

## Clinical and Laboratory Features of *Mycobacterium mageritense*

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**Six clinical isolates of the nonpigmented, rapidly growing species *Mycobacterium mageritense* were recovered from sputum, bronchial wash, blood, sinus drainage, and two surgical wound infections from separate patients in Texas, New York, Louisiana, and Florida. The isolates matched the ATCC type strain by PCR restriction enzyme analysis of the 65-kDa *hsp* gene sequence of Telenti, high-performance liquid chromatography, biochemical reactions, and partial 16S rRNA gene sequencing. These are the first isolates of this species to be described in the United States and the first isolates to be associated with clinical disease. Susceptibility testing of all known isolates of the species revealed all isolates to be susceptible or intermediate to amikacin, cefoxitin, imipenem, and the fluoroquinolones and sulfonamides but resistant to clarithromycin. Because of their phenotypic and clinical similarity to isolates of the *Mycobacterium fortuitum* third biovariant complex (sorbitol positive), isolates of *M. mageritense* are likely to go undetected unless selected carbohydrate utilization or molecular identification methods are used.**

In 1997 Domenech et al. (4) described a new nonpigmented species of rapidly growing mycobacterium that they named *Mycobacterium mageritense*. The name was derived from Magerit, the old Arabic name of Madrid, where four of the five isolates had been recovered. The isolates were from sputum and came from two hospitals in Spain. None were known to be clinically significant. The isolates were nonpigmented and had similarities to both *M. smegmatis* and the *M. fortuitum* complex (1, 4, 10, 18).

Utilizing PCR restriction enzyme analysis (PRA) of the Telenti fragment (11, 14) of the 65-kDa *hsp* gene, we identified six clinical isolates whose PRA patterns matched the pattern of *M. mageritense*. We compared these strains to the initial strains from Spain by using standard growth and biochemical tests, partial 16S rRNA gene sequencing, PRA of the 65-kDa *hsp* gene, high-performance liquid chromatography (HPLC), and determination of antimicrobial susceptibilities.

### MATERIALS AND METHODS

**Organisms.** Eleven isolates of *M. mageritense*, including the five original Spanish isolates described in 1997 (ATCC 700351<sup>T</sup>, strains 1635, 1470, 1636, and 1336) (4), three isolates from Texas (strains 1852, 1582, and 2056), one from Florida (strain 1301), one from New York (strain 2048), and one from Louisiana (strain 1846), were studied. *M. fortuitum* ATCC 6841<sup>T</sup> and *M. smegmatis* ATCC 19420<sup>T</sup> were used as control strains for HPLC and PRA, while *Staphylococcus aureus* ATCC 29213 and *M. peregrinum* ATCC 700686 were used as controls for susceptibility testing.

**Growth and biochemical characterization.** All 11 isolates identified as *M. mageritense* were examined for colony morphology, pigmentation, and growth

within 7 days on Trypticase soy agar and Middlebrook 7H10 agar at 30, 35, and 45°C, as well as for arylsulfatase activity at 3 days (graded from 0 to 4+), nitrate reductase activity (graded from 0 to 5+), iron uptake, and urease activity (graded from 0 to 4+) (5). Carbohydrate utilization testing included D-mannitol, i-myoinositol, citrate, D-galactose, L-rhamnose, D-trehalose, D-glucitol (sorbitol), D-xylose, and L-arabinose (10, 15). Acetamide (Becton Dickinson Biosciences, Sparks, Md.) utilization was also determined in addition to susceptibility to kanamycin, cephalothin, and polymyxin B by using a disk diffusion technique on Mueller-Hinton agar (17, 20).

**Susceptibility testing.** Susceptibility to 10 antimicrobial agents was determined by broth microdilution in cation-supplemented Mueller-Hinton broth (2, 3, 12, 13, 17, 23). The antimicrobials tested were amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, minocycline, sulfamethoxazole, tobramycin, and linezolid. Because not all isolates were tested simultaneously, some isolates were not tested against minocycline or linezolid. Breakpoints were those suggested by the National Committee for Clinical Laboratory Standards for rapidly growing mycobacteria (7), except with linezolid and minocycline. Breakpoints for minocycline (not addressed by the National Committee for Clinical Laboratory Standards) were made the same as those for doxycycline, which is addressed in the document (7), and linezolid breakpoints were those recently proposed for rapidly growing mycobacteria (19). Quality control was performed with *S. aureus* ATCC 29213 and *M. peregrinum* ATCC 700686 (6).

**HPLC.** Mycolic acids were prepared as described previously (1). They were then dissolved in 200  $\mu$ l of chloroform containing 200  $\mu$ g of 4-bromomethyl-6,7-dimethoxycoumarin and 200  $\mu$ g of 18-crown-6 ether and transferred to a 2-ml autosampler vial into which 100  $\mu$ l of a 2% potassium bicarbonate solution had been evaporated previously. The vial was heated at 60°C for 15 min and allowed to evaporate. The mycolic esters were dissolved in 500  $\mu$ l of chloroform containing internal standards and analyzed by fluorescence detection HPLC (FL-HPLC) as described previously (1).

**PRA.** All isolates of *M. mageritense* were subjected to PCR amplification of a 439-bp segment of the 65-kDa *hsp* gene, as originally described by Telenti et al. (14). Ground cell supernatants were used as DNA templates (11, 22) by using the appropriate positive and negative controls (14).

Seven restriction endonucleases (*Bst*EII, *Hae*III, *Aci*I, *Hha*I, *Msp*I, *Hinf*I, and *Bsa*HI) were used. Restriction fragments were electrophoresed on 3% metaphor agarose (4-bp resolution; FMC Bioproducts, Rockland, Maine) containing ethidium bromide in a Mini-SubCell electrophoresis system (Bio-Rad, Hercules, Calif.) at 95 V for 1.5 to 2.0 h. Fragment sizes (in base pairs) were estimated on a computerized Bio Image system (Millipore, Bedford, Mass.) (1).

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TABLE 1. Clinical features of the six United States isolates of *M. mageritense*

Isolate	Age/sex <sup>a</sup>	Source specimen	Location	Disease	Other disease	Treatment; outcome
1852	37/F	Surgical wound (thigh)	Texas	Wound infection following liposuction	None	Doxycycline, ciprofloxacin; surgery, cured
1582	Unknown/F	Sputum	Texas	Unknown	Unknown	Unknown
1301	32/F	Blood	Florida	Catheter-related sepsis	Immune suppression	Amikacin, linezolid
2048	51/F	Sinus	New York	Severe sinusitis	Unknown	Amikacin (topical), ciprofloxacin, clarithromycin
2056	25/M	Surgical wound, knee effusion	Texas	Wound infection, possible osteomyelitis	Recent open femur fracture with open reduction	Amikacin, imipenem; surgery, improved
1846	42/M	Bronchial wash	Louisiana	Clinical significance unknown	HIV	Macrolide

<sup>a</sup> Age given in years. F, female; M, male.

**16S rRNA partial gene sequencing.** 16S rRNA gene sequencing was performed by using standard techniques (8). Sequencing analysis of the first 500 bp in the 16S rRNA gene that includes the hypervariable A region was performed with an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, Calif.) and a MicroSeq 16S 500 16S rDNA sequencing module. Data analysis was carried out with MicroSeq version 1.4 (Applied Biosystems).

## RESULTS

**Organisms.** Eleven isolates of *M. mageritense* were studied. Five isolates were isolated in Spain from 1987 to 1989 and described in 1997 (4). One of the five isolates was submitted to the American Type Culture Collection and is designated ATCC 700351<sup>T</sup>. The six United States isolates were submitted for susceptibility testing and identification from 1999 to 2002 to the Mycobacteria/Nocardia Laboratory of the University of Texas Health Center at Tyler. The first isolate was recovered from sputum culture, the second was recovered from a bronchoscopy sample, the third was obtained from a thigh wound culture from a woman with localized cellulitis following a liposuction procedure, the fourth was from the blood of an immune-suppressed patient with a central catheter and clinical sepsis, the fifth was from a patient with severe sinusitis, and the sixth was from a patient with a wound infection and, probably, osteomyelitis following an open fracture which had undergone open reduction and internal fixation (Table 1). The history of the second patient is provided below.

**Case summary.** The patient was a 37-year-old previously healthy white female referred by a local plastic surgeon in July 2000. She had a liposuction procedure in her medial thigh area in January 2000. At an instrument insertion site she had a "sore that never healed." She continued to have drainage off and on from this area despite therapy with oral cephalosporins. A small nodule remained in the thigh. The thigh lesion began to drain more profusely. On 17 July 2000, a culture of this drainage grew an acid-fast bacillus on routine media that was subsequently identified as *M. mageritense*. She was referred to one of the authors (J.T.B.) on 21 July, and a soft tissue infection involving a rapidly growing mycobacterium was suspected. Her physical exam was normal except for a small, 2-cm circular nodule on the left medial thigh with a scant amount of serous drainage. She was started on doxycycline and ciprofloxacin. She was seen again on 29 August 2000. The small nodule was still present but much improved, and no drainage was present.

At her request, the antibiotic therapy was discontinued. She was seen again in October 2000, after she had noticed about a week of drainage from the same thigh nodule. She was re-started on antibiotics. An elliptical excision of the nodule was performed on 8 November 2000. A culture showed no growth. She was continued on ciprofloxacin alone at that time. She was seen again 17 January 2001 and had persistent drainage, although the nodule was much smaller. This area was excised again with excellent results. She was placed on ciprofloxacin following that last visit and was doing well with no evidence of recurrence 3 months later.

**Growth and biochemical characterization.** All 11 isolates of *M. mageritense* produced nonpigmented smooth or rough colonies within 7 days on Trypticase soy agar and Middlebrook 7H10 agar at 30 and 35°C (Table 2). Growth at 45°C was variable. Isolates were strongly positive for 3-day arylsulfatase production, nitrate reductase, iron uptake, and urease. All five of the Spanish isolates and the six United States isolates utilized D-mannitol, i-myo-inositol, L-rhamnose, D-glucitol (sorbitol), D-trehalose, and acetamide. Approximately 50% of isolates from both groups utilized D-galactose, and most or all of the isolates were unable to utilize L-arabinose, citrate, or D-xylose.

**Susceptibility testing.** All 11 isolates of *M. mageritense* were susceptible to ciprofloxacin and sulfamethoxazole. All isolates were susceptible or intermediate in susceptibility to amikacin, linezolid, imipenem, and cefoxitin and were intermediate or resistant to tobramycin. All 11 isolates were resistant to clarithromycin, with MICs for 8 of 11 being >32 µg/ml. By disk diffusion testing, only 50% of the Spanish isolates other than the type strain were susceptible to polymyxin B while all of the United States isolates were susceptible. For kanamycin, all of the Spanish isolates were susceptible while all of the United States isolates were resistant. None of the isolates were inhibited by cephalothin. Susceptibility results for all drugs are shown in Tables 2 and 3.

When grown simultaneously to stationary phase with the lipids extracted after the same incubation time, the ATCC type strain of *M. mageritense* (ATCC 700351<sup>T</sup>), the remaining four Spanish strains, and five of the six United States isolates had matching FL-HPLC patterns that were distinct from those produced by *M. smegmatis* and *M. fortuitum*. The *M. mag-*

TABLE 2. Laboratory features of the ATCC type strain, Spanish isolates, and United States clinical strains of *M. mageritense*

Feature	Presence (+), absence (-), or type of feature for indicated strain or isolate(s)								
	ATCC 700351 <sup>T</sup>	Spanish isolates (%)	United States Isolates						
			1582	1852	1301	2048	2056	1846	
Pigment (early/late)	-/-	-/- (0)	-/-	-/-	-/-	-/-	-/-	-/-	-/-
Colony morphology	Smooth	Smooth	Smooth	Smooth	Smooth	Rough	Rough	Rough	Rough
Growth (2 wk)									
<7 days	+	+(100)	+	+	+	+	+	+	+
30°C	+	+(100)	+	+	+	+	+	+	+
35°C	+	+(100)	+	+	+	+	+	+	+
42°C	+	ND <sup>a</sup>	+	+	+	+	+	ND	ND
45°C	+/- <sup>b</sup>	+/- (50)	-	-	+/-	+	+	+	+/-
Growth on MacConkey agar without crystal violet	+	+(100)	ND	+	ND	ND	ND	ND	ND
Arylsulfatase (3 days)	+(4+)	+(100)	+(3+)	+(3+)	+(4+)	+(4+)	+(4+)	+(4+)	+(4+)
Catalase (68°C)	-	-(0)	-	-	-	-	-	-	-
Nitrate reduction	+(5+)	+(100)	+(5+)	+(5+)	+(5+)	+(5+)	+	+	+(5+)
Iron uptake	+	+(100)	+	+	+	+	+	+	+
Urease	+(4+)	+(100)	+(4+)	+(4+)	+(4+)	+(4+)	+(4+)	+(4+)	+(4+)
Carbohydrate utilization									
L-Arabinose	+/-	-(0)	-	-	-	-	-	-	-
Citrate	-	-(0)	+/-	-	+/-	+	+	+/-	+/-
D-Galactose	-	+/- (50)	+	+	+	+	+	+	+
i-myo-Inositol	+	+(100)	+	+	+	+	+	+	+
D-Mannitol	+	+(100)	+	+	+	+	+	+	+
L-Rhamnose	+	+(100)	+	+	+	+	+	+	+
D-Glucitol (sorbitol)	+	+(100)	+	+	+	+	+	+	+
D-Trehalose	+	+(100)	+	+	+	+	+	+	+
D-Xylose	-	-(0)	ND	-	-	+	+/-	-	-
Acetamide	+	+(100)	+	+	+	+	+	+	+
Inhibition									
Polymyxin B	-	+/- (50)	+	+	+	+	+	+	+
Cephalothin	-	-(0)	-	-	-	-	-	-	-
Kanamycin	+	+(100)	-	-	-	-	-	-	-

<sup>a</sup> ND, not determined.<sup>b</sup> +/-, weak or variable.

*eritense* isolates had a relatively flat first set of peaks and a single major peak in the second set of peaks (Fig. 1). As previously described (1), a minor difference in peak retention time permitted a distinction between *M. mageritense* and *M. fortuitum*; all but one of the *M. mageritense* strains produced a peak at approximately 5.64 min, whereas the corresponding *M. fortuitum* peak eluted 0.04 min earlier. One isolate (2048)

exhibited a different pattern, with two major peaks and somewhat different retention times.

**PRA.** By PRA, the 11 isolates of *M. mageritense* gave identical restriction patterns with the seven restriction enzymes, except that three of the six clinical isolates from the United States had an extra cutting site for *Bsa*HI (this enzyme commonly exhibits intraspecies variation with mycobacteria and

TABLE 3. Susceptibility results of isolates of *M. mageritense*

Drug	Resistance breakpoint (μg/ml) <sup>a</sup>	MIC (μg/ml) against:										
		Spanish isolates					United States isolates					
		ATCC 700351 <sup>T</sup>	1635	1470	1636	1336	1852	1582	1301	2048	2056	1846
Amikacin	≥64	2	2	2	2	8	32	32	32	32	32	32
Cefoxitin	≥128	16	8	16	16	16	32	32	16	16	32	16
Ciprofloxacin	≥4	≤0.125	0.25	0.25	0.25	0.5	0.25	0.25	≤0.25	0.5	0.5	0.25
Clarithromycin	≥8	>64	16	>64	64	16	>64	>64	8	>32	>32	>64
Doxycycline	≤16	4	0.5	1	2	2	4	8	2	>64	16	32
Imipenem	≥16	2	≤1	4	2	4	4	8	≤1	2	2	ND <sup>b</sup>
Linezolid	≥32	1	ND	ND	ND	ND	4	8	≤2	16	8	8
Minocycline	≥16	4	0.5	1	2	2	ND	ND	ND	ND	ND	ND
Sulfamethoxazole	≥64	2	2	4	2	2	32	8	32	8	≤4	8
Tobramycin	≥16	8	8	8	8	8	>16	16	>16	>16	>16	>16

<sup>a</sup> See reference 7.<sup>b</sup> ND, not determined.

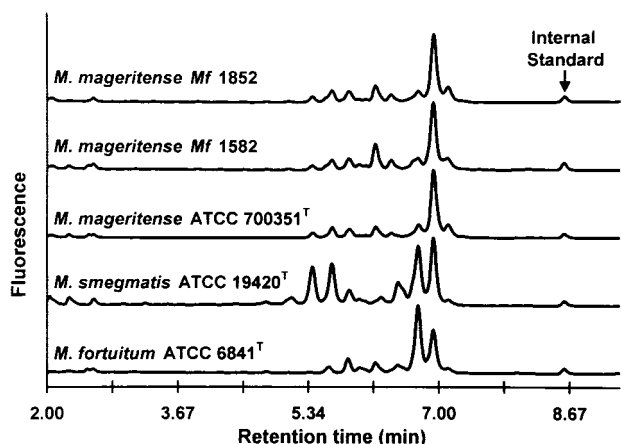


FIG. 1. FL-HPLC patterns of three strains of *M. mageritense* and two rapidly growing mycobacterial reference strains.

other aerobic actinomycetes) (R. W. Wilson and R. J. Wallace, Jr., unpublished data). Their pattern was unique among those of all species in our current database (Fig. 2).

**16S rRNA sequencing.** The ATCC type strain of *M. mageritense* (ATCC 700351<sup>T</sup>) and four of the clinical isolates (1852, 1582, 2048, and 1846) had identical 500-bp sequences that included the hypervariable region A. Two isolates (1301 and 2056) differed from the other five isolates by a single G→A nucleotide change at position 446 (99.8% identify with the ATCC type strain).

**DISCUSSION**

These are the first cases of clinical disease due to *M. mageritense* to be published and the first six isolates of the species

to be identified in the United States, and this is the first report of drug susceptibilities for this species. We have also further detailed laboratory characteristics of this new species.

The clinical disease produced by *M. mageritense* is similar to disease produced by the *M. fortuitum* third biovariant complex (17, 21) and includes skin and soft tissue infections as well as health care-associated infections. From a therapeutic standpoint, United States isolates of *M. mageritense* are generally more difficult to treat, as amikacin MICs for all six isolates to date have been high (32 µg/ml), 50% of the isolates were resistant to doxycycline, and all were resistant to clarithromycin. Drugs that offer therapeutic potential include imipenem, cefoxitin, sulfonamides, linezolid, and the newer quinolones, although quinolone monotherapy should be avoided when possible because of the risk of developing mutational resistance (16); in addition, there is no published experience with the use of linezolid for the *M. fortuitum* group. Surgical debridement can be useful (as in case 2) but is not required for most diseases given the drugs available for therapy.

The susceptibility patterns of the 11 isolates of *M. mageritense* were very similar. However, there were differences in aminoglycoside susceptibilities between the Spanish isolates and the United States isolates. The six United States isolates were all intermediate to amikacin, with amikacin MICs of 32 µg/ml, and resistant to kanamycin, while the five Spanish isolates were susceptible to both aminoglycosides. These two different aminoglycoside susceptibility patterns have previously been shown to be present in isolates of the *M. fortuitum* third biovariant complex (17). The isolates were also uniformly resistant to clarithromycin, a finding previously noted for all isolates of the sorbitol-positive *M. fortuitum* third biovariant complex (of which most isolates have been tentatively designated *M. houstonense* [17]). High-level resistance to clarithromycin (MIC of ≥32 µg/ml) is seen only in these two taxonomic groups among all members of the *M. fortuitum* group. With

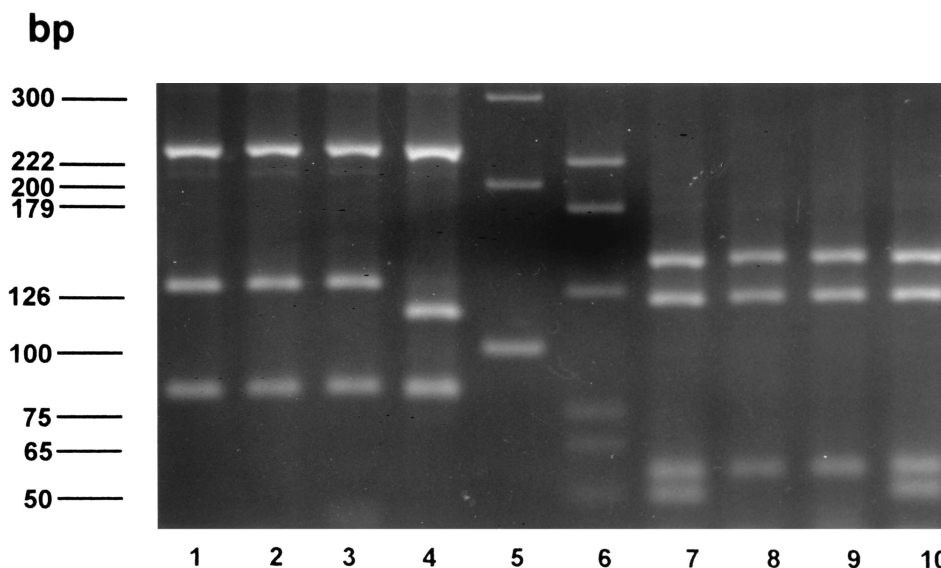


FIG. 2. PRA restriction fragment length polymorphism pattern from isolates of *M. mageritense* and an *M. fortuitum* control. Lanes 1 to 4: *BstEII* digests from *M. mageritense* ATCC 700351<sup>T</sup>, clinical isolates 1582 and 1852, and control isolate *M. fortuitum* ATCC 6841<sup>T</sup>, respectively. Lanes 5 and 6: 100-bp and pGEM markers, respectively. Lanes 7 to 10: *HaeIII* digests with the same isolates as those used for the *BstEII* digests.



susceptibility patterns, the two groups are distinguishable by cefoxitin susceptibility, as the cefoxitin MICs for all of the sorbitol-positive third biovariant isolates are  $\geq 64$   $\mu\text{g/ml}$  (17) while those for all of the isolates of *M. mageritense* are  $\leq 32$   $\mu\text{g/ml}$  (Table 3).

The first study of *M. mageritense* suggested that it was phenotypically most closely related to *M. smegmatis*, based on growth at 45°C and a variable 3-day arylsulfatase reaction (4). The present study, however, showed that many isolates of the species grow poorly at 45°C and all had a positive 3-day arylsulfatase reaction when tested by the method of Kent and Kubica (5). These features, combined with the absence of pigmentation and growth on MacConkey agar without crystal violet (10), result in responses identical to those of other species currently grouped within the *M. fortuitum* complex. According to results of the additional recommended tests for evaluating rapidly growing mycobacteria, namely those of nitrate reductase, iron uptake, and carbohydrate utilization of D-mannitol, i-myo-inositol, and citrate (10), the isolates of *M. mageritense* have reactions identical to those of the *M. fortuitum* third biovariant complex. They are also sorbitol positive, as are approximately 40% of the members of this complex (17).

Initial phenotypic studies suggested that the *M. fortuitum* third biovariant complex (defined as isolates that matched the growth and biochemical characteristics of *M. fortuitum* but were also D-mannitol and i-myo-inositol positive) (10, 17) was heterogeneous and likely comprised multiple taxa. They were subdivided into two groups according to D-glucitol (sorbitol) utilization (17), but studies of the electrophoretic patterns of their  $\beta$ -lactamase showed multiple patterns among isolates which were cefoxitin susceptible in both sorbitol groups, suggesting that multiple taxa might be present (24). The data on the previous susceptibilities to cefoxitin of isolates in the sorbitol-positive group showed the MICs to be low ( $\leq 32$   $\mu\text{g/ml}$ ) for 4 of 33 isolates (12%) according to the same broth susceptibility method (17) as that used in the present study. In retrospect, these four isolates may well have been isolates of *M. mageritense*, and this suggests that 10 to 15% of sorbitol-positive isolates will belong to this taxon. In contrast, with isolates of *M. fortuitum*, *M. chelonae*, and *M. abscessus*, each species had a single unique  $\beta$ -lactamase pattern. The recently described *M. septicum* (9) and now *M. mageritense* support the concept of this group being a complex, as both of these new species meet prior phenotypic definitions of the third biovariant complex. Additionally, ongoing studies suggest that at least five more species are present in this complex, including *M. houstonense* and *M. bonicki* (M. F. Schinsky, R. E. Morey, M. P. Douglas, A. G. Steigerwalt, R. W. Wilson, M. M. Floyd, M. I. Daneshvar, B. A. Brown-Elliott, R. J. Wallace, Jr., M. M. McNeil, D. J. Brenner, and J. M. Brown, submitted for publication).

Given that the isolates of *M. mageritense* have the growth, biochemical, and drug susceptibility patterns of *M. fortuitum* third biovariant complex sorbitol-positive isolates (except for cefoxitin MICs) (17), it is easy to see how isolates of the former could be missed. One carbohydrate not routinely tested that would separate the two taxa is L-rhamnose, as the *M. mageritense* isolates were all positive and  $< 5\%$  of *M. fortuitum* third biovariant complex isolates have previously been reported to be positive (R. J. Wallace, Jr., unpublished data). One method

TABLE 4. Carbohydrate utilization for recognition of species and taxa presently within the *M. fortuitum* group

Species or taxon	Presence (+) or absence (-) of utilization of indicated carbohydrate			
	Mannitol	Inositol	Sorbitol	Rhamnose
<i>M. fortuitum</i>	-	-	-	-
<i>M. peregrinum</i>	+	-	-	-
<i>M. fortuitum</i> third biovariant				
Sorbitol positive	+	+	+	-
Sorbitol negative	+	+	-	-
<i>M. mageritense</i>	+	+	+	+

for separation of the species and/or taxa of the *M. fortuitum* group is to screen them with mannitol. Most isolates within this group will be *M. fortuitum*, which produces a negative test. These can be identified without any additional testing. All other members of the *M. fortuitum* group are mannitol positive. For isolates that are mannitol positive, the additional sugars of inositol, sorbitol, and rhamnose will readily separate these four remaining species and/or taxa (Table 4). The combination of intermediate amikacin MICs, resistance to kanamycin, and low cefoxitin MICs ( $\leq 32$   $\mu\text{g/ml}$ ) is also highly suggestive of this new taxon. The most accurate methods for identification are molecular, with PRA and partial 16S rRNA gene sequencing readily separating the two taxa. The use of molecular studies, greater attention to susceptibility patterns, or carbohydrate utilization with L-rhamnose should allow for increased recognition of this species as a human pathogen.

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