First report of isolation of *Mycobacterium elephantis* from bronchial lavage of a patient in Asia

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DECLARATIONS

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This case represents the first report on the isolation of a difficult-to-identify clinical isolate of *Mycobacterium elephantis* from Asia.

Case presentation

A 72-year-old woman was admitted to hospital because of chronic respiratory disease for approximately 2 months. Her major symptoms included dry cough and nocturnal fever (to 38°C). Her outpatient record indicated that she was prescribed amoxicillin by her general practitioner but no improvement was obtained. She had no history of infection with mycobacteria or contact with an elephant. Results of physical examination were unremarkable. The patient's tuberculin test was negative. Routine laboratory testing on admission revealed an elevated C-reactive protein (CRP) of 34 mg/L, and an erythrocyte sedimentation rate of 45 mm/hr. The chest X-ray examination showed a diffuse shadow in the right apex suggestive of pulmonary tuberculosis. The initial microbiological examination of bronchial lavage by the hospital laboratory revealed the presence of small coccobacillary acid-fast organisms. The bronchial lavage specimen was cultured on Löwenstein-Jensen medium for mycobacterial isolation. Since the growth of the isolate on Löwenstein-Jensen medium was slower than that for the majority of rapidly growing mycobacteria, and due to apical pulmonary lesions the patient was misdiagnosed as having tuberculosis and entered into the TB Register. After one month of antituberculosis therapy, the patient discontinued treatment due to extreme weakness and severe abdominal pain. Meanwhile the isolate was referred to our laboratory for definitive identification. Further investigation on repeated specimens revealed that the infecting micro-organism was a rapidly growing mycobacteria. The patient's treatment was changed from the antituberculosis regimen to a combination therapy consisting of amikacin and ciprofloxacin. After two months of treatment she recovered and has remained well ever since.

The conventional identification and drug susceptibility of the Iranian isolate, namely, 'M202' was achieved according to the standard procedures described previously.[1,2] The conclusive identification included molecular testing, i.e. the PCR restriction fragment length polymorphism analysis (PRA algorithm) of the *hsp*65[3] and direct sequencing analysis of almost full length of 16S rDNA[4] and 16S–23S internal transcribed spacer (ITS)[5] as well as partial sequencing of *hsp*65[3] and *rpo*B genes.[6] The GenBank accession numbers of *M. elephantis* determined in this work are as follow: GU142921, HM229788-90 for 16S rDNA, *rpo*B, *hsp*65 and ITS genes, respectively.

Based on the phenotypic characteristics, M202 was a scotochromogenic rapidly growing mycobacterial species which grew at 37°C and 45°C as well as on Löwenstein-Jensen medium with 5% NaCl. It was positive for the key biochemical tests including catalase, nitrate, urease, tween hydrolysis and tellurite reduction tests. It was resistant to rifampicin but susceptible to amikacin, clarithromycin, ciprofloxacin, ethambutol, isoniazid, doxicyclin, streptomycin, cefoxitin, sulfamethoxazol and imipenem. The main microbiological traits of the Iranian isolate resembled those of the human clinical strains of *M. elephantis* characterized by Turenne *et al.*[7] and Tortoli *et al.*[8]

In PRA method the isolate exhibited a unique pattern that was distinct from the previously

Reviewer
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Figure 1
Alignment of selected stretches of 16S rDNA gene of *Mycobacterium elephantis* strains

Alignment of selected stretches of 16SrDNA gene of *Mycobacterium elephantis* strains

			16S rDNA positions according to *E.coli* numbering system																										
Strain	Accession number	Source	18	19	20	464	466	467	601	602	603	1127	1128	1129	1257	1258	1259	1267	1268	1269	1293	1294	1295	1315	1316	137
DSM 44368[T]	AJ010747*	Elephant	C	A	T	C	T	G	T	T	C	G	T	C	T	T	C	T	C	A	G	T	T	T	C	C
F1-13900	AJ 536100**	Clinical specimen	C	C	.	.	.	A	.	C	.	.	C	.	.	G	.	.	G	.	.	G	.	.	G	.
GN-12198	GQ924944**	Clinical specimen	-	-	-	.	.	.	.	C	.	.	C	.	.	G	.	.	G	.	.	G	.	.	G	.
FI05187	DQ142668**	Clinical specimen	-	-	-	T	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NRCM 01-17	AF385898*	Clinical specimen	C	C	.	.	.	.	.	C	.	.	C	.	.	G	.	.	G	.	.	G	.	.	G	.
M 202	GU142921*	Clinical specimen	C	.	.	.	.	.	.	C	.	.	C	.	.	G	.	.	G	.	.	G	.	.	G	.

"*":Genbank; "**": EMBL
Dashes indicate: not determined; "." Indicates that the base pair was identical to that of type strain.

published patterns[3] as well as from those in our in-house library.

The 16S rDNA, *rpo*B and *hsp*65 gene sequences of the isolate showed 100%, 98.7% and 96.2% similarities with those of the validly published reference strains of *M. elephantis*, respectively. The hypervariable signature sequences of 16S rDNA of M202 were identical to those of the type strain of *M. elephantis* (Figure 1). The ITS sequence of M202 was unique compared with those of other validly published mycobacteria.

Discussion

Further to the phenotypic features, all molecular tests used in the current study provided the evidence that the Iranian isolate belongs to *M. elephantis* species.

M. elephantis is a rapidly growing mycobacteria first isolated from a lung abscess in a Sri Lankan elephant that died from chronic respiratory disease.[4] Human isolation of this species has been reported from the developed countries including Canadian, Italian and Belgian patient specimens.[7–9] It belongs to a group of clinical isolates of mycobacteria which has been evaluated as difficult-to-identify organisms by Springer *et al.*[10]

Our case represents the first report of isolation of *M. elephantis* from the human clinical specimen in a developing country. The elderly patient had no apparent underlying diseases. However, the risk factors for mycobacterial infection could not completely be excluded in the patient due to age.

The etiologic role of the isolate M202 might be inferred from the fact that acid fast bacilli were microscopically observed in two different bronchial lavage specimens of the patient. Furthermore, the isolate was recovered from the pure culture. Also, no such isolate was made in our laboratory in the past and during the same period of time. As for 15 previously reported clinical strains,[7–9] the clinical relevance of *M. elephantis* remains difficult to ascertain since the isolate is very rare and hard to identify. Increased awareness of this species might help to distinct this species from closely related mycobacteria to more exactly determine the role of *M. elephantis* in human infection.

In conclusion, this case re-affirms the fact stated by Tortoli *et al.*[8] that the environment is the most probable reservoir of *M. elephantis* for either human or animal infections. It is not limited to a certain geographic area. Furthermore, the key molecular markers when combined with the major microbiological traits provide a conclusive evidence for identification of the rare clinically significant non-tuberculous mycobacteria.

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