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Case Report

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Isolation of *Mycobacterium frederiksbergense* From Redundant Tap Water: A Case Report

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Abstract
Non-tuberculosis mycobacteria (NTM) are saprophytic bacteria in environmental resources such as water and soil. The presence of atypical mycobacteria in hospital resources may lead to infections and the spread of aerosol particles through ventilation systems, wind, and even drinking water. Therefore, control of contamination of environmental resources in hospitals is one of the most important approaches to reduce and manage NTM nosocomial infections. This study reported the isolation of *Mycobacterium frederiksbergense* from a tap water sample, which is considered important for clinical and biodegradation aspects. The isolated bacterium was identified using phenotypic features and 16S rRNA sequencing. This report verified the necessity to identify the presence of NTM in water and to find a solution for controlling such contaminations.

Keywords: Mycobacterium frederiksbergense, 16S rRNA sequencing, Biodegradation, Infection

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1. Introduction

Non-tuberculosis mycobacteria (NTM), also known as atypical mycobacteria, are environmental microorganisms or saprophytic bacteria that live in environmental resources such as soil, dust, water, lake, river, and on plants and animals. They have been recognized since 1950s as a cause of human diseases especially immune disorders. In recent years, we have seen the rising numbers of case reports on NTM infection in the world (1-3).

Mycobacterium frederiksbergense is a rapidly-growing *Mycobacterium* species which was isolated from coal tar contaminated soil in Denmark as biodegrading *Mycobacterium* species in 2001 by Willumsen et al (4). Based on the literature, *M. frederiksbergense* was isolated from environmental resources as a candidate for the degradation of contaminants in soil and clinical specimens from cutaneous to disseminated infections in immunodeficiency patients (4-6). Given that there was no evidence for person to person transmission of NTM infection, it seems that environmental resources in hospitals are responsible for outbreak of infection in hospitalized patients and invasion to susceptible hosts. Therefore, control of hospital-acquired infections in healthcare facilities depends on the identification of the source of infections (1,7). Thus, isolation, identification, and differentiation of NTM are important for decomposition or biodegradation of contaminants, final diagnosis, appropriate treatment, and epidemiological investigations (1,4,7).

Despite a lot of studies on the isolation of NTM from clinical samples, this study reported the isolation of an uncommon NTM species which survives in a severe and adverse condition such as tap water; a condition on which there is few reports. The present report described an environment–isolated species which was identified as a novel strain of *M. frederiksbergense* using phenotypic and molecular tests. This might be useful in determination of the natural habitat of this uncommon species. Based on the results of the study, it can be assumed that environmental resources, especially water is possible to play a key role in the transmission of NTM infections.

2. Materials and Methods

The water samples were collected between April and July 2017 from water resources of Isfahan, Iran (including hospitals, public places, drinking water, and tap water) and treated with cetylpiridinium chloride (CPC) based on the previous reports (8). At first, the samples were transported to the laboratory in cool and sterile condition and disinfected with CPC 0.05% and filtered using sterile syringe filters; hydrophilic membrane with a 0.45 µm pore size. The filters were transferred in 5 mL sterile double distilled water and centrifuged at 10000 rpm for 5 minutes, then sediments were transferred to Lowenstein-Jensen (LJ) medium, and incubated at 37°C and 5% CO_2. One isolate coded as WM24 was subjected to identification by conventional tests including acid-fast staining, growth at different temperatures, growth

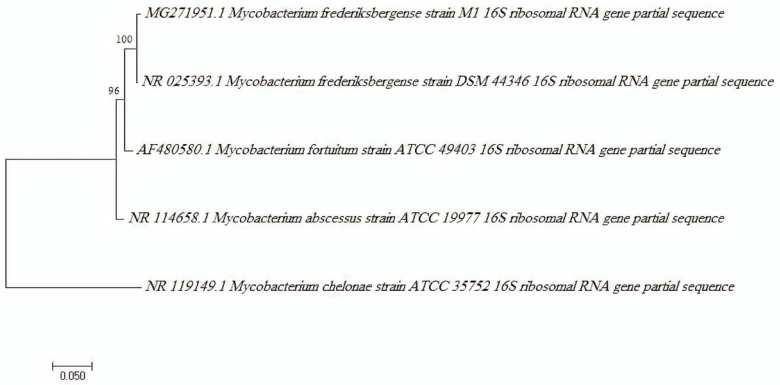

Fig. 1. 16S rRNA sequence-based phylogenetic tree of the water isolate of *Mycobacterium frederiksbergense* compared with those of closely-related rapidly-growing mycobacterial species, calculated by the neighbor-joining method and K2P distance.

rate, pigment construction, Tween 80 hydrolysis, growth on MacConkey agar, nitrate reduction, iron uptake, urease activity, pyrazinamidase, arylsulfatase (3 days), niacin accumulation, heat labile catalase, and heat stable catalase tests (9). Chromosomal DNA was extracted using a simple boiling method. In brief, mycobacterial suspension was added to TE buffer and boiled at 100°C for 7 minutes, centrifuged at 13000 rpm for 10 minutes and the supernatant was transferred to a new Eppendorf microtube as DNA for polymerase chain reaction (PCR) amplification. The genus and species of WM24 was identified using a genus-specific PCR based on the full length study of the 16S rRNA gene as recommended by Rogall and colleages followed by the direct sequencing (10). Sequencing was performed by Bioneer Corporation (South Korea) and the sequence data received was aligned manually with the existing sequences of non-tuberculosis mycobacterial species recovered from GenBank database. This isolate was a novel strain of *M. frederiksbergense*. The GenBank accession number of this isolate was MG271951 as *M. frederiksbergense* M1.

3. Results and Discussion

The *M. frederiksbergense* isolate, or WM24, was isolated from tap water, sampled from a public place. It was grown on LJ medium. According to the conventional features, the isolate was gram-positive, acid-fast, rapid growth, scotochromogenic mycobacterial species which had yellow pigment, positive results for catalase, Tween 80 hydrolysis and nitrate reductase, while negative for urease and growth on MacConkey agar tests. Given the phenotypic features, WM24 is similar to rapid-growing mycobacteria. The approximately full length of 16S rDNA gene sequences (1080 base pairs) of WM24 showed 99% similarity with *M. frederiksbergense* strain DSM 44346 [NR_025393.1] (4). This value corresponds to 6 nucleotide differences at positions 97, 98, 292, 494, 587, 824 and 969 compared with the corresponding sequences of *M. frederiksbergense* (4). Furthermore, existence of the short helix 18 in the 16S rRNA gene of the isolated species showed the molecular exclusive marker of rapidly growing mycobacterial species (11). The relationship between our isolates and *M. frederiksbergense* was supported by phylogenetic tree of 16S rDNA and by the high bootstrap value obtained using the neighbor-joining method (Fig. 1).

NTM are ubiquitous; several species of NTM were isolated from various clinical specimens which could cause different infections including localized cutaneous, pulmonary, wound, catheter-related, and disseminated infections (1,2). According to the similarity of clinical manifestations and radiological findings between NTM pulmonary infections and tuberculosis, identification and differentiation of NTM pulmonary infection from tuberculosis is necessary (9). In developing countries, NTM infection is diagnosed as tuberculosis; and considering that treatment of tuberculosis and NTM infections are different, these patients are not treated and considered as drug resistant tuberculosis patients (9,12).

Mycobacterium frederiksbergense isolate was recovered from redundant tap water of public places in Isfahan city between April and July 2017. The recorded temperature and pH of this water sample were 19°C and 7.54, respectively. *M. frederiksbergense* was first identified in 2001. It is an aerobic, gram-positive, acid-fast, scotochromogenic colony, yellow pigment, which grows between 15°C and 37°C. It shows a positive reaction against catalase and nitrate reduction, but is negative against urease. Tween 80 is hydrolysed but no growth occurs on MacConkey agar. *M. frederiksbergense* has an exclusive 16S rRNA gene sequence and unique mycolic acid pattern (4). According to the results of conventional features and molecular test, the isolate WM24 known as *M. frederiksbergense is* based on the highest similarity of the 16S rRNA sequence. Isolation and identification of NTM species plays a key role in bioremediation and biodegradation process and elimination of contaminants from soil. NTM species are important in improvement of public health, especially *M. frederiksbergense* which is capable in degrading polycyclic aromatic hydrocarbons (PAHs), anthracene, heavy

metals, and different contaminants (4,13).

4. Conclusion

Based on the findings of this study, environmental resources especially water sources are probably one of the most important natural habitats of NTM and the route for transmission of mycobacterial infections to patients with immune disorders, even healthy individuals. Moreover, identification and differentiation of NTM using conventional tests are time-consuming, laborious and need trained technicians whereas, molecular methods such as PCR-RFLP and sequencing are reliable, rapid and accurate in identifying NTM species and useful for management of NTM infections.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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