Bahrain Medical Bulletin, Vol. 32, No. 1, March 2010

The Occurrence of *Nocardia* Species among Patients with Pulmonary Infection

Mogahid M El Hassan, PhD* Eman Magzoob, B.Sc, M.Sc** Maha F El Rahman, B.Sc, M.Sc*** Nageeb S Saeed, PhD*** Mohamed E Hamid, PhD****

Objective: The aim is to determine the frequency of *Nocardia* spp. among tuberculous patients in Khartoum state.

Design: Prospective study.

Setting: Abu-Anga Teaching Hospital, El-Shaab Teaching Hospital and the Tuberculosis Reference Laboratory at the National Health Laboratory, Khartoum, Sudan.

Method: Three hundred and twenty-nine patients were included in this study during the period from October 2004 to January 2006. The patients were examined for the presence of acid-fast bacilli. Thin bacterial smears were prepared and stained by Ziehl Neelsen (ZN) stain. Two tubes of the Lowenstein-Jensen (LJ) medium were inoculated with 20 µl of the neutralized sputum sample that was obtained from the digestion and decontamination. One of the two tubes contained glycerol while the other tube contained pyruvic acid to isolate *M. bovis* species if encountered. All cultures were incubated at 37°C for 8 weeks. The growth was monitored up to the 8th week. Phenotypic characterization was performed by using different biochemical tests.

Result: Ten isolates showed rapid growth pattern within 2-3 days after inoculation. Further conventional methods suggested that all these isolates were belonging to the family Nocardiaceae.

Conclusion: *Nocardia* spp revealed considerable occurrence among patients with pulmonary infections (3.3%). This finding suggested that pulmonary nocardiosis might occur in patients who suffer from chronic lung disease in Sudan.

Bahrain Med Bull 2010; 32(1):

---

* Consultant of Medical Laboratory Practice
Department of Microbiology, College of Medical Laboratory Science
University of Science and Technology, Sudan
** Researcher, Department of Microbiology, College of Veterinary Medicine
University of Khartoum
*** Researcher, National Health Laboratory
**** Chief Director, Dept of Pathology, Federal Ministry of Health
University of Khartoum
*****Tuberculosis Research Group
Department of Microbiology, College of Medicine
King Khalid University, Saudi Arabia
Email: Greensudan70@yahoo.com

The genera *Mycobacterium* and *Nocardia* have been grouped into the family Mycobacteriaceae within the order Actinomycetales based upon similarities in staining and motility, lack of spore formation, and catalase production. These genera are characterized by the presence of mycolic aids, the side chains (R1 and R2) vary in length according to the genus; several species produce disease in humans[1,2].

There are approximately 71 recognized or proposed species in the genus *Mycobacterium*[3]. These species produce a spectrum of infection in humans and animals ranging from localized lesions to disseminated disease. Although some species cause only human infection, others have been isolated from a wide variety of animals. Many species are also isolated from water and soil[4].

Nocardiae are known to cause a variety of localized and disseminated infections in humans and animals[5,6]. They are in the same family of *Mycobacterium, Streptomyces, Corynebacterium* and *Rhodococcus*[7]. The most commonly reported pathogenic species are *Nocardia asteroides, N. farcinica* and *N. nova*, followed by *N. brasiliensis, N. otitidiscaviarum, N. pseudo brasiliensis* and *N. transvalensis*. Infections caused by *Nocardia* species are infrequent but challenging to clinicians. In recent years, the number of case reports has been increasing, and this can be attributed to the improvements in diagnostic capabilities and the higher clinical index of suspicion in immune-suppressed patients[6,8].

Although nocardiosis had been reported from around the world, but it is well established that *nocardiae* can be easily overlooked under routine culture and smear examinations. Moreover, its incidence in many countries remains unknown. It was also found that nocardiosis, although infrequent, is an important cause of morbidity and mortality in patients with cancer (immunocompromised). It had pleomorphic clinical manifestations and it can be seen in breakthrough infections. Cytomegalovirus co-infection influences the outcome of patients with cancer and nocardiosis[9].

Tuberculosis (TB) is a major public health problem worldwide. It is estimated that some 7-8 million new cases and 2-3 million deaths occurs annually in the world[10]. Its elimination will be impossible as long as poverty, overpopulation, and malnutrition characterize many countries[11].

METHOD

Three hundred and twenty-nine patients were examined for the presence of acid-fast bacilli during the period of October 2004 to January 2006. They were suspected of having tuberculosis based on their symptoms. Most of the patients had either not responded to treatment with antitubercular drugs or had responded and then relapsed. Sputum samples were collected according to WHO criteria in sterile, plastic wide-mouthed, strong leak-proof containers. Following the treatment with the digestion-decontamination procedure of Roberts et al, the sputum samples were concentrated by centrifugation and the resultant preparations were used to inoculate Lowenstein-Jensen (LJ) slopes, which were incubated at 37°C for 14 days and then used to make smears[12,13]. The smears were examined with standard Ziehl-Neelsen acid-fast stain.

Ten of the LJ slopes supported the growth of small orange filamentous colonies, which were considered typical of *Nocardia*. The isolates, which were designated SD1001, SD1002, SD1002, SD1003, SD1004, SD1005, SD1006, SD1007, SD1008, SD1009 and SD1010, were

subcultured and maintained on glucose-yeast, extract agar (GYEA) slopes at room temperature[14]. The ten isolates were examined for a range of the phenotypic properties described by Isik et al[15]. Standard procedures were used for the extraction and analysis of mycolic acids and two known *Nocardia africana* strains were used as controls[16].

RESULT

Two hundred and forty-one (73.3%) were males and 88 (26.7%) were females. The majority of the patients were new, 218 (66.3%), the rest were old 62 (18.8%). Relapse cases were 32 (9.7%) and treatment failure were 17 (5.2%), *Nocardia* spp. were isolated from four (1.2%) of them.

All the smear positive specimens showed positive growth on LJ medium after 2-21 days post inoculation. The colonial morphology of 319 (97%) appeared as rough, friable, warty, granular and grey in color with irregular margins and showed the appearance of AFB when stained again (indirect smear) with ZN for confirmation. The 319 isolates were initially identified as members of the *Mycobacterium tuberculosis* complex.

Ten (3%) of the LJ slopes revealed the growth of small orange filamentous colonies, which were tentatively considered to be *nocardiae*.

On GYEA, all 10 isolates showed orange, creamy and yellow wrinkled colonies. Some colonies were embedded into agar but some were relatively smooth and easily detached. The aerial hyphae were seen. Diffusible pigments were not formed, see Figure 1.

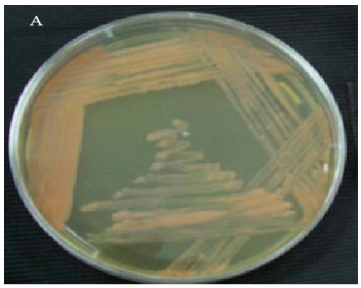

Figure 1 (a): SD1001

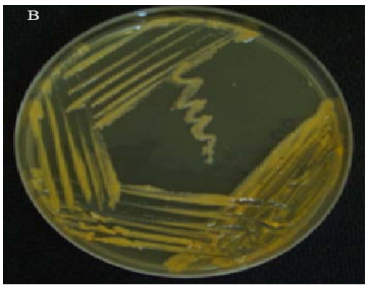

Figure 1 (b): SD1005

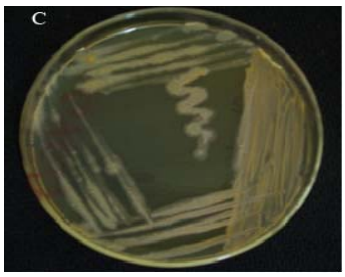

Figure 1 (c): SD1008

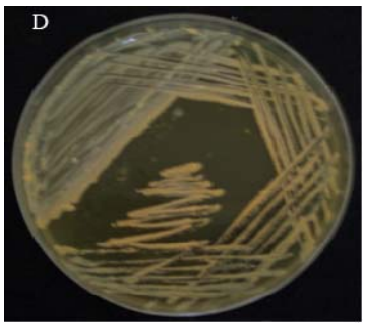

Figure 1 (d): SD1009

Figure 1 (a,b,c,d): Growth of *Nocardia* Species on GYEA Medium (7 Days Old Culture)

Selected biochemical tests were performed, see Table 1. The result of these tests showed that all the strains utilize glucose by oxidation pathway and that they were all catalase and urea positive. Seven out of the ten were positive (70%) growth at 45°C. Two (20%) out of ten were positive for mannitol and rhamninose as well as starch whilst all the isolates were negative for xanthine, casein, tyrosine, sorbitol, arabinose and citrate. Eight (80%) out of ten showed the standard patterns of mycolic acid components using thin layer chromatographic technique, see Figure 2. The tested strains were found to have phenotypic properties typical of members of the genus *Nocardia*, see Table 1.

Table 1: Morphological, Cultural, Biochemical and Physiological Properties of *Nocardia* and *Nocardia*-like Species

Isolate	Partial acid fast	Gram stain	Colonies			Colonies color			Biochemical tests											Growth at 45°C	Myc Acid
			R	E	Re	O	Y	Cr	C	T	X	S	M	Ma	So	Rh	A	U	Ci	Ca	+	+
SD 1001	+	+C	+	-	+	+	-	-	-	-	-	-	Ox	-	-	-	-	+	+	+	+	+
SD 1002	+	+C	+	+	+	-	-	+	-	-	-	-	Ox	-	-	-	-	+	+	+	ND	+
SD 1003	+	+C	+	-	+	+	-	-	-	-	-	-	Ox	-	-	-	-	+	+	+	+	+
SD 1004	+	+C	+	-	+	+	-	-	-	-	-	-	Ox	-	-	-	-	+	+	+	+	+
SD 1005	+	+C	+	-	+	+	-	-	-	-	-	-	Ox	-	-	-	-	+	-	+	ND	+
SD 1006	+	+C	+	-	+	+	-	-	-	-	-	-	Ox	-	-	-	-	+	+	+	+	+
SD 1007	+	+C	+	-	+	+	-	-	-	-	-	+	Ox	-	-	-	-	+	+	+	+	+
SD 1008	+	+C	+	-	+	-	-	+	-	-	-	+	Ox	-	-	-	-	+	-	+	+	ND
SD 1009	+	+C	+	-	+	-	-	+	-	-	-	-	Ox	-	-	-	-	+	+	+	+	+
SD 1010	+	+C	+	-	+	-	+	-	-	-	-	-	Ox	-	-	-	-	+	-	+	ND	ND

R=Raised, E=Embedded, Re=Regular, O=Orange, Y=Yellow, Cr=Creamy
C= Casein, T=Tyrosine, X=Xanthine, S=Starch, M=Metabolism of glucose, Ma=Mannitol, So=Sorbitol, Rh= Rhamnose, A=Arabinose, U=Urea, Ci=Citrate, Ca=Catalase
ND: Not Determined. +C: Coccobacilli. Ox: Oxidative

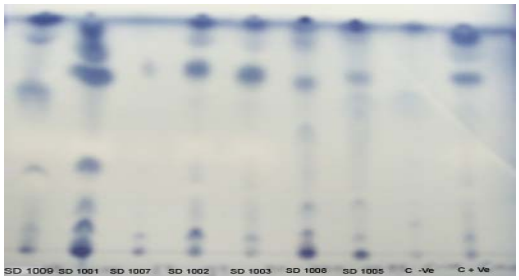

Figure 2: Thin Layer Chromatography (TLC) (10x10cm), Run Twice in a Solvent Containing Toluene-Acetone (97:3, V/V), C +Ve*: N. africana* SD 925, C –Ve: *S. aureus*

DISCUSSION

All the patients included in this study were smear positive using ZN stain; more confirmation was obtained when all the sputum samples were cultured on LJ medium because culturing technique is required for the definitive diagnosis of TB[17]. The importance of an effective strategy for routine TB cultures during bronchoscopy in patients from non-endemic areas in whom TB is not suspected -especially those with pulmonary mass - was reported[18].

In a study of tuberculosis suspected population, 37% of sputum samples showed obvious growth on Lowenstein-Jensen medium although they were smear negative; the finding confirms the importance of culture in the diagnosis of tuberculosis, especially in a low incidence area[19].

The colonial morphology of 319 (97%) appeared as rough, friable, warty, granular and grey in color with irregular margins and showed the appearance of AFB when stained again (indirect smear) with ZN for confirmation.

The data obtained from the patients revealed that the number of ZN positive males was significantly higher than that of females (73.3%:26.7%), this phenomenon was also observed previously and was attributed to an X chromosome susceptibility gene, which might contribute to the excess of males with tuberculosis observed in many different populations[20].

There was a significant number of the study subjects 17 (5.2%), who did not respond to the empirical anti-tuberculosis therapy (treatment failure). Similar findings were previously reported in the Sudan and world wide[21,10]. Among the 17-treatment failure, there were 4 patients (25%) from whom *Nocardia* species were isolated (SD1001, SD1003, SD1004 and SD1008). Hence, a reliable antibacterial agent to which *Nocardia* is known to be susceptible should be described by the physician in such cases[22].

CONCLUSION

Recent increases in the reported frequency of human nocardial infections can be attributed to the widespread use of immunosuppressive drugs, improved selective isolation procedures, and increased clinical and microbiological awareness.

Nevertheless, in some developing countries where other chronic lung diseases particularly tuberculosis are prevalent, *nocardiae* are either missed or misidentified in laboratory specimens. It is important, therefore, that clinicians in Chest Units should consider this condition, especially when patients with respiratory infections failed to respond to antitubercular therapy.

Identification of clinically significant *nocardiae* to the species level is important for establishing the spectrum of disease produced by members of each species and for predicting antimicrobial susceptibility.

REFERENCES

1. Beaman BL, Beaman L. Nocardia Species: Host Parasite Relationships. Clin Microbiol Rev 1994; 7: 253-64.
2. Bloom BR, Murray JL. Tuberculosis: Commentary on a Re-Emergent Killer. Science J 1992; 257: 1055-64.
3. Shinnick TM, Good RC. Mycobacterial Taxonomy. Eur J Clin Microbiol Infect Dis 1994; 13: 884.

4. Betty AF, Daniel FS, Alice SW. Diagnostic Microbiology. 10th ed, London: Mosby, 1998; 715-6.
5. Goodfellow M. Nocardia and Related Genera; 2nd ed, In: Ballows, Duerden, eds. Topley and Wilsons Microbiology and Microbial Infection. 9th ed, Systemic Bacteriology, London: Edward Arnold, 1998: 463- 89.
6. Laurent F, Provost F, Boiron P, et al. Rapid Identification of Clinically Relevant Nocardia Species to Genus Level by 16S rRNA Gene PCR. J Clin Microbiol 2000; 37(1): 99-102.
7. McNeil MM, Brown JM. The Medically Important Aerobic Actinomycetes: Epidemiology and Microbiology. Clin Microbiol Rev 1994; **7**: 357-417.
8. Corti ME, Villafane-Fioti MF. Nocardiosis: A Review. Int J Infect Dis 2003; 7: 243-50.
9. Torres HA, Reddy BT, Raad II, et al. Nocardiosis in Cancer Patients. Medicine 2002; 81(5): 388-97.
10. WHO. Global Tuberculosis Control Report 2009. http://www.who.int/tb/publications /global_report/2009/update/en/index.html Accessed on 12.12.2009.
11. Raviglione MC. The TB Epidemic from 1992 to 2002. Tuberculosis 2003; 83(1): 4-14.
12. Roberts GD, Koneman EW, Kim YK. Mycobacterium. In: A Balows, Hausler Jr, KL Herrmann, HD Isenberg, HJ Shadomy, eds. Manual of Clinical Microbiology, 5th ed. Washington DC: American Society for Microbiology, 1991: 304-39.
13. Plansky F, Viznerova A, Hejdova E, et al. Comparison of Laryngeal Smear and A Modified Petroff's Method. Cesk Epidemiol Mikrobiol Immunol 1971; 1: 43-7.
14. Hamid ME, Maldonado L, Sharaf Eldin GS, et al. Nocardia Africana sp. Nova, a New Pathogen Isolated from Patients with Pulmonary Infection. J Clin Microbiol 2001; 39(2): 625-30.
15. Isik KJ, Chun YC, Goodfellow M. Nocardia Salmonicida nom. rev., a Fish Pathogen. Int J Syst Bacteriol 1999; 49: 833-7.
16. Minnikin DE, Alshamaony L, M Goodfellow. Differentiation of Mycobacterium, Nocardia and Related Taxa by Thin-Layer Chromatographic Analyses of Whole-cell Methanolysates. J Gen Microbiol 1975; 88: 200-4.
17. Jensen KA. Reinzuch und Typen Bestimmung von Tuberkelbazillensta ¨mmen. Zentbl. Bakteriol 1932; 125: 222-39.
18. Shitrit D, Vertenshtein T, Shitrit AB, et al. The Role of Routine Culture for Tuberculosis during Bronchoscopy in a Nonendemic Area: Analysis of 300 Cases and Review of the Literature. Am J Infect Control 2005; 33(10): 602-5.
19. Kherad O, Herrmann FR, Zellweger J, et al. Clinical Presentation, Demographics and Outcome of Tuberculosis (TB) in a Low Incidence Area: A 4-year Study in Geneva, Switzerland. BMC Infectious Diseases 2009; 9: 217.
20. Bellamy R, Beyers N, McAdam K, et al. Genetic Susceptibility to Tuberculosis in Africans: A Genome-Wide Scan. Proc Natl Acad Sci 2000; 97: 8005-9.
21. El-Sony AI, Mustafa SA, Khalis AH, et al. Conséquences de la décentralisation sur les services de tuberculose dans trois Etats du Soudan. Int J Tuberc Lung Dis 2003; 7(5): 445-0.
22. El Hassan MM, Hamid ME. Antimicrobial Sensitivity Testing of *Nocardia africana* Recently Isolated from Clinical Specimens in Sudan. Bahrain Medical Bulletin 2005; 27(1): 31-4.