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First report of isolation of *Nocardia otitidiscaviarum* from hospital water

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ABSTRACT

Nocardia otitidiscaviarum DSM43242 was first isolated as a novel species in 1924 from infected middle ear of guinea pig. Since then, there have only been a lot number additional reports for example from Spain, the USA, French and Japan, on the isolation of this species from clinical specimens such as pulmonary infection, disseminated infection, cutaneous infection and primary brain abscess. *Nocardia* infection is mainly associated with immunocompromised patients, but sometimes may appear in healthy humans. We herein present the first report on isolation of this organism from hospital water, which represents the environmental resources as a source of risk for people especially the hospital environment. The isolate NR4 was subjected to identification by conventional phenotypic tests such as resistance to lysozyme and molecular tests including genus-specific PCR for *Nocardia* based on partial *16S rDNA* gene and sequencing analysis of 16S rDNA. Our finding of *16S rDNA* gene sequence of the studied strain were identical and showed 100% similarties with those of *Nocardia otitidiscaviarum*.

1. Introduction

Nocardia species are Gram-positive, non-spore, filamentous, branching, obligatory aerobic and relatively slow-growing bacteria[1]. *Nocardia otitidiscaviarum* DSM43242 (*N. otitidiscaviarum*) was first isolated as a novel species in 1924 from infected middle ear of guinea pig[2]. *Nocardia* species are found worldwide, in soil and water. These organisms mainly enter the human body via the inhalation, but sometimes infections are caused by traumatic percutaneous inoculation, causing primary cutaneous disease. *Nocardia* species are disseminated to many organs, specially brain, through the blood circulation. This mainly happens in immunocomprimised patients[3,4]. Reports indicate a wide range of diseases which are caused by *N. otitidiscaviarum* such as pulmonary infection in Spain, disseminated infection in the USA, cutaneous infection in French and primary brain abscess in Japan[5-8].

The current report described that environmental isolate was identified as *N. otitidiscaviarum* by a variety of phenotypic and molecular tests.

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2. Materials and methods

2.1. Water sampling

The water sample was collected between November 2013 and May 2014 from hospitals in the Isfahan Province. The strain (NR4) isolated from the tap water in children department of specialized chemotherapy hospital.

The hospital water had a temperature of 29 °C with 0.1 mg/L chlorine residual, 412 mg/L total dissolved solids and 7.5 pH.

The water sample was processed according to described methods[9]. In brief, each 1000 mL sample was transported at 4 °C in our research laboratory and processed within 24 h and it was filtered by using 45 μm pore size cellulose nitrate filter (Sartorius AG, Gottingen, Germany). The filter was rinsed and macerated in 3 mL sterile distilled water. Aliquots (0.1 mL) was transfered into McClung media with paraffin and incubated at 28, 37 and 42 °C.

2.2. Microbiological analysis

Canyon staining of colony was confirmed as partial acid-fast coco bacilli and filament, and those colonies were subcultured on Sauton's media to obtain a pure color for identification. The *Nocardia* species isolated was subjected to identication by conventional phenotypic tests, standard culture and biochemical assay[1].

2.3. Molecular analysis

For molecular identification, the chromosomal DNA was extracted as described earlier with specific modification to facilitate the cell wall lysis of *Nocardia*[9]. For the *Nocardia*-specific PCR amplification protocol targeting a 596-bp region, partial of the *16S rDNA* gene [genus-specific primers (NG1 and NG2)] was carried out according to optimal PCR amplification condition recommended by Laurent *et al*.[10].

The amplification and direct sequence analysis of 16S rDNA were carried out as described previously[11]. The obtained sequence was aligned manually with all exisiting sequence of rapidly growing *Nocardia* retrieved from Genbank database and compared by using Ribosomal Database Project[12].

3. Results

N. otitidiscaviarum isolate, *i.e.*, NR4 was identified in one sample of the 75 hospital water samples. The isolate was recovered on Sauton's media after direct culture of water samples. Based on phenotypic characteristics, the isolate was growing at 28, 37 and 42 ℃, and had the white pigmentation, positive tests for resistance to lysozyme and decomposition of xanthine and hypoxanthine, but negative for tyrosine. The genus-specific PCR amplification recommended by Laurent *et al*. reliably produced a 596-bp amplification partial of the *16S rDNA* gene [genus-specific primers (NG1 and NG2)] of the genus, which was confirmed that the isolate belonged to genus *Nocardia*[10]. The *16S rDNA* gene sequence of the studied strain was identical and showed 100% similarities with those of *N. otitidiscaviarum*. The relationship between our isolate and *N. otitidiscaviarum* was supported by phylogentic tree of 16S rDNA and the high bootstrap value obtained using the neighbor-joinning method.

4. Discussion

Nocardia species are found worldwide, maintaining a saprophytic existence in soil and water, these opportunistic pathogenic bacteria cause infections in humans and animals[13,14]. It is important to specify the source of potentially pathogenic *Nocardia*, as the management and the performance of epidemiological control method must reverberate the natural monastery and mode of transmition of *Nocardia* species encountered, so that we can prevent deadly diseases such as brain abscess in humans[14].

N. otitidiscaviarum was first isolated as a novel species in 1924 from infected middle ear of guinea pig. It is an aerobic, Gram-positive, partial acid alcohol fast, nonmotile organism, which optimally grows in at 28 ℃ and has white pigmentation, positive tests for resistance to lysozyme and decomposition of xanthine and hypoxanthine, but was negative for tyrosine. The organism has uniqe *16S rDNA* gene sequence and distinct fatty and mycolic acid patterns[2]. Consistent with the phenotypic feature, the molecular tests used in the current study provided an evidence that the isolate NR4 belongs to genus *Nocardia* and shows the highest similarities in term of 16S rDNA sequence to those of type strain of *N. otitidiscaviarum.*

5. Conculsion

We isolated *N. otitidiscaviarum* from water hospital first, because water plays a major role in the transmission of microorganisms. And because of illness in the elderly are immunocompromised, their identification is important in water resources, which in turn might be helpful in understanding of the dynamic of transmission and infection in human.

Moreover, the molecular techniques are more sensitive, specific and faster compared with phenotypic methods in the diagnosis of *Nocardia* species. Therefore, in addition to genus-specific primers (NG1 and NG2) for confirming and the application of a more complicated molecular tests, 16S rDNA sequence showed conclusive in accurate identification of the species isolated.

Conflict of interest statement

We declare that we have no conflict of interest.

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**: 259-82.

[2] Leibniz Institute Deutsche Sammlung von Mikroorganismen und Zellkulturen. Leibniz Institute DSMZ-German collection of microorganisms and cell cultures. Braunschweig: Leibniz Institute Deutsche Sammlung von Mikroorganismen und Zellkulturen; 2014. [Online] Available from: https://www.dsmz.de/ [Accessed on 21st Apr, 2016]

[3] Clark NM, Braun DK, Pasternak A, Chenoweth CE. Primary cutaneous *Nocardia otitidiscaviarum* infection: case report and review. *Clin Infect Dis* 1995; **20**(5): 1266-70.

[4] Lerner PI. Nocardiosis. *Clin Infect Dis* 1996; **22**: 891-903.

[5] Pelaez AI, Garcia-Suarez Mdel M, Manteca A, Melon O, Aranaz C, Cimadevilla R, et al. A fatal case of *Nocardia otitidiscaviarum* pulmonary infection and brain abscess: taxonomic characterization by molecular techniques. *Ann Clin Microbiol Antimicrob* 2009; **8**: 11.

[6] Sharma M, Gilbert BC, Benz RL, Santoro J. Disseminated *Nocardia otitidiscaviarum* infection in a woman with sickle cell anemia and end-stage renal disease. *Am J Med Sci* 2007; **333**(6): 372-5.

[7] Dekeyser S, Corroyer-Simovic B, Cachia M, Gillot C, Senneville E, Descamps D. [*Nocardia otitidiscaviarum*, cutaneous infection in a patient receiving long-term corticosteroid treatment]. *Ann Biol Clin (Paris)* 2003; **61**(2): 219-22. French.

[8] Ishihara M, Takada D, Sugimoto K, Oguro H, Gonoi T, Akiyama Y, et al. Primary brain abscess caused by *Nocardia otitidiscaviarum*. *Intern Med* 2014; **53**(17): 2007-12.

[9] Bafghi MF, Eshraghi SS, Heidarieh P, Habibnia S, Nasab MR. DNA extraction from *Nocardia* species for special genes analysis using PCR. *N Am J Med Sci* 2014; **6**(5): 231-3.

[10] Laurent FJ, Provost F, Boiron P. Rapid identification of clinically relevant *Nocardia* species to genus level by *16S rRNA* gene PCR. *J Clin Microbiol* 1999; **37**: 99-102.

[11] Shojaei H, Heiderieh P, Hashemi A, Feizabadi MM, Daei Naser A. Species identification of neglected nontuberculous mycobacteria in a developing country. *Jpn J Infect Dis* 2011; **64**: 265-71.

[12] Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, et al. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Res* 2009; **37**: D141-5.

[13] Cercenado E, Marín M, Sánchez-Martínez M, Cuevas O, Martínez-Alarcón J, Bouza E. *In vitro* activities of tigecycline and eight other antimicrobials against different *Nocardia* species identified by molecular methods. *Antimicrob Agents Chemother* 2007; **51**(3): 1102-4.

[14] Kurup PV, Sandhu RS. Isolation of *Nocardia caviae* from soil and its pathogenicity for laboratory animals. *J Bacteriol* 1965; **90**(3): 822-3.