First report of osteomyelitis caused by the novel species *Mycobacterium mantenii*

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P1609

BACKGROUND

Mycobacterium mantenii is a slow growing, scotochromogenic NTM species, most closely related to *Mycobacterium scrofulaceum* that was described firstly in 2009 [1]. Little is known about its clinical significance, natural habitat and virulence. We report for the first time a case of chronic osteomyelitis caused by *M. mantenii* in an immunocompromised patient with sarcoidosis and diabetes mellitus.

CASE PRESENTATION

Case report: A 59 -year old woman was referred to the outpatient clinic of Infectious Disease Division, for the management of a chronic uncured inflammation on left forefinger nail, over the last eight months.
The patient had a history of sarcoidosis and liver failure caused from extensive hepatic granulomatus focuses, with an episode of hepatic encephalopathy one year ago. She also had mild renal failure as a complication of diabetes mellitus. Her medications included insulin, corticosteroids and thyroxin. She worked as housewife. She hadn't travelled outside Greece during the last three years and hadn't owned pets. No contact with swimming pool water was mentioned.
On physical examination, the patient appeared well. There was no fever, jaundice or lymphadenopathy. The cardiovascular and respiratory systems were intact. The hand radiograph revealed a destructive process at the final phalanx of the left forefinger (sequestra).

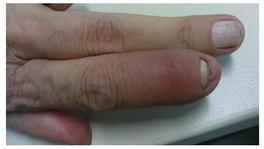

Fig 1. Picture of the left forefinger show the inflammation of the nail.

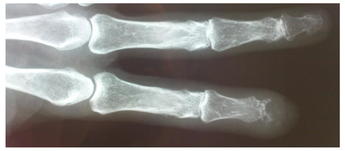

Fig 2. Radiograph of the left forefinger show the bony destruction of the final phalanx.

- A fine needle aspiration was performed from the lesion area and cultured for common bacteria fungi and mycobacteria.
- Cultures for common bacteria fungi were negative.
- Regarding mycobacterial cultures the specimen was processed by standard procedures [2] and incubated into Lowenstein- Jensen (LJ) slants at 30º C and 37ºC and into the MGIT960 automated system (Becton Dickinson).
- Ziehl-Neelsen stain was negative.
- After fifteen days of incubation, acid-fast bacilli were recovered from the MGIT960 system (strain GR-5098) (Fig 3)
- No growth was observed in both LJ slants after 84 days.

REFERENCES

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RESULTS

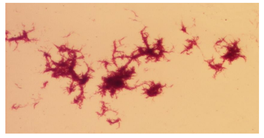
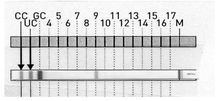


Fig 3. Ziehl-Neelsen stain of *Mycobacterium mantenii* strain grown in the MIGT960 tubes (6000x)

✓ **The isolate identified as *M. intracellulare* by the Genotype Mycobacterium CM (Hain, Lifescience)**



Genotype CM: 1,2,3, 9

Fig 4. Results of the Genotype Mycobacterium CM assay for the recovered strain

➢ **A 439-bp fragment of the 65-kDa-heat shock protein (hsp65) gene was amplified using the protocol and the primers Tb11 and TB12 [3]. The PCR product digested with HaeIII and BsteII (New England Biolabs).**

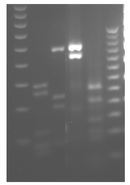


Fig 5. Agarose (3.5%) gel electrophoresis of fragments produced by digestion of PCR amplification products of the *hsp65* gene of *M. intracellulare* (lane 1,2) and *Mycobacterium* strain GR-5098 (lane 3,4) with *BstEII* (lane 2,3) and *HaeIII* (lane 1,4). The restriction mixture was run at 100 V. M1: GeneRuler 50 bp DNA ladder (Fermentas) M2: GeneRuler 25 bp DNA ladder (Fermentas).

➢ **For Mycobacterium strain GR-5098 the *BstEII* digestion produced two fragments of 235 and 210 bp and the *HaeIII* digestion produced four fragments of 130, 105, 70 and 50 bp while for *M.intracellulare BstEII* digestion produced three fragments 234, 117, 100 and *HaeIII* digestion produced at least three fragments of 145, 130 and 60 bp.**

✓ **According to results of PCR-RFLP strain GR-5098 was different than *M. intracellulare***

✓ **To resolve this discrepancy, a 1248 bp product containing a fragment of the 16S rRNA gene, a 439-bp fragment of the 65-kDa heat shock protein (hsp65), a 340 bp (region III) fragment of the rpoB gene were sequenced with the previously described primers [3,4,5]. Moreover, sequence analysis of another portion (region V position 2573-3337), of the rpoB gene was performed [6].**

Target	GenBank Accession Number	GenBank comparison results	% similarity
16S rRNA	GU827992	*M. mantenii NLA000401474*	100
hsp65	GU827993,	*M. mantenii NLA000401474*	100
rpoB (region III)	JN661704	*M. mantenii NLA000401474*	100
rpoB (region V)	JN935397	*M. mantenii NLA000401474*	100

Table 1. Molecular identification results of *Mycobacterium mantenii* strain GR-5098

RESULTS

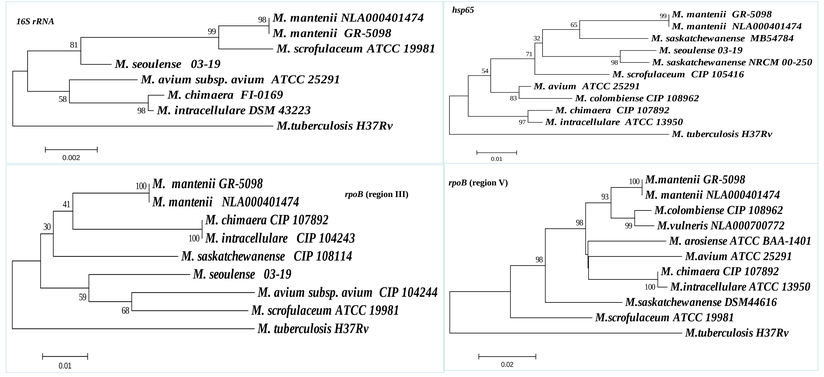


Fig 6. Phylogenetic relationship of *M. mantenii* strain GR-5908 and related species of the genus *Mycobacterium* based on 16S *rRNA*, *hsp65*, *rpoB* (region III) and *rpoB* (region V) gene sequences. Neighbor-joining tree was created, bootstrapped 1000x and visualized with MEGA 6.0. Bootstrap values are indicated at the nodes. The tree was rooted with the use of *M. tuberculosis H37Rv* as the outgroup. The scale bar represents a 0.2-2% difference in nucleotide sequences.

✓ **According to sequencing results the strain was identified as *M. mantenii***

➢ **The minimum inhibitory concentration (MICs) in μg/ml of drugs selected for their activity on slowly growing mycobacteria were determined using commercially available microdilution plates (SLOMYCOI, TREK Diagnostic systems) following the Clinical and Laboratory Standards Institute (CLSI) recommendations [7].**

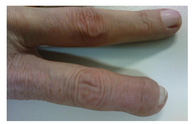
Agent	MIC (μg/ml)	Result (S: susceptible, R:resistant)
Clarithromycin	0.25	S
Rifampin	1	S
Moxifloxacin	2	S
Linezolid	4	S
Rifabutin	<0.25	S
Ethambutol	4	S
Ciprofloxacin	16	R
Amikacin	8	S
Trimethoprim-Sulfamethoxazole	4/76	R
Isoniazid	>8	-
Streptomycin	32	-
Doxycycline	4	-
Ethionamide	10	-

Table 2. Susceptibilities to different antimicrobial agents

The strain was considered:

✓ **Susceptible to clarithromycin, rifabutin, rifampin, ethambutol, amikacin, linezolid and moxifloxacin**

✓ **Resistant to ciprofloxacin, isoniazid, and trimethoprim-sulfamethoxazole**



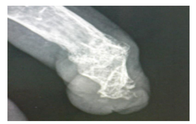


Fig 7. Picture and radiograph of the left forefinger after six months of therapy.

A triple therapy with clarithromycin, moxifloxacin and ethambutol for 5 weeks was initiated and moxifloxacin plus ethambutol followed for six months. The patient experienced considerable clinical improvement (Fig.7) and a follow-up fine needle specimen, three months after the initiation of therapy was negative in both liquid and solid culture media.

CONCLUSIONS

Our findings suggest that the osteomyelitis case in the immunocompromised patient was caused by the novel species *M. mantenii*. Sequencing analysis of the genes *hsp65*, 16S *rDNA* and *rpoB* allowed the identification of this uncommon mycobacterial species, which was misidentified by commercial probes.