***Mycobacterium septicum* sp. nov., a new rapidly growing species associated with catheter-related bacteraemia**

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Rapidly growing mycobacteria are capable of causing several clinical diseases in both immunosuppressed and immunocompetent individuals. A previously unidentified, rapidly growing mycobacterium was determined to be the causative agent of central line sepsis in a child with underlying metastatic hepatoblastoma. Four isolates of this mycobacterium, three from blood and one from the central venous catheter tip, were studied. Phenotypic characterization, HPLC and genetic analysis revealed that while this organism most closely resembled members of the *Mycobacterium fortuitum* complex and *Mycobacterium senegalense*, it differed from all previously described species. Phenotypic tests useful in differentiating this species from similar rapidly growing mycobacteria included: growth at 42 °C, hydrolysis of acetamide, utilization of citrate, production of arylsulfatase (3-d), acidification of D-mannitol and i-myo-inositol, and susceptibility to erythromycin, vancomycin and tobramycin. The name *Mycobacterium septicum* is proposed for this new species. The type strain has been deposited in Deutsche Sammlung von Mikroorganismen und Zellkulturen as DSM 44393^T and in the American Type Culture Collection as strain ATCC 700731^T.

Keywords: *Mycobacterium septicum* sp. nov., *Mycobacterium fortuitum* complex, sepsis/bacteraemia, DNA–DNA hybridization, 16S rRNA gene sequence

INTRODUCTION

Rapidly growing mycobacteria have been increasingly recognized as a cause of human infection during the last two decades. Specifically, the diagnoses of *Mycobacterium fortuitum* complex infections have risen in part because of an increased awareness of their potential as human pathogens. In the immunosuppressed patient, these organisms can cause serious infections such as catheter-related bacteraemia or disseminated disease. Disseminated disease can be life-threatening, although it usually presents as a chronic

illness associated with skin abscesses, cellulitis and multiple subcutaneous nodular lesions that primarily involve the extensor surfaces of the arms and legs (Sack, 1990).

It has become apparent that the genus *Mycobacterium* is more diverse than was previously realized and includes a significant number of emerging pathogens (Domenech *et al.*, 1997; Haas *et al.*, 1997; Wallace *et al.*, 1991). Recently, an unidentified *Mycobacterium* species was isolated from three blood cultures and a central venous catheter tip after its removal from a child diagnosed with metastatic hepatoblastoma (Hogg *et al.*, 1999). These isolates were phenotypically and genetically identical. By standard biochemical tests, they were phenotypically similar to the *M. fortuitum* complex and could not be differentiated from related members of the complex on the basis of

Abbreviations: NCCLS, National Committee for Clinical Laboratory Standards; RRT, relative retention times.

The GenBank accession number for the 16S rRNA sequence of *Mycobacterium septicum* W4964^T is AF111809.

mycolic acid profiles obtained using HPLC. The 16S rRNA gene of a representative isolate was sequenced and showed that these isolates were related but not identical to *M. fortuitum*, *Mycobacterium peregrinum* and *Mycobacterium senegalense*. An extensive polyphasic study that used additional biochemical tests, antimicrobial susceptibility analysis, ribotyping and DNA relatedness indicated that this micro-organism was not a species of *M. fortuitum*, *M. peregrinum* or *M. senegalense*. The results of this study showed that this previously unidentified mycobacterium belongs to a new species for which the name *Mycobacterium septicum* is proposed.

METHODS

Bacterial strains. Isolates W4962, W4963 and W5064 were cultured from the blood of a 2-year-old child with metastatic hepatoblastoma. Isolate W4964^T was cultured from the tip of the patient's centrally placed Hickman catheter upon its removal (Hogg *et al.*, 1999). *M. fortuitum* ATCC 6841^T and *M. peregrinum* ATCC 14467^T were provided by Richard J. Wallace, Jr, Department of Microbiology, The University of Texas Health Sciences Center at Tyler, TX, USA. *M. senegalense* ATCC 35796^T was obtained from the ATCC.

Phenotypic analysis. Macroscopic morphological studies were performed on colonies grown on heart infusion agar with rabbit blood (blood agar) (BBL Prepared Media, Becton-Dickinson, Microbiology Systems) and incubated at 35 °C for 2 d. A stereomicroscope (10×) was used to determine the presence of aerial mycelium. Microscopic morphology and acid-fastness were studied by using Gram and modified Kinyoun acid-fast stains, respectively (Berd, 1973). Standard biochemical tests routinely used in Actinomycete and Mycobacteriology Laboratories at the Centers for Disease Control and Prevention were performed (Berd, 1973; Silcox *et al.*, 1981). In addition, hydrolysis of acetamide (BBL), utilization of citrate (BBL) and growth at 42 °C were tested using the methods of Wallace *et al.* (1991).

Antimicrobial susceptibility tests were performed by a previously described broth microdilution method using cation-supplemented Mueller–Hinton broth (Wallace *et al.*, 1991). The drugs tested were amikacin, amoxicillin-clavulanate, ampicillin, cefotaxime, ceftriaxone, ciprofloxacin, doxycycline, erythromycin, imipenem, minocycline, sulfamethoxazole, trimethoprim-sulfamethoxazole and vancomycin. Plates were incubated for 72 h at 35 °C. Since the methods of testing and the breakpoints for resistance for rapidly growing mycobacteria have not been standardized or approved by the National Committee for Clinical Laboratory Standards (NCCLS), the breakpoints for resistance used were those of the NCCLS for organisms that grow aerobically (National Committee for Clinical Laboratory Standards, 1997). Additionally, antimicrobial susceptibilities were determined by using a disk-diffusion method for cefamandole (30 µg), tobramycin (10 µg), streptomycin (10 µg), gentamicin (10 µg), neomycin (30 µg) and kanamycin (30 µg), as previously described (Grange & Stanford, 1974). The zone sizes for resistance used were those of Grange & Stanford (1974).

HPLC. The mycolic acid pattern analysis was performed according to the procedure of Butler *et al.* (1991). Briefly, whole cells were saponified in methanolic potassium hydroxide solution. After acidification, chloroform extracts

containing the mycolic acids were separated from the cellular materials and mycolic acids were derivatized to bromophenacyl esters. Chromatography was performed with a Beckman chromatograph (Beckman Instruments) controlled by Beckman System Gold software and equipped with a Beckman reverse phase C-18 column (4.6 × 75 mm), a Beckman column heater (35 ± 1 °C) and a variable wavelength UV detector adjusted to 260 nm. A 10-min methanol and methylene chloride gradient was used as previously described (Butler *et al.*, 1996). Patterns generated with this method were visually matched to HPLC patterns of authentic species by using the relative retention time (RRT) ratios as calculated by the software. These ratios were derived using a high-molecular-weight standard (Rib ImmunoChem Research) as the reference peak (Butler *et al.*, 1992).

DNA purification. Strains were subcultured from Lowenstein–Jensen slants into 1.5 l Mueller–Hinton broth containing 22.5 g glycine (Sigma) and 7.5 ml Tween 80 (Sigma) and grown for 1–2 d at 35 °C before being harvested by centrifugation. DNA was purified from lysed protoplasts as described previously by Lasker *et al.* (1992). To obtain optimal yields, repeat extractions were performed with of 20% (w/v) SDS (Roche) extractions to improve the DNA yield as adapted from Loeffelholz & Scholl (1989).

Ribotyping. Genomic DNA (1–2 mg ml⁻¹) was digested with 20 U *Sma*I and *Sal*I (Roche) for 8 h at 35 °C in the buffer recommended by the manufacturer. DNA fragments were separated by electrophoresis in a 0.85% (w/v) agarose gel (GIBCO). DNA fragments were then transferred to a nylon membrane (Nytran; Schleicher & Schuell) and were hybridized with a digoxigenin-labelled set of five oligonucleotides at 35 °C as previously described (Regnault *et al.*, 1997). rDNA-containing fragments were visualized according to the 'Genius' kit (Roche) protocol.

16S rRNA sequencing. Purified genomic DNA was diluted to 1 µg ml⁻¹ in sterile water. Diluted DNA (10 µl) was used in a 100 µl PCR containing 200 µM dNTPs, 1 mM MgCl₂, 1 × PCR buffer II (Perkin-Elmer), 0.1 µM M104F primer (5'-GAACGGGTGAGTAACACG-3'), 0.1 µM M1510R primer (5'-GGCTACCTTGTTACGACTT-3') and 2.5 U AmpliTaq DNA polymerase (Perkin-Elmer). M104F and M1510R were derived from mycobacterial 16S genes published in GenBank. The parameters for amplification were 94 °C for 5 min, 35 cycles of 15 s at 94 °C, 15 s at 50 °C, 1.5 min at 72 °C, and a 72 °C extension for 5 min before cooling to 4 °C.

The results of the PCR reaction were checked by running 10 µl of each reaction mix on a 1% (w/v) agarose gel. The 16S DNA from isolate W4964^T was then purified and concentrated by using a PCR Purification kit from Qiagen; 60 ng PCR product was used for each sequencing reaction. The sequencing reaction consisted of 16S DNA, 8 µl ABI PRISM dye terminator cycle sequencing ready reaction kit (Perkin-Elmer) and 3.2 pmol of primer; sterile water was added to a final amount of 20 µl. The primer set used for sequencing was derived from those designed by Stackebrandt & Charfreitag (1990). The manufacturer's instructions for the cycle sequencing kit were followed for the thermal cycler conditions. The extension products from each sequencing reaction were purified through a Centriscap column (Princeton Separations) and dried in a vacuum centrifuge for 20 min. Amplifications were performed on a Perkin-Elmer 9600 thermocycler.

The sequencing reactions were resolved on a 4.2% (w/v)

acrylamide/8 M urea gel electrophoresed on an ABI 377 automated sequencer (Perkin-Elmer). The sequence data were edited and compiled by using the Wisconsin Sequence Analysis Package (Genetics Computer Group). The 16S sequences were aligned by using the Wisconsin Sequence Analysis Package program PILEUP with 16S sequences of 18 *Mycobacterium* spp. and an outgroup sequence retrieved from GenBank. The multiple sequence alignment was edited manually. The edited alignment was used in PHYLIP (version 3.5, J. Felsenstein) to derive a phylogenetic dendrogram using the nucleotide substitution model of Jukes & Cantor (1969) and the neighbour-joining method of Saitou & Nei (1987). The plotfile from PHYLIP 3.5 was reformatted for the HP Laserjet 4 printer by using PRINTGL software (Ravitz Software).

DNA relatedness and G + C determination. DNA labelling and DNA relatedness experiments by the hydroxyapatite method have been described previously (Brenner *et al.*, 1982). DNA was labelled *in vitro* with [³²P]dCTP by using a nick-translation kit (GIBCO). The temperature used for optimal hybridization was 75 °C and the percentage divergence was calculated to the nearest 0.5% (Brenner *et al.*, 1983). The G + C content of DNA from *M. septicum* isolate W4964^T was determined spectrophotometrically by using the thermal denaturation method as previously described (Mandel *et al.*, 1970).

RESULTS AND DISCUSSION

Phenotypic properties

The four isolates from the patient all exhibited the same phenotypic profile. Microscopic morphological studies showed that they were Gram-positive, pleomorphic coccobacilli, lacking spores and capsules, that frequently exhibited longer filamentous forms. No true branched filaments were observed. Upon examination with a low-power stereomicroscope, colonies of cultures incubated on blood agar plates at 35 °C for 2 d showed slightly beige, cerebriform and irregular-edged colonies (1 mm in diameter) that did not demonstrate aerial hyphae. The isolates were acid-fast by the modified Kinyoun method.

The isolates grew on Lowenstein–Jensen medium at 35 °C in less than 7 d; grew at 28 °C in 5% NaCl and on MacConkey agar without crystal violet; were negative for growth in lysozyme, utilization of citrate and hydrolysis of acetamide; and were positive for iron uptake, nitrate reduction and 14-d arylsulphatase production. The isolates produced acid from D-fructose, D-glucose, glycerol, i-myo-inositol, D-mannitol, D-mannose, salicin, D-trehalose and D-xylose. They did not produce acid from adonitol, L-arabinose, cellobiose, dulcitol, i-erythritol, galactose, lactose, maltose, melibiose, raffinose, L-rhamnose, D-sorbitol, starch or sucrose. All of the isolates were resistant to ampicillin, cefamandole, cefotaxime, ceftriaxone and streptomycin. The isolates showed susceptibility to the remaining 14 antimicrobial agents tested.

Fig. 1. Comparison of representative mycolic acid profiles as determined by HPLC of *Mycobacterium septicum* (four isolates), *Mycobacterium fortuitum*, *Mycobacterium peregrinum* and *Mycobacterium senegalense*. The last peak, labelled STD, corresponds to the internal standard.

HPLC analysis

RRT ratios (± 0.01 min) were used to compare corresponding peaks among chromatograms (Butler *et al.*, 1992). The mycolates for the isolates appeared within the expected range for the rapidly growing mycobacteria, which elute after 5 min by the HPLC method. Comparison of the RRT ratios for these isolates with strains of *M. fortuitum*, *M. peregrinum* and *M. senegalense* revealed that these species are not easily differentiated by HPLC profiles (Fig. 1). The RRT ratios for initial eluting peaks for each of the species studied were the same, but *M. senegalense* exhibited some differences in the RRT ratios for the late eluting peaks. The late eluting peaks for the isolates, *M. fortuitum* and *M. peregrinum* exhibited the same RRT with slight differences in relative heights. Visual recognition by HPLC clearly distinguished

Fig. 2. Ribotype patterns from *SalI*-digested genomic DNA of *Mycobacterium septicum* and the other members of the *Mycobacterium fortuitum* complex. Lanes: M, λ -DNA molecular size marker digested with *EcoRI* and *HindIII*; 1, *Mycobacterium senegalense* ATCC 35796^T; 2, *Mycobacterium peregrinum* ATCC 14467^T; 3, *Mycobacterium fortuitum* ATCC 6841^T; 4–7, *Mycobacterium septicum* sp. nov. isolates W4962, W4963, W4964^T and W5064, respectively. Molecular sizes are given in kilobases (kb).

these isolates from *Mycobacterium chelonae* and *Mycobacterium abscessus*, which were previously included in the *M. fortuitum* complex. HPLC analysis alone was not conclusive; therefore, additional test methods were required to differentiate the isolates from the members of the *M. fortuitum* complex.

Genotypic description and evaluation

Ribotyping is useful in species identification, especially when strains are difficult to analyse phenotypically, by detecting minor genetic differences and providing information for species determination on the basis of common bands (Kiehlbauch *et al.*, 1991). Moreover, ribotyping has been a potent molecular technique in species differentiation, particularly when combined with other genetic tools such as 16S rRNA gene sequencing and DNA–DNA hybridization (Popovic *et al.*, 1993). As shown in Fig. 2, *SalI*-digested genomic DNA hybridized with five digoxigenin-labelled oligonucleotides gave identical hybridization bands between the catheter tip and the blood isolates. This banding pattern differed significantly from those of *M. fortuitum*, *M. peregrinum* and *M. senegalense*. Similar results were obtained with *SmaI* (results not shown). The isolate obtained from the catheter tip and the three blood isolates were identical in phenotypic tests including morphological and physiological tests and antimicrobial susceptibilities; these four isolates had

identical RFLP patterns of the chromosomal DNA digested with *SalI*, *SmaI*, *PvuII* and *PstI* (results not shown) demonstrating they are of clonal origin. In addition, the catheter tip and the three blood isolates differed from *M. fortuitum* third biovariant complex (sorbitol-positive) reference strain ATCC 49403 and *M. fortuitum* third biovariant complex (sorbitol-negative) reference strain ATCC 49935 as presented in Hogg *et al.* (1999).

A 1387 bp region of a 16S rRNA gene corresponding to *Escherichia coli* positions 109–1509 (GenBank accession number J01859) was determined for isolate W4964^T. The W4964^T sequence was aligned with 18 other mycobacterial 16S rRNA sequences and with *Nocardia otitidiscaviarum* as the outgroup. The GenBank accession numbers and strain designations for the sequences used in the alignment are given in Table 1. *Mycobacterium mageritense* was not included in the alignment because of its incomplete 16S sequence (634 bases). Regions in the alignment that were not represented in the majority of sequences were removed, resulting in a 971 base sequence consisting of regions 125–642, 892–1081 and 1109–1377 (*E. coli* numbering system). The multiple alignment was used as the input to a phylogeny inference software package (PHYLP) and a phylogenetic tree was generated (Fig. 3). W4964^T was placed on a branch with *M. peregrinum*, and in a group with *M. senegalense* and *M. fortuitum*. Bootstrap analysis indicated that the branch consisting of W4964^T and *M. peregrinum* was robust (94% of all possible errors), whereas the branch occupied by *M. fortuitum*, *M. senegalense*, isolate W4964^T and *M. peregrinum* occurred in 70% of the trees. The distance matrix generated from the edited alignment indicated that the *M. peregrinum* sequence was the most similar to that of W4964^T (99.58%); the *M. senegalense* sequence similarity value was 99.26% and *M. fortuitum* was 98.85% similar. The remainder of the mycobacterial 16S sequences were 93.58–98.22% similar to W4964^T. In a pairwise comparison, *M. mageritense* was 97.10% similar to W4964^T over a 621 base region.

The corrected G + C content for isolate W4964^T was 64 mol %, placing it within the 61–71 mol % range for *Mycobacterium* species as given by Takeuchi & Hatano (1998).

The results of DNA-relatedness studies are given in Table 2. The levels of DNA hybridization between *M. septicum* catheter tip isolate W4964^T and the blood isolates were between 82% and 98% with a divergence of 0%. The percentage relatedness between W4964^T and the type strains of *M. fortuitum*, *M. peregrinum* and *M. senegalense* was 25% or less with divergence of 5.0–5.5%. These results indicated that the four isolates represent a unique species, according to the phylogenetic definition of 'approximately 70% or greater DNA–DNA relatedness and with 5% or less ΔT_m ' as given by Wayne *et al.* (1987). The name *Mycobacterium septicum* is proposed for the new species.

Table 1. Strain designation and GenBank accession numbers for sequences in 16S rRNA tree

Organism	Strain	GenBank accession no.
<i>Mycobacterium abscessus</i>	ATCC 19977 ^T	X82235
<i>Mycobacterium aichiense</i>	ATCC 27280 ^T	X55598
<i>Mycobacterium aurum</i>	ATCC 23366 ^T	M29558
<i>Mycobacterium avium</i>	DSM 43216	X52918
<i>Mycobacterium chubuense</i>	ATCC 27278 ^T	X55596
<i>Mycobacterium chelonae</i>	ATCC 35752 ^T	X82236
<i>Mycobacterium diernhoferi</i>	ATCC 19340 ^T	X55593
<i>Mycobacterium fortuitum</i>	ATCC 6841 ^T	X52933
<i>Mycobacterium gilvum</i>	ATCC 43909 ^T	X55599
<i>Mycobacterium mucogenicum</i>	ATCC 49650 ^T	X80771
<i>Mycobacterium neoaurum</i>	ATCC 25795 ^T	M29564
<i>Mycobacterium obuense</i>	ATCC 27023 ^T	X55597
<i>Mycobacterium peregrinum</i>	ATCC 14467 ^T	AF130308
<i>Mycobacterium phlei</i>	ATCC 11758 ^T	M29566
<i>Mycobacterium senegalense</i>	ATCC 35796 ^T	M29567
<i>Mycobacterium septicum</i>	W4964 ^T	AF111809
<i>Mycobacterium smegmatis</i>	ATCC 14468 ^T	X52922
<i>Mycobacterium tuberculosis</i>	NCTC 7416 ^T H37Rv	X5889
<i>Mycobacterium ulcerans</i>	Unknown	X58954
<i>Nocardia otitidiscaviarum</i>	ATCC 14629 ^T	M59056

Fig. 3. Phylogenetic tree based on 16S rRNA sequences showing the position of *Mycobacterium septicum* strain W4964^T. The tree was rooted by using *Nocardia otitidiscaviarum* as the outgroup. *Mycobacterium mageritense* is not shown. Scale bar, 1% difference in sequence.**Table 2.** DNA relatedness of *Mycobacterium septicum* sp. nov. isolate W4964^T to the other *Mycobacterium septicum* isolates and *Mycobacterium fortuitum*, *Mycobacterium peregrinum* and *Mycobacterium senegalense*

Source of unlabelled DNA	RBR*	Divergence†
<i>M. septicum</i> isolate W4962	98	0.0
<i>M. septicum</i> isolate W4963	96	0.0
<i>M. septicum</i> isolate W5064	82	0.0
<i>M. fortuitum</i> isolate ATCC 6841 ^T	25	5.5
<i>M. peregrinum</i> isolate ATCC 14467 ^T	22	5.0
<i>M. senegalense</i> isolate ATCC 35796 ^T	23	5.0

* Relative binding ratio [(percentage heterologous DNA bound to hydroxyapatite/percentage homologous DNA bound by hydroxyapatite) × 100] at 75 °C. All reactions were done at least twice.

† Divergence is the decrease in thermal stability (in degrees Celsius) of heterologous DNA duplexes compared with those of homologous DNA duplexes.

Characteristics that differentiate *M. septicum* from related mycobacteria

M. septicum can be distinguished from the type strains of *M. fortuitum*, *M. peregrinum* and *M. senegalense* by using a variety of biochemical and antimicrobial susceptibility tests. *M. septicum* differs from the type strain of *M. fortuitum* in that the former does not grow at 42 °C, does not hydrolyse acetamide, does not have 3-d arylsulfatase activity, but produces acid from D-

Table 3. Phenotypic differences between the *Mycobacterium septicum* isolates and *Mycobacterium fortuitum*, *Mycobacterium peregrinum* and *Mycobacterium senegalense*

+, Positive; —, negative.

Characteristic	<i>M. fortuitum</i> ATCC 6841 ^T	<i>M. peregrinum</i> ATCC 14467 ^T	<i>M. senegalense</i> ATCC 35796 ^T	<i>M. septicum</i> isolates (n = 4)
Growth at 42 °C	+	—	+	—
Hydrolysis of acetamide	+	—	+	—
Arylsulfatase activity at 3 d	+	+	+	—*
Utilization of citrate	—	—	+	—
Acid production from:				
D-Mannitol	—	+	+	+
i-myo-Inositol	—	—	—	+
Resistance to:†				
Erythromycin (MIC ≥ 8 µg/ml)	+	+	+	—
Minocycline (MIC ≥ 16 µg/ml)	—	+	—	—
Sulfamethoxazole (MIC ≥ 32 µg/ml)	—	—	+	—
Doxycycline (MIC ≥ 16 µg/ml)	—	+	—	—
Vancomycin (MIC ≥ 32 µg/ml)	+	+	—	—
Kanamycin (zone size < 15 mm)	—	—	+	—
Gentamicin (zone size < 20 mm)	—	—	+	—
Tobramycin (zone size < 20 mm)	+	+	+	—
Neomycin (zone size < 20 mm)	—	—	+	—

* All isolates are positive for arylsulfatase activity at 14 d. Positive reactions may be detected at 3 d with other methods.

† The MIC breakpoints that were used are those of the National Committee for Clinical Laboratory Standards (1997). Disk contents were as follows: kanamycin (30 µg), tobramycin (10 µg) and neomycin (30 µg). Zone sizes for resistance are those of Grange & Stanford (1974).

mannitol and i-myo-inositol. Furthermore, *M. septicum* is susceptible to erythromycin, vancomycin and tobramycin. *M. peregrinum* and *M. septicum* differ in that *M. peregrinum* has 3-d arylsulfatase activity, does not produce acid from i-myo-inositol and is resistant to erythromycin, minocycline, doxycycline, vancomycin and tobramycin. *M. septicum* and *M. senegalense* can be differentiated in that *M. senegalense* grows at 42 °C, hydrolyses acetamide, utilizes citrate, has 3-d arylsulfatase activity, does not produce acid from i-myo-inositol, and is resistant to erythromycin, sulfamethoxazole, kanamycin, gentamicin, tobramycin and neomycin. The phenotypic differences between *M. septicum* and the members of the *M. fortuitum* complex are given in Table 3. In addition, genetic techniques such as ribotyping and 16S rRNA sequencing can be used to differentiate *M. septicum* as shown in Figs 2 and 3, respectively.

Description of *Mycobacterium septicum* sp. nov.

Mycobacterium septicum (sep'ti.cum. M.L. adj. for spread of bacteria in blood, referring to the condition from which this species was isolated).

This organism was isolated from and was the aetiological agent of catheter-related bacteraemia in a 2-year-old child diagnosed with metastatic hepatoblastoma (Hogg *et al.*, 1999). The cells are acid-fast, Gram-positive, pleomorphic coccobacilli. Longer filamentous forms are often observed but spores and capsules are absent. Colonies are cerebriform, slightly beige, with an irregular edge and do not demonstrate aerial hyphae. Growth occurs on Lowenstein-Jensen medium at 35 °C in less than 7 d, at 28 °C in 5 % NaCl and on MacConkey agar without crystal violet. The isolates reduce nitrate, have arylsulfatase activity by 14 d and exhibit iron uptake. They do not hydrolyse acetamide, utilize citrate or grow in lysozyme. *M. septicum* produces acid from D-fructose, D-glucose, glycerol, i-myo-inositol, D-mannitol, D-mannose, salicin, D-trehalose and D-xylose, but not from adonitol, L-arabinose, cellobiose, dulcitol, i-erythritol, galactose, lactose, maltose, melibiose, raffinose, L-rhamnose, D-sorbitol, starch or sucrose. This organism is resistant to ampicillin, cefamandole, cefotaxime, ceftriaxone and streptomycin but susceptible to amikacin, amoxicillin-clavulanate, ciprofloxacin, doxycycline, erythromycin, imipenem, minocycline,

sulfamethoxazole, trimethoprim-sulfamethoxazole, vancomycin, tobramycin, gentamicin, neomycin and kanamycin. *M. septicum* has a G+C content of 64 mol%. The nearest phylogenetic neighbours of *M. septicum* by 16S rRNA gene sequencing are *M. peregrinum*, *M. senegalense* and *M. fortuitum*. The type strain of *M. septicum* is W4964^T, which was isolated from the catheter tip of a 2-year-old child with metastatic hepatoblastoma. This strain has been deposited in the DSMZ as DSM 44393^T and in the ATCC as ATCC 700731^T.

ACKNOWLEDGEMENTS

We thank Robbin S. Weyant for his encouragement and support and Winfried Barchet for his assistance in sequencing. We thank Tanja Popovic, Centers for Disease Control and Prevention, Division of Bacterial and Mycotic Diseases, Meningitis and Special Pathogens Branch, for providing the digoxigenin-labelled set of five oligonucleotides that are complementary to bacterial 16S+23S rRNA gene sequences.

Use of trade names is for identification only and does not imply endorsement by the US Public Health Service or the US Department of Health and Human Services.

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