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First isolation of *Mycobacterium setense* from hospital water

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ABSTRACT

Objective: To present the findings of a study on isolation of four unrelated environmental strains of *Mycobacterium setense* (*M. setense*) from hospital environment and help to assess the natural habitat and the mode of transmission in man.

Methods: The water samples were collected from hospital departments and cultured on Löwenstein-Jensen and Sauton's media. The isolates, *i.e.*, AW3-2, AW5, AW11 and AW18 were subjected to identification by conventional and molecular tests including sequencing analysis of 16S rRNA.

Results: The water isolates revealed the phenotypic and molecular features which were consistent with *M. setense* including a genus specific amplicon of the *hsp65* gene and 99.6% similarities with those of *M. setense* CIP: 109395T 16S rRNA gene sequences.

Conclusions: The current report will contribute to a better understanding of the pathogenesis and path of transmission of this opportunistic pathogen to human.

1. Introduction

In recent years, nontuberculous mycobacteria (NTM) have emerged as an important cause of opportunistic nosocomial infections and pseudo-infections[1-4]. *Mycobacterium setense* (*M. setense*) is an emerging organism of the *Mycobacterium fortuitum* (*M. fortuitum*) group that was added to the growing list of rapidly growing mycobacteria isolated from humans in 2008. It was firstly isolated and characterized from a patient with a post-traumatic chronic skin abscess associated with osteitis[5]. Apart from two clinical reports from France and Iran, there have been no ecological data in the natural habitat of this species since its original isolation and characterization[6,7].

This investigation presents the findings of a study on isolation of four unrelated environmental strains of *M. setense* from hospital environment and helps to assess the natural habitat and the mode of transmission in man.

2. Materials and methods

2.1. Water samplings and microbiological analysis

The water samples were collected between September 2011 and December 2012 and processed according to the standard methods[8]. In brief, the samples were transported to the laboratory and decontaminated with cetylpiridinium chloride, and filtered with sterile microbiological cellulose nitrate filters before rinsing in sterile distilled water. The samples were then inoculated on Lowenstein-Jensen and Sauton's media, and incubated at 25 °C, 32 °C and 37 °C. The four isolates named AW3-2, AW5, AW11

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and AW18 were then identified by conventional phenotypic and biochemical tests including Ziehl-Neelsen staining, growth rate, pigment production, Tween opacity, nitrate reduction, iron uptake, urease, pyrazinamidase and niacin accumulation tests[9].

Molecular identification chromosomal DNA was obtained by a modified method of Pitcher *et al.*[10]. A genus specific PCR amplification for mycobacteria detecting a 228-bp fragment of the 65-kDa heat shock protein (*hsp*) gene was carried out as what was recommended by Khan and Yadav[11]. The species identification of the isolates were determined by amplification and direct sequence analysis of partial length of 16S rRNA as described previously[4]. The obtained sequences were aligned with the relevant sequences of the rapidly growing mycobacteria and analyzed using the jPHYDIT program[12]. The GenBank accession number for the 16S rRNA of the representative isolate AW3-2 determined in this study is KF019693.

3. Results

The *M. setense* isolates were isolated from four different hospitals in two cities, AW3-2, AW5 and AW11 were recovered between September 2011 and December 2012 respectively from the tap running water in an otolaryngology department, and from the hot tap water of uncirculated tanks in the neurosurgery department in Isfahan City, the isolate AW18 was recovered from the tap running water of a pediatrics department in Khoram Abad City.

The recorded temperature, pH, chlorine residual and total dissolved solids for the water samples from which the strains, *i.e.*, AW3-2, AW5, AW11 and AW18, were isolated were 22, 20, 38 and 26 ˚C; 7.1, 7, 7.6 and 6.8; 0.2 , 0.4, 0.3 and 0.6 mg/L; and 415, 380, 363 and 392 mg/L, respectively.

The isolates were rapidly growing (< 7 days) non-pigmented species growing at 25 ˚C, 32 ˚C and 37 ˚C with white color colonies, positive reactions for arylsulfatase, catalase, urease, tellurite tests and pyrazinamidase and growth in 5% NaCl and was resistant to *p*-nitro-benzoic acid but negative reaction for nitrate reductase, Tween opacity and niacin. They were unable to grow on MacConkey agar without crystal violet. Based on the phenotypic profile, the isolates were considered rapidly growing non-pigmented mycobacteria (Table 1).

All isolates produced a genus specific 228-bp amplicon of the *hsp65* gene that confirmed the identity of the isolates as *Mycobacterium*. The partial 16S rRNA gene sequences (1012 bp) of the isolate revealed 99.6% similarities with those of *M. setense* CIP 109395^T (DSM 45070^T). This represents four nucleotide differences at positions 481, 493, 917 and 918 (*Escherichia coli* numbering) compared to the corresponding sequences of the type strain of *M. setense*. The first hypervariable signature sequences of the isolates (positions 128-270 *Escherichia coli* numbering) were identical to those of the type strain of *M. setense*. Moreover, the presence of the short helix 18 in the *16S rRNA* gene of the isolated represented the typical molecular signature of rapidly growing mycobacteria[13]. The phylogenetic tree constructed using 16S rRNA sequences and the high bootstrap value obtained using the neighbor-joining method indicated the close relationship between our isolates and *M. setense* (Figure 1).

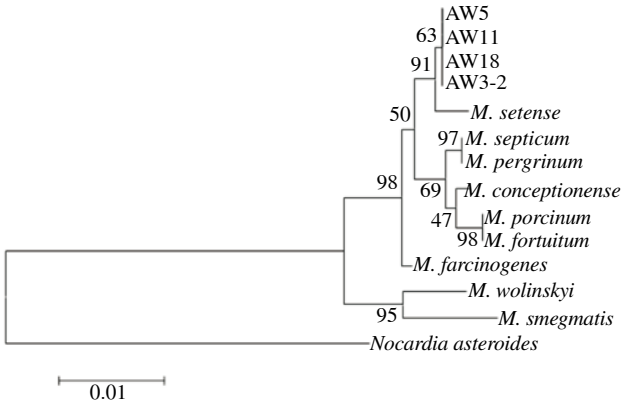

Figure 1. 16S rRNA sequence based phylogenetic tree for water isolates of *M. setense* and closely related reference species of rapidly growing mycobacteria by using the neighbor-joining method. The figures at each node represent bootstraping values. The tree was rooted with *Nocardia asteroides*. *M. septicum*: *Mycobacterium septicum*; *M. pergrinum*: *Mycobacterium pergrinum*; *M. conceptionense*: *Mycobacterium conceptionense*; *M. porcinum*: *Mycobacterium porcinum*; *M. farcinogenes*: *Mycobacterium farcinogenes*; *M. wolinskyi*: *Mycobacterium wolinskyi*; *M. smegmatis*: *Mycobacterium smegmatis*.

Table 1
Differential phenotypic characteristics of Iranian *M. setense* (AW3-2, AW5, AW11 and AW18) and related rapidly growing mycobacteria.

Test	Arylsulfatase (in 3 days)	Citrate utilization	Nitrate reduction	Growth in the presence of 5% NaCl	High catalase activity (> 45 mm)	Pyrazinamidase
M. fortuitum CIP 104534^T	+	-	+	+	+	+
Mycobacterium peregrinum CIP 105382^T	+	-	+	+	+	+
Mycobacterium conceptionense CIP 108544^T	+	-	+	+	+	+
Mycobacterium chelonae ATCC 19977^T	+	+	-	+/-	+	+
Mycobacterium setense DSM 45070^T	+	-	+	+	+	+
Iranian isolates of *M. setense* (AW3-2, AW5, AW11 and AW18)	+	-	-	+	+	+

4. Discussion

NTM are ubiquitous in the environment. Several species of NTM are now recognized to be facultative parasites which are capable of causing chronic granulomatous diseases in human and animals. The human infections are clinically indistinguishable from tuberculosis and the causative organisms are microbiologically hard to differentiate from *Mycobacterium tuberculosis*[14]. NTM have also been associated with hospital acquired infection in health care settings[15]. However, little is known about the situations in which transmission occurs. The control of hospital acquired infection in the health care setting depends on persevering surveillance and identification of the source of infection[16].

This reports deals with the detection of a rare NTM, *M. setense*, in hospital water systems. It is an aerobic, Gram-positive, weakly acid-alcohol fast, asporogenous, non-motile non-pigmented organism growing at 30 ˚C and 37 ˚C. The organism differentiates from other mycobacteria by a unique 16S rRNA gene sequence and distinct fatty and mycolic acid patterns[5]. A battery of phenotypic and molecular tests particularly 16S rRNA sequencing applied in our study confirmed the identity of the isolates AW3-2, AW5, AW11 and AW18 as *M. setense* species. Until now, *M. setense* have not been recovered from the environment but only from clinical specimens. The presence of this rare *Mycobacterium* in hospital settings signifies the importance of implementation of effective control measures including high level of disinfection of water and critical medical devices for assuring the safety and integrity of patient care equipment in preventing transmission of such rare and resistant opportunistic pathogens.

Conflict of interest statement

We declare that we have no conflict of interest.

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