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CONCISE ARTICLE

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Mycobacterium elephantis: Not an Exceptional Finding in Clinical Specimens

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Abstract Following the recent report of new 16S rDNA sequences of *Mycobacterium elephantis*, three clinical strains suspected to belong to such species were investigated using biochemical and cultural tests, high performance liquid chromatography of cell wall mycolic acids and genetic sequencing. Antimicrobial susceptibility was also determined. The findings confirmed recent data concerning human isolates of this new mycobacterium and identified a new 16S rDNA sequevar for this species.

Introduction

The accuracy of mycobacterial identification has greatly improved in recent years along with advancements made to molecular techniques [1]. *Mycobacterium elephantis* is a newly described species first isolated from the lung of an elephant [2]. At first, this species appeared to be little more than a curiosity, but a recent report [3] focused attention on the genetic microheterogeneity present within its 16S rRNA gene, leading to its inclusion among the strains presenting a nucleotide sequence identical to the one deposed in the publicly available databases since 1996 under the name MCRO 17 [4]. Three strains isolated between 1995 and 2000 in two Italian laboratories that were previously assigned to the taxonomic group MCRO 17 [5] are characterized here.

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Materials and Methods

The three Italian strains studied, coded FI-15495, FI-8298 and FI-13900, were isolated from the respiratory specimens of three separate patients (2 from sputum and 1 from a bronchial aspirate) processed according to the standard NALC decontamination procedure [6]. The isolates were identified and further tested using the following methods. Conventional investigations included all the most frequently performed biochemical and cultural tests [7]. Cell wall lipidic composition was determined by high performance liquid chromatography (HPLC) analysis according to the procedure recommended by the Centers for Disease Control and Prevention for ultraviolet detection of mycolic acid [8]. Antimicrobial susceptibility to six major antimycobacterial agents was investigated by determining the minimal inhibitory concentrations (MICs) on Middlebrook 7H11 agar. The complete sequence of both strands of the 16S rDNA was determined as reported previously [9].

Results and Discussion

As shown in Table 1, the culture results revealed a very homogeneous pattern, the salient features of which include (i) the intermediate growth rate (average, 10 days) at temperatures ranging from 25°C to 45°C, (ii) the pale yellow scotochromogenic pigmentation of the colonies, which are smooth, and (iii) the ability to grow on Lowenstein-Jensen medium containing 5% NaCl but not on MacConkey agar. Among the biochemical tests, nitrate reduction, 68°C catalase, Tween 80 hydrolysis and

Table 1 Comparison of biochemical and culture test results for clinical strains of *Mycobacterium elephantis*

Test	Result (% positive)		
	FI-15495, FI-8298, FI-13900	"Clinical strains" [3]	MCRO 17, MCRO 20 [4]
Niacin	−(0)	−(0)	−(0)
Nitrate reduction	+(100)	+(100)	+(100)
68°C catalase	+(100)	+(100)	ND
Catalase >45 mm	+(100)	+(100)	+(100)
β-glucosidase	−(0)	+(100)	ND
Tween 80 hydrolysis	+(100)	+(100)	+(100)
Tellurite reduction	V (33)	−(0)	V (50)
Arylsulfatase (3d)	−(0)	−(0)	ND
Urease	+(100)	+(100)	+(100)
Growth at 25°C	+(100)	+(100)	ND
Growth at 37°C	+(100)	+(100)	ND
Growth at 45°C	+(100)	ND	ND
Pigmentation	S (100)	S (100)	S (100)
Colony morphology	smooth (100)	smooth (100)	ND
Growth rate (days)	7–14	5–7	>12
Growth on MacConkey agar	−(0)	−(0)	ND
Growth on Lowenstein Jensen agar with 5% NaCl	+(100)	+(100)	−(0)
Tolerance			
Thiophene 2-carboxylic hydrazide (5 µg/ml)	+(100)	ND	ND
Thiacetazone (10 µg/ml))	+(100)	ND	ND
Hydroxylamine-HCl (500 µg/ml)	−(0)	ND	ND
Isoniazid (1 µg/ml)	+(100)	ND	ND
Oleic acid (250 µg/ml)	V (33)	ND	ND
p-nitrobenzoic acid (500 µg/ml)	V (33)	ND	ND

V, variable; S, scotochromogenic; ND, not done (or not reported)

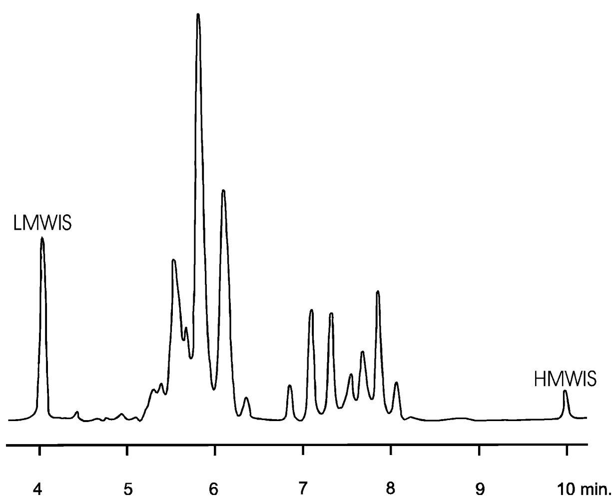

Fig. 1 Mycolic acid pattern of *Mycobacterium elephantis* by high performance liquid chromatography. LMWIS, low molecular weight internal standard; HMWIS high molecular weight internal standard

urease were positive, while semiquantitative catalase was over 45 mm. HPLC chromatograms obtained for the three strains were practically identical (Fig. 1) and were characterized by two clusters of peaks, with the first one being consistently higher.

The MICs were very homogeneous (Table 2). Interpreted according to the suggestions of Heifets [10], they featured susceptibility to amikacin, ciprofloxacin and streptomycin and resistance to clarithromycin and rifampicin, with only ethambutol being variable.

Table 2 Minimal inhibitory concentrations of six antimicrobial agents against strains FI-15495, FI-8298 and FI-13900

Agent	FI-15495	FI-8298	FI-13900
Amikacin	≤2	≤2	≤2
Ciprofloxacin	≤1	≤1	≤1
Clarithromycin	>32	32	>32
Ethambutol	≤2	≤2	8
Rifampin	>8	>8	>8
Streptomycin	≤2	≤2	≤2

The 16S rDNA sequence covered a stretch of 1,515 bases, corresponding to the sequence of *Escherichia coli* between positions 4 and 1,540. The trait that could be compared with published sequences of clinical isolates of *Mycobacterium elephantis* [3, 4] revealed a complete overlap for two strains (FI-15495 and FI-8298), while the last strain (FI-13900) presented one base mismatch at *Escherichia coli* position 458 (EMBL accession number AJ536100).

Clinical records revealed lung cancer for one patient. The other two patients had pulmonary symptoms compatible with an infectious origin, and one of them yielded two positive sputa; however, this was not sufficient to fulfill the minimum criteria for clinical significance recommended by the American Thoracic Society [11].

	12	455	597	1,522	
	ATCATGG	CGTGAGG	TGTTCGT	GCGGCTGG	AJ010747 (GenBanK)
5′	...C...	....	...C...	...--...	FI-15495, FI-8298
5′	...C...	...A...	...C...	...--...	FI-13900, (EMBL acc. n. AJ536100)
5′	...C...	....	...C...		"clinical strains" (3), (AF385898)

Fig. 2 Alignment of regions of interest in the 16S rDNA. Numbers indicate the base position on the *Escherichia coli* 16S rDNA

Apart from the two strains labeled MCRO 17 and MCRO 20 [4] investigated before the description of *Mycobacterium elephantis* as a new species, this is the second report of the isolation of *Mycobacterium elephantis* from human clinical samples. To the 11 isolates reported recently [3], we now add three others, all coming from respiratory specimens.

While the sp. nov. description of *Mycobacterium elephantis* [2] provides a very limited number of characters, exhaustive data were reported by Turenne et al. [3]. The agreement between our strains and the clinical isolates characterized by Turenne et al. [3] is almost complete for all biochemical and cultural traits, with only the results for β-glucosidase conflicting (Table 1). It must be noted, however, that in the case of pigmented strains, the interpretation of the test, relying on the yellow product liberated by the enzyme [12], may be biased by the color of the bacterial suspension.

Three chromatograms were reported by Turenne et al. [3] for different strains of *Mycobacterium elephantis*; their differences, in our opinion, fall within the normal range of variability of the test. The profiles of our strains (Fig. 1), which were very similar to each other, are compatible and most closely resemble the one attributed by Turenne et al. [3] to colony type 2 of the reference strain DSM 44368^{T}, but the first cluster of peaks is consistently higher.

Except for clarithromycin, the antimicrobial susceptibility profiles we obtained are fully compatible with those reported by Turenne et al. [3], despite the use of different media. They also confirm the susceptibility to ciprofloxacin, reported as inactive in the sp. nov. description [2].

In the report of Turenne et al. [3], which corrects several errors present in the sequence AJ010747 available in GenBank for reference strain DSM44368^{T}, three different sequevars emerge for *Mycobacterium elephantis*. The sequevars referred to colony types 1 and 2 (isolated from the reference strain), which differ from each other in six bases, and the colony type for clinical strains, which differs from the others in five bases and one base, respectively. In comparison with AJ010747, the 16S rDNA sequences of our strains reveal further differences, i.e., a cytosine in place of adenine at *Escherichia coli* position 15 and a two-base deletion at positions 1,525–1,526 (Fig. 2).

The first mismatch, although not cited by Turenne et al. [3], is present in the relevant sequence (AF385898) deposed in GenBank, while the second remains undisclosed because of its location near the 3′ end of the gene, outside of the trait sequenced there. When the latter deletion is ignored, two of our isolates (FI-15495 and FI-8298) fully overlap with the clinical strains [3], while the other (FI-13900) suggests a new sequevar differing from these in one base at *Escherichia coli* position 458 (Fig. 2). However, it must be pointed out that among the genetic variants, the one featured by the type strain is very rare in comparison with the ones isolated from humans; these, in fact, represent a quite homogeneous cluster, with only 1 of the 16 strains reported so far presenting a one-base discordance.

From the phylogenetic point of view, *Mycobacterium elephantis* clusters with thermotolerant rapid growers, as inferred by the one-cytosine insertion within the helix 10 of 16S rDNA [1] at *Escherichia coli* position 181.

As for 10 of the 11 previously reported clinical strains [3], the clinical relevance of *Mycobacterium elephantis* isolation appears unlikely. However, far from considering the identification of *Mycobacterium elephantis* unnecessary, we think a distinction from other more virulent strains is needed and is useful in terms of increasing the poor awareness of this species.

Despite its first isolation from an elephant, the most probable reservoir of *Mycobacterium elephantis*, as for other nontuberculous mycobacteria, is the environment, and it is likely that this has been the source of infection for both humans and animals. We conclude, therefore, that *Mycobacterium elephantis* is not isolated exclusively from clinical samples, in particular from the respiratory tract, and it is not pathogenic in most cases. Once more, the nucleotide sequencing of 16S rDNA, although complicated by the presence of different genetic variants (risen to 4 with that described here), is the method of choice for identification. An equally valid option, however, is HPLC, since *Mycobacterium elephantis* presents a very characteristic profile, different from any other reported so far.

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