⁶ *N. farcinica* was also found evenly throughout the USA, even though it was less prevalent than *N. asteroides*.⁶ *N. asteroides* complex was the predominant pathogen in humans. Eighty-five percent of pulmonary infections were caused by *N. asteroides*, while *N. brasiliensis* caused cutaneous and subcutaneous infections.^{3,5,15} Additionally, a study in the Republic of China showed that *N. asteroides*, *N. brasiliensis*, *N. otitidiscaviarum*, and *N. farcinica* caused diseases in humans. Of these studies, approximately 90 percent of infections were caused by *N. asteroides*, 7 percent by *N. brasiliensis*, and 3 percent by *N. otitidiscaviarum*, but the infections caused by *N. farcinica* were very rare.¹⁶ In Thailand, a study of 37 *Nocardia* spp. isolated from AIDS patients in 1995 found that 25 isolates belonged to *N. asteroides* sensu stricto (48%) and *N. farcinica* (52%).¹² Other species included *N. otitidiscaviarum*, *N. brasiliensis*, and *N. transvalensis*.¹² Two patients in our study had human immunodeficiency virus infection.

Interestingly, in Germany the changing species distribution of *Nocardia* spp. causing human diseases was recently observed; *N. farcinica* was predominantly isolated.¹⁷ A study from Belgium, using 16S rRNA gene sequencing found that among 86 isolates of *Nocardia*, 83 (96%) isolates belonged to

only six species including *N. farcinica* (44%), *N. nova* (22%), *N. cyriaciorgica* (15%), *N. brasiliensis* (6.9%), *N. abscessus* (5.8%), and *N. paucivorans* (2.3%).¹⁸ In Japan, a large study of 303 *Nocardia* spp. isolated between 1992 and 2001 found that *N. farcinica* was the most frequent isolate.¹⁰ All of these results are in contrast with our findings, probably due to geographic variations.

Since the 1940s, sulfonamides have been the agent of choice for treatment of nocardiosis.^{15,19,20} Prior to the introduction of these agents, the fatality rate of pulmonary nocardiosis was nearly 100 percent.¹⁵ Despite the development of several new members and classes of antimicrobials during the past 60 years, sulfonamides continue to be the recommended treatment option for most cases of nocardiosis.^{6,13} The drug most commonly used is trimethoprim-sulfamethoxazole.¹⁵ We did not perform the antimicrobial susceptibility test according to the recommendation by the National Committee for Clinical Laboratory Standards (NCCLS). However, the Clinical Laboratory Standard Institute (CLSI) recently published the first approved recommendations for antimicrobial susceptibility testing of aerobic actinomycetes including *Nocardia* spp. by broth microdilution and with cation-supplemented Mueller Hinton broth.²¹ On the basis of previous studies by many investigators, it is interesting to discuss some of their reports. Their results showed that *N. asteroides* isolates were susceptible to amikacin and susceptible or moderately susceptible to amoxicillin-clavulanate. The activity of amikacin against all *Nocardia* spp. at clinically achievable serum levels, confirms that it may be another drug of choice in the therapy of all forms of nocardial infection.^{5,22,23} These results support previous observations that amoxicillin-clavulanate was moderately active against many isolates of *N. asteroides*, *N. farcinica*, and *N. brasiliensis*.¹³ Moreover, the effectiveness of 13 antimicrobial agents

against 51 clinical isolates of *Nocardia* spp. was determined in the USA. Amikacin inhibited more than 90 percent of the isolates, and showed good correlation between minimal inhibitory concentrations and sizes of zones of inhibition around the disk.²⁴

Uniquely, a high degree of drug resistance makes treatment of *N. farcinica* more problematic than other *Nocardia* spp. It is interesting that *N. farcinica* is resistant to most beta-lactam antibiotics including all cephalosporins such as cefamandole (100%), cefotaxime (100%), and ceftriaxone (80%). It is also resistant to aminoglycosides, including tobramycin (>90%) and kanamycin (100%); but is susceptible to amikacin (100%), sulfamethoxazole (100%), imipenem (82%), ciprofloxacin (88%); and only 5 percent and 3 percent resistant to ampicillin and erythromycin, respectively.¹⁵

Lastly, we would like to mention that the genus *Nocardia* has undergone a taxonomic revolution during the last 10 years. Only 12 species were described between 1888, when the genus was first isolated by Nocard²⁵, whereas more than 50 species¹⁵ are now recognized to exist, mostly by molecular techniques such as 16S rRNA gene sequencing.²⁶⁻²⁹ Therefore, identification of *Nocardia* to the species level will become increasingly difficult in the future without the use of molecular techniques. Some new *Nocardia* spp. have been collected from clinical specimens; others have been isolated only from environmental specimens. Determination of the species of *Nocardia* is useful to define the spectrum of diseases caused by each species and the pathogenesis for different patient groups. Fortunately, most commonly isolated species from humans, such as *N. asteroides* sensu stricto, *N. brasiliensis*, *N. farcinica*, and *N. pseudobrasiliensis*, can be reliably identified by extensive study of their phenotypes and biochemical characteristics.^{12,15}

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