Eur Respir J 1998; 11: 975–977
DOI: 10.1183/09031936.98.11040975

European Respiratory Journal
ISSN 0903 - 1936

CASE STUDY

Pulmonary infection due to *Mycobacterium szulgai*, case report and review of the literature

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Pulmonary infection due to Mycobacterium szulgai, case report and review of the literature. E. Tortoli, G. Besozzi, C. Lacchini, V. Penati, M.T. Simonetti, S. Emler. ©ERS Journals Ltd 1998.

ABSTRACT: We describe the case of a patient with a chronic pulmonary infection due to a mycobacterium tentatively identified as *Mycobacterium flavescens*, but finally shown to be *Mycobacterium szulgai*; this is the first *M. szulgai* infection reported in Italy. The patient responded to treatment with multiple antituberculosis drugs only after two cycles of 10 and 6 months, respectively. The literature concerning previous case reports in which *M. szulgai* is involved is revised and the difficulty concerning the identification of this rare mycobacterium, along with its *in vitro* and *in vivo* susceptibility, are discussed.

Eur Respir J 1998; 11: 975–977.

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Keywords: Case report, identification, *Mycobacterium szulgai*, pulmonary infection, review

Received: March 26 1997
Accepted after revision September 2 1997

Mycobacterium szulgai is an uncommon nontuberculous mycobacterium (mycobacteria other than tuberculosis (MOTT)) whose isolation from clinical specimens is usually accompanied with evidence of disease [1]. Since the first description of this new species, in 1972 [2], only 38 cases have been reported; several of which have been in patients with acquired immunodeficiency syndrome (AIDS) [3–6]. The lungs remain the main locality for pathological manifestation caused by this relatively unknown organism.

We have identified as *M. szulgai* the isolates from a long lasting pulmonary mycobacteriosis, which were initially thought to be *Mycobacterium flavescens*. This is the first case of *M. szulgai* mycobacteriosis reported in Italy. We present here the case, discussing the difficulty for an accurate diagnosis, with a review of the relevant literature.

Patient and methods

Case report

The patient, a 48 yr old male, has undergone periodic pneumological examination (table 1). Since 1968 he has been known to have apical bilateral scleroses as a result of a previous untreated lung tuberculosis. In 1989 acid-fast bacilli were detected in his sputum and a scotochromogenic mycobacterium was isolated; this was initially identified as *M. flavescens*. Skin tests were positive with purified protein derivative (PPD) and with sensitins for *Mycobacterium avium* and *Mycobacterium scrofulaceum*. Because of the identification of the mycobacterium as *M. flavescens*, a nonpathogenic environmental species, rarely, if ever, involved in human infections, no treatment was undertaken at that time. However, after repeated isolation of the same mycobacterium, treatment with rifampin (600 mg), ofloxacin (600 mg) and isoniazid (300 mg) was started; 2 months later, isoniazid was replaced with ethambutol (1,500 mg). After 8 months of such a therapeutic regimen a pleural effusion developed and the treatment was interrupted. Three months later the patient was hospitalized and bronchoscopic examination was undertaken. A temporary worsening was noted, with heavy production of purulent secretions. After a second 6 month cycle of treatment with rifampin, ethambutol and isoniazid the patient finally improved: chest radiography showed reduction of

Table 1. – Summary of isolations and treatment

Period	Positive smears†	Positive cultures††	Identification			Treatment		
			conventional	by HPLC	genetic	drugs	beginning	duration
May 1985–September 1988	0/8	0/8				none		
February 1989–April 1989	1/3	3/3	*M. flavescens*	*M. szulgai*	*M. szulgai*	INI, RIF, OFL	July 1989	2 months
Sept. 1989–December 1989	1/4	4/4	*M. flavescens*	ND	ND	RIF, OFL, ETA	Sept. 1989	6 months
January 1990–March 1990	0/3	3/3	*M. flavescens*	ND	ND	RIF, ETA	February 1990	2 months
May 1990	0/1	1/1	*M. flavescens*	*M. szulgai*	ND	INI, RIF, ETA	July 1990	6 months
October 1990–March 1992	0/6	0/6				none		

Sept.: September; †: positive smears/smears done; ††: positive cultures/cultures done. ND not done; *M. flavescens*: *Mycobacterium flavescens*; *M. szulgai*: *Mycobacterium szulgai*; INI: isoniazid; RIF: rifampin; OFL: ofloxacin; ETA: ethambutol.

dystrophic bullae. Cultures remained negative. At the present time the patient is still alive, and no relapses have occurred.

Microbiology

Cultures of sputum were performed on Lowenstein-Jensen medium using standard procedures [7].

The identification, first attempted by means of conventional methods [7], was subsequently repeated by the more sophisticated techniques of high performance liquid chromatography (HPLC) and sequencing of the 16S ribosomal deoxyribonucleic acid (16S rDNA).

HPLC of cell wall lipids was carried out as previously described [8], on mycolic acids extracted with chloroform and derivatized to their bromophenacyl esters.

A portion (600 bp) of the gene coding for the mycobacterial 16S rRNA was amplified by polymerase chain reaction and regions containing species specific variations were subsequently sequenced [9].

Lacking a standard procedure for antimicrobial susceptibility testing of MOTTs, the assay was first carried out following the proportion method recommended for *Mycobacterium tuberculosis* [10], on egg-based media. The test was subsequently repeated, in consideration of the close similarity in growth kinetics between *M. szulgai* and *M. avium*, by resorting to the broth macrodilution method proposed for the latter [11].

Results

The isolates were initially identified on the basis of conventional tests as *M. flavescens*.

Susceptibility testing revealed, on solid media, resistance to isoniazid, kanamycin and pyrazinamide, and susceptibility to amikacin, ethambutol and rifampin. Minimal inhibitory concentrations, determined later in broth, outlined a situation characterized by low values for all antimicrobials tested. The isoniazid result is the only discrepancy between the two tests (table 2).

Some years later, when identification of unusual isolates or isolates uncertainly identified in a laboratory collection was undertaken, the strains revealed a mycolic acid pattern not compatible with *M. flavescens* but identical to that of *M. szulgai*. This result was confirmed by the 16S rDNA sequence analysis yielding the distinctive sequence of *M. szulgai*.

Table 2. – Susceptibility pattern of the isolate of *Mycobacterium szulgai*

Drug	Qualitative results on Lowenstein-Jensen	MICs $\mu g \cdot mL^{-1}$ in liquid radiometric medium
Amikacin	S	<2
Ciprofloxacin	ND	<1
Clarithromycin	ND	<2
Ethambutol	S	<2
Isoniazid	R	<0.2
Kanamycin	R	ND
Pyrazinamide	R	ND
Rifabutin	ND	<0.5
Rifampin	S	<0.5
Sparfloxacin	ND	<0.5
Streptomycin	I	<2

MICs: minimal inhibitory concentrations; S: susceptible; R: resistant; I: intermediately susceptible; ND: not done.

The clinical significance of the strain [12], in the presence of medical and radiological signs of disease, is microbiologically supported by the repeated isolations from sputum samples of heavy mycobacterial charges during a 16 month period.

Discussion

As with other MOTTs, there is no evidence of human-to-human transmission of *M. szulgai*, and the environment appears to be its most likely source. In line with this hypothesis, we have recently identified as *M. szulgai* a strain isolated from the water of a swimming pool. A worldwide distribution of cases emerged from literature reports, Africa being the only continent from which no *M. szulgai* isolation has been described so far.

Identification of *M. szulgai* on the basis of conventional biochemical and cultural tests is a matter of chance; it was in fact only thanks to the use of thin layer chromatographic analysis of cell wall lipids that, in 1972, *M. szulgai* could be first distinguished from the other nontuberculous mycobacteria known at that time [2]. Identification difficulties might be responsible for an underestimation of this species, but its rarity seems to be confirmed by the low recovery rates in laboratories implementing identification approaches appropriate to its recognition, such as lipid analyses or 16S rDNA sequencing. In our laboratory, among over a thousand MOTTs identified, only the above mentioned environmental *M. szulgai* was detected, in addition to the present strain.

The inadequacy of conventional tests for the identification of *M. szulgai* is confirmed by the initial misidentification of our isolates, which are deceptive because they lack nitrate reductase activity, normally present in *M. szulgai* [7]. Apart from *M. flavescens*, as in the present case, *M. szulgai* can be erroneously assigned to many other scotochromogenic species, like *M. scrofulaceum* and *Mycobacterium gordonae*, and the recently described *Mycobacterium interjectum* and *Mycobacterium lentiflavum* as well. These differ from *M. szulgai* in no more than one or two phenotypic characters and may become indistinguishable from it in the presence of anomalous features (like nitrate reductase in our case) or because of the low reliability of a test. A highly significant feature for the speciation of *M. szulgai* is represented by its singular pigmentation: colonies grown at 37°C are scotochromogenic, whilst grown at 25°C they appear photochromogenic [13]. Unfortunately incubation in the dark is routinely performed only at 37°C, as we did initially; the photochromogenicity at 25°C was tested and highlighted only after the isolates were suspected to be *M. szulgai*.

Pulmonary diseases account for 27 of the 38 reported cases of *M. szulgai* infection [1–3, 13–27]; other localizations include: three olecranon bursites [2, 15]; three skin infections [28–30]; two cases of osteomyelitis [4, 31]; and one each of cervical adenitis [2] and renal disease [5]. Pulmonary involvement was the prominent pathology also in two [3, 6] of the four reported cases of patients with AIDS.

Patients suffering from pulmonary *M. szulgai* infection are middle-aged (mean 50 yrs, range 26–62), with a large prevalence of males (83%). Main risk factors for lung infection appear to be chronic obstructive pulmonary pathologies, smoking and alcoholism [32].

M. szulgai is characterized by a good *in vitro* susceptibility to most standard antituberculosis drugs, with isoniazid, rifampin and ethambutol being the most frequently tested. In the literature susceptibility percentages of 74% for isoniazid, 72% for rifampin and 68% for ethambutol are reported; information concerning newer drugs like quinolons and macrolides is minimal. Clarithromycin was found active *in vitro* on the only isolate tested [4], in addition to ours. Further investigations are needed to confirm, for *M. szulgai* too, the high efficacy of this drug against other MOTTs.

Despite the enormous increase in the isolation of MOTTs in recent years, a consensus has not been reached as to how well *in vitro* susceptibility predicts a favourable response to therapy. This is true for the very frequently encountered mycobacteria like the ones of the *M. avium* complex, and is therefore an even greater problem for the less common species and even more so for the rarely isolated *M. szulgai*.

No standard recommendation for the treatment exists so far. In general triple therapies are reported to warrant a low rate of relapses and to allow sterilization of cultures within a mean of 3 months [15]; however occasional relapses are reported even several years later. Isoniazid (85%) is the most frequently adopted drug followed by rifampin (77%) and ethambutol (73%).

In the case reported here a longer treatment was needed for the eradication of infection, despite the *in vitro* efficacy of the drugs used.

Acknowledgement: The authors would like to thank P. Urbano (Institute of Microbiology, University of Florence, Florence, Italy) for critically reviewing the manuscript.

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