

## Case Report

# Isolation of a rare *Nocardia wallacei* from an HIV-positive patient with pulmonary infection in southern Saudi Arabia

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### ABSTRACT

هدفت هذه الدراسة لتحديد التصنيف لبكتيريا نادرة من "الشعيات" والتي تم عزلها من مريض في منطقة عسير بجنوب المملكة العربية السعودية يعاني من مضاعفات رئوية ومصاب بفيروس نقص المناعة المكتسبة. أظهرت نتائج الخصائص المظهرية أن هذه العترة تنتمي لجنس النوكارديية. كما أوقعت نتائج تحليل الحامض النووي الرايبوسومي (16S rRNA) هذه العترة مع "النوكارديية الواليسية" في الفرع من شجرة الوراثة الذي يضم مجموعة "النوكارديية الترانسفالية" المقاومة لعقار الاميكاسين. نستنتج أن داء النوكاردييات غالباً ما يتم تجاوزه في العيادة أو يفشل المختبر في التعرف عليه. تخلص الدراسة أيضاً إلى أن إمكانية التعرف على وتمييز أنواع النوكارديية بالطرق المظهرية غالباً ما يكون مضني ولا يحدد الأنواع بشكل قاطع ولكن التمييز الفاعل يتم بواسطة الطرق الجزيئية.

This study aimed to clarify the taxonomic position of an actinomycete isolated from an HIV-positive male patient with pulmonary complications in Asir, southern region of Saudi Arabia. The strain was found to have phenotypic properties typical of nocardiae and 16S rRNA gene analysis clustered the isolate with *Nocardia wallacei* (accession KC677696) in the phylogenetic branch of the amikacin resistance *Nocardia transvalensis* complex. We consider that nocardiosis is usually missed or misdiagnosed clinically and recognition of these bacteria based on phenotypic tests is strenuous, but definitive identification is attainable by molecular methods.

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*Nocardia* is the most important aerobic actinomycetes and nocardiosis should be included in the differential diagnosis of any chronic pneumonia that does not respond to empirical or common antimicrobial regimens.<sup>1</sup> Several species of the genus *Nocardia* are the causal agents of many diseases mainly with pulmonary and cutaneous manifestations. *Nocardia transvalensis* (*N.transvalensis*) was first isolated in 1927 from a mycetoma in a South African.<sup>2</sup> *Nocardia transvalensis* and related species were found to have high levels of antibiotic resistance, especially to the aminoglycosides.<sup>3</sup> Attention from clinicians and laboratory technicians should be paid, as well, towards isolating and identifying infrequent and fastidious pathogens such as *Nocardia*. Such pathogens are easily misdiagnosed or passed undiagnosed or being overgrown by rapidly growing bacteria and saprophytic fungal contamination<sup>4</sup> This study aims to clarify the taxonomic position of an actinomycete isolated from a male HIV-positive patient with pulmonary complications in Asir, Kingdom of Saudi (KSA).

**Case Report.** A 54-year-old male was presented to Asir Central Hospital, Abha, KSA, with chronic pulmonary illness during 2007. The patient was HIV positive and the chest x-rays revealed multiple nodular lesions, non-symmetrical interstitial, and airspace infiltrates and consolidation. Bronchoalveolar lavage specimen was submitted to microbiology laboratory and processed according to standard methods.<sup>5</sup> Growth of the causative agent was obtained in mycosel gar (BBL Microbiology Inc, USA) after 3 days under aerobic condition at 30°C, and subsequently sub-cultured for identification onto bacteriological media including blood agar, glucose malt extract yeast extract agar, tryptic soya agar and Lowenstein Jensen media. The colonial morphological properties of the grown culture indicated an actinomycete-type of organisms with chalky grey-white, rough, wrinkled deeply embedded into agar

and dry colonies which produced aerial hyphae (Figure 1A). Smears made from grown culture revealed gram positive and partially acid-fast branching filamentous organism when stained with modified Ziehl-Neelsen method. The filaments fragment into short chains of rods and coccobacillary elements which is characteristic for members of the genus *Nocardia* (Figure 1B).

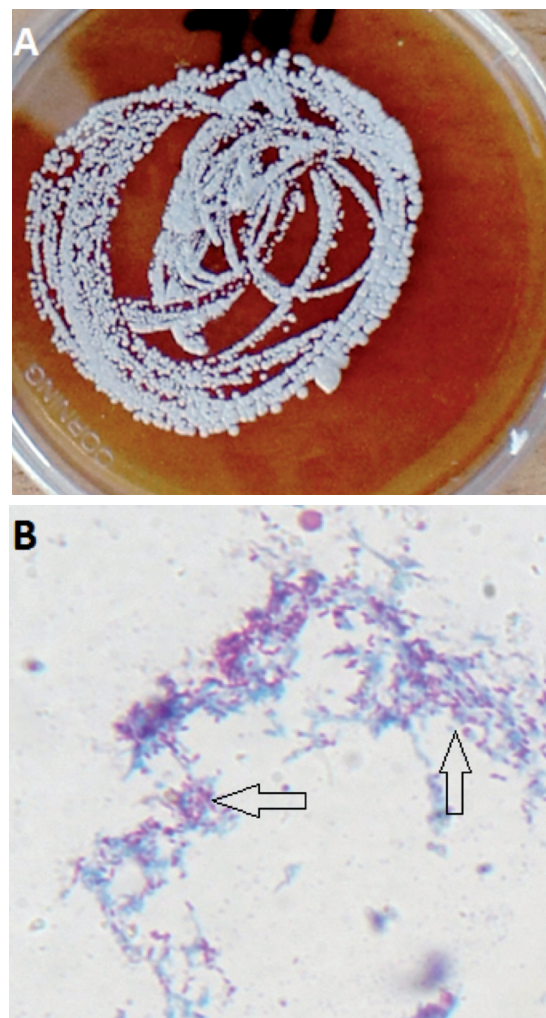
The organism was catalase and urease positive, liquefied gelatine, did not hydrolyze citrate, and was esculin positive. It fermented glucose but not arabinose, inositol, mannitol, rhamnose, sorbitol, or sucrose. The isolate was found susceptible to cefepime, ciprofloxacin, co-trimoxazole, and moderate susceptibility to ampicillin, cephalothin and tetracycline; but resistant to amikacin, aztreonam, bacitracin, ceftazidime, chloramphenicol, fusidic acid, gentamicin, imipenem, methicillin, nalidixic acid, neomycin, nitrofurantoin, norfloxacin, penicillin G, polymyxin B, and vancomycin. The complete 16S rRNA gene sequence was determined by direct sequencing of PCR-amplified 16S rRNA. Genomic DNA extraction was carried out using the MasterPure™ Gram Positive DNA Purification Kit (Epicentre Biotechnologies, USA) according to the manufacturer's instructions. Polymerase chain reaction mediated amplification of the 16S rDNA and purification of the PCR product was carried out as described previously.<sup>6</sup> Purified PCR products were sequenced using the CEQTM DTC-Quick Start Kit (Beckmann Coulter, Krefeld, Germany) as directed in the manufacturer's protocol. Sequence reactions were electrophoresed using the CEQTM 8000 Genetic Analysis System.

Obtained 16S rDNA nucleotide sequences data (1514 bp; accession no. KC677696) were tested on the BLAST electronic system (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to establish a quick phylogenetic position. Following an assignment of the isolate with *Nocardia* spp. in the BLAST system, the sequences were analyzed using PHYDIT for Windows (Version 3.1, J. Chun) and in comparison to all known sequences of *Gordonia* spp. found in GenBank database (<http://www.ncbi.nlm.nih.gov/nucleotides>).

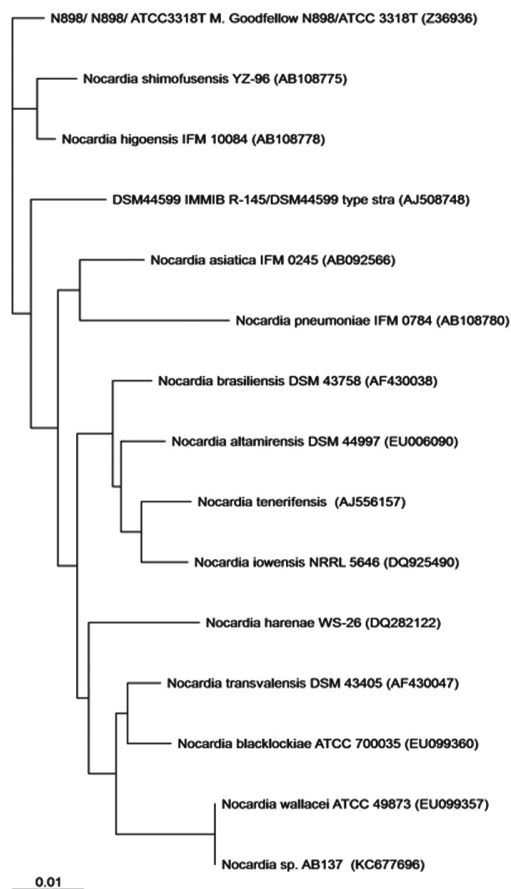
Comparison of the complete 16S rDNA sequences of the isolate AB137 with corresponding nucleotide sequences of representatives actinomycetes confirmed that they belong to the genus *Nocardia*. 16S rDNA gene sequencing data indicated that the strain fall within the phylogenetic branch that accommodates members of the genus *Nocardia*. The strain has a 100% similarity value with *Nocardia wallacei* (*N. wallacei*) in the 16S rDNA

gene. It revealed high similarities to the *N. transvalensis* (98.6%) and *Nocardia blacklockiae* (*N. blacklockiae*) (97.9%) (Figure 2).

**Discussion.** The isolation of *Nocardia* from a respiratory specimen is indicative of pulmonary nocardiosis.<sup>4</sup> In the present study, a species of *Nocardia* was isolated from a 54-year-old HIV positive patient who suffered a chronic pulmonary disease with multiple nodular lesions and consolidations. The patients failed to respond to empirical antifungal drugs since initial diagnosis was considered a fungal



**Figure 1** - Growth of isolate AB137 (DSM45846) on malt extract yeast extract agar after 7 days under aerobic condition. **A)** The growth indicates actinomycete-type of organisms with chalky grey-white, rough, wrinkled colonies which are embedded into agar. **B)** Smears made from grown culture revealed partially acid-fast branching filaments which fragmented into short chains of coccoid elements (arrows).



**Figure 2** - A phylogenetic tree based on 16S rDNA sequences showing relationships and position of *Nocardia* spp. isolates AB137 to closely related members of the genus *Nocardia* and the *Nocardia transvalensis* complex. The scale bar indicates 0.01 substitutions per nucleotide position

infection. The symptoms of the patient in coincidence with the failure to respond to antifungal drugs and a successful treatment (empirically) with sulphonamide, are coherent with the observation that the patient was suffering from pulmonary nocardiosis, not mycosis. The *in vivo* treatment agreed with the *in vitro* antimicrobial testing results. These findings are also in line with earlier reports that a substantial proportion of patients that exhibit chronic lung diseases in many developing countries are suffering from pulmonary nocardiosis.<sup>1</sup>

*Nocardia wallacei* is a newly described species.<sup>7</sup> It is the most commonly isolated member of the *N. transvalensis* complex in the United States.<sup>3,7</sup> No record of isolation neither of *N. wallacei* nor of other member of the *N. transvalensis* complex in Saudi Arabia. In their review of 10 years, Al-Jahdali et al,<sup>8</sup> concluded that nocardiosis is common in KSA and cases are not restricted to immuno-compromised individuals. In

a review of nocardiosis in KSA, Hakawi and Rabiah<sup>9</sup> identified *Nocardia asteroides* (58%), *Nocardia brasiliensis* (21%), and *Nocardia otitidiscaviarum* (21%) as common causal agents of nocardiosis predominantly from lungs and from patients with renal transplant. Our isolate from Asir region in KSA, represents a first record of such pathogen in Saudi Arabia. It showed a 100% similarity with *N. wallacei* and share all the tested phenotypic characteristics notably resistance to amikacin and imipenem, susceptibility to ciprofloxacin and ceftriaxone; hydrolysis of esculin and urea; and inability to hydrolyse casein, tyrosine and xanthine. Differentiation between members of the *N. transvalensis* complex and other species of the genus *Nocardia* using morphological or physiological tests is considered inadequate. As the identification was based on phenotypic characterization is laborious and time-consuming and in many situations is not conclusive, definitive identification is achieved by sequence analysis notably 16S rDNA gene.<sup>10</sup>

Strains of *N. wallacei* that have been described by Conville et al<sup>7</sup> and the present strain AB137 are resistant to the frequently used antimicrobial agents notably amikacin and the other agents listed above. Therefore, accurate diagnosis and *in vitro* testing results of *Nocardia* spp. of clinical origin is particularly important for patient's health. As it has been argued previously<sup>3,7</sup> there are considerably variations between results both at inter-laboratory and intra-laboratories levels. *Nocardia transvalensis* complex, like other nocardiae, infects both compromised and non-immune compromised individuals.<sup>8</sup> This report represents the second one after a first from USA.<sup>7</sup> Therefore, such pathogen is regarded as "a rare" or could be misidentified in routine laboratories given the fact that routine isolation and biochemical characterization are usually not enough to establish species names. The strain AB137 was safely deposited in the German collection of microorganisms and cell cultures (Braunschweig, Germany) and was given the accession number DSM45846.

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## References

1. Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ Jr. Clinical and laboratory features of the *Nocardia* spp. based on current molecular taxonomy. *Clin Microbiol Rev* 2006; 19: 259-282.



2. Pijper A, Pullinger BD. South African nocardiasis. *J Trop Med Hyg* 1927; 32: 153-156.
3. Wilson RW, Steingrube VA, Brown BA, Blacklock Z, Jost KC Jr, McNabb A, et al. Recognition of a *Nocardia transvalensis* complex by resistance to aminoglycosides, including amikacin, and PCR-restriction fragment length polymorphism analysis. *J Clin Microbiol* 1997; 35: 2235-2242.
4. Saubolle MA, Sussland D. Nocardiosis: review of clinical and laboratory experience. *J Clin Microbiol* 2003; 41: 4497-4501.
5. Isenberg HD, editor. *Essentials Procedures for Clinical Microbiology*. Washington (DC): ASM Press; 1998. p. 171-359. 1998.
6. Rainey FA, Ward-Rainey N, Kroppenstedt RM, Stackebrandt E. The genus *Nocardopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage; proposal of *Nocardopsisaceae* fam. nov. *Int J Sys Bacteriol* 1996; 46:1088-1092.
7. Conville PS, Brown JM, Steigerwalt AG, Brown-Elliott BA, Witebsky FG. *Nocardia wallacei* sp. nov. and *Nocardia blacklockiae* sp. nov., human pathogens and members of the "Nocardia transvalensis complex". *J Clin Microbiol* 2008; 46: 1178-1184.
8. Al-Jahdali H, Baharoon S, Alothman S, Memish Z, Waness A. Nocardiosis in a tertiary care hospital in Saudi Arabia. *J Glob Infect Dis* 2011; 3: 128-132.
9. Hakawi AM, Al Rabiah FA. Clinical pattern of nocardiosis in Saudi Arabia: a case series. *East Mediterr Health J* 2008; 14: 966-971.
10. Trujillo ME, Goodfellow M. Numerical phenotypic classification of clinically significant aerobic sporoactinomycetes and related organisms. *Antonie Van Leeuwenhoek* 2003; 84: 39-68.

#### Related Articles

Parveen S, Khan HS, Sabzposh NA. Screening of human immunodeficiency virus positive women for abnormal cell cytology by Pap smear. *Saudi Med J* 2011; 32: 1078-1079.

Alim A, Artan MO, Baykan Z, Alim BA. Seroprevalence of hepatitis B and C viruses, HIV, and syphilis infections among engaged couples. *Saudi Med J* 2009; 30: 541-545.

Arslan U, Tuncer I, Uysal EB, Inci R. Pleuropulmonary and soft tissue *Nocardia cyriacigeorgici* infection in a patient with Behcet's disease. *Saudi Med J* 2007; 28: 1435-1437.