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Polyphasic Characterization Reveals that the Human Pathogen *Mycobacterium peregrinum* Type II Belongs to the Bovine Pathogen Species *Mycobacterium senegalense*†

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Mycobacterium peregrinum **consists of two taxa: types I and II. We evaluated 43 clinical type II strains from throughout the United States. They were responsible for soft-tissue and bone infections, catheter-related infections, and possible pneumonitis. By carbohydrate utilization, they were indistinguishable from type I strains, being D-mannitol and trehalose positive. However, they had a distinct susceptibility pattern that included intermediate ciprofloxacin MICs but low clarithromycin and doxycycline MICs of ≤1 μg/ml. These features were also shared by reference isolates of** ***Mycobacterium senegalense*** **from African bovine cases of "farcy." By 16S rRNA gene sequencing, the type II isolates shared 100% sequence identity with** ***M. senegalense*****. Partial sequencing of the type II** ***hsp65*** **gene (441 bp) revealed four sequevars showing ≥98.4% identity with each other and ≥98.6% identity with the sequence of five bovine strains of** ***M. senegalense*****. There was ≤97.1% identity with** ***M. peregrinum*** **type I isolates and other** ***Mycobacterium fortuitum*** **group species. Sequencing of additional gene targets including the 16S–23S rDNA internal transcribed spacer region and the** ***rpoB*** **gene (partial sequence) revealed a similar phylogenetic grouping. DNA-DNA hybridization showed 76 to 99% relatedness between the bovine and human strains. These studies demonstrate that type II isolates are not isolates of** ***M. peregrinum*** **but represent human strains of** ***M. senegalense*****. This study is the first to demonstrate this species as a human pathogen. Representative human** ***M. senegalense*** **strains include ATCC 35755 and newly submitted strains ATCC BAA-849, ATCC BAA-850, and ATCC BAA-851.**

In 1962, Bojalil and colleagues described a new species of nonpigmented, rapidly growing mycobacteria which they named *Mycobacterium peregrinum*, a name derived from the Latin meaning "strange" or "foreign" (4). Presumably, the name was chosen based on the observation that the isolate was unusual or differed from the common *Mycobacterium fortuitum*. The type strain of the species was ATCC 14467^T. A study by Rudolph Bönicke in 1966 was one of the first to note different biochemical groups within the species *Mycobacterium fortuitum* using carbohydrate studies (5). Bönicke noted that one group, which he called group A, was negative for carbohydrates; a second group, which he called group B, was positive for mannitol only; and a third group, which he called group C, was positive for mannitol and inositol.

A detailed study of rapidly growing mycobacteria by Kubica et al. and the International Working Group on Mycobacterial Taxonomy was published in 1972 (20). The authors noted that the biochemical results of Bojalil et al. with ATCC 14467^T (4) could not be reproduced, likely because the authors of the earlier study utilized sugar fermentations which produce erratic results in taxa such as this one that produce complete sugar hydrolysis. They did observe that ATCC 14467^T and related strains formed a unique taxonomic cluster with groupings similar to those of Bönicke. They positioned the three groups of Bönicke as biovariants of *M. fortuitum.* Bönicke's group B, which was positive for mannitol only among the commonly tested sugars, was named *Mycobacterium fortuitum* biovariant *peregrinum.* The other two groups became known as *Mycobacterium fortuitum* biovariant *fortuitum* (group A) and *M. fortuitum* third-biovariant complex (group C).

In 1981, Silcox et al. described laboratory criteria for the three biovariants and noted recovery of all three taxa from patient sources highly suggestive of clinical disease (32). Sub-

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sequent DNA-DNA homology studies (2, 22, 24) resolved the issue of the status of Bojalil's ATCC 14467^T; they established this strain and Bonicke's original group B as a species. The original name of *M. peregrinum* proposed by Bojalil et al. (4) remains its accepted designation (22). The first complete and accurate 16S rRNA gene sequence of the type strain of *M. peregrinum* was submitted to GenBank in 2000 and updated in 2002 (accession number AF130308).

Compared to the other two Bönicke groups recognized within *M. fortuitum* (now also species, with the third biovariant comprising multiple species), little has been documented in the literature about *M. peregrinum.* It is known to be a human pathogen, but no large study of clinical disease has been reported, and isolates described prior to the 1980s were from sputum and of unknown clinical significance. Silcox et al. reported nine isolates in 1981 associated with cavitary lung disease and wounds of the sternum and foot (32). In 1983 Wallace et al. described 64 cases of human disease due to the *M. fortuitum* group, 3 of which were due to *M. peregrinum* and associated with skin or soft-tissue disease (52). One pseudooutbreak of respiratory disease due to a contaminated ice machine (23) and one cluster of sternal wound infections in Hong Kong (54) due to *M. peregrinum* have been described since that time. No detailed laboratory investigation of the species has been reported since the species description by Kusunoki and Ezaki in 1992 (22).

The possibility that the species *M. peregrinum* might include more than one taxonomic group was first noted in 1985. Wallace and colleagues evaluated the β-lactamase patterns of *M. peregrinum* using polyacrylamide gels (49, 55). Two major patterns were seen, one represented by ATCC 14467^T and the other represented by ATCC 35755. In a disk diffusion study of the susceptibility of the *M. fortuitum* group to an early quinolone known as pipemidic acid, Steele and Wallace (36) noted that clinical strains and the same two ATCC reference strains of *M. peregrinum* could be divided into two groups: one susceptible and one resistant to pipemidic acid. The resistant group represented 3.1% of 162 consecutive clinical isolates of the *M. fortuitum* group and was comparable in prevalence to the susceptible group. Subsequent studies of isoenzymes (3), mycobactin patterns (6), ribotyping (54), and PCR restriction enzyme analysis (PRA) patterns for *M. peregrinum* using the *groEl* heat shock protein gene (25) showed similar groupings, though the relationship to pipemidic acid susceptibility was not noted.

In a 1995 description of the use of PRA of the 441-bp Telenti fragment of the *hsp65* gene (40) for identification of rapidly growing mycobacteria, Steingrube et al. noted that two restriction fragment length polymorphism patterns were present among isolates that met the current phenotypic definition of *M. peregrinum* published by Silcox et al. (32, 37). These two groups corresponded to the pipemidic acid-susceptible and -resistant groups and also differed in other characteristics, including growth at 45°C and use of acetamide as a carbon source (36, 37). These two groups then became known as the pipemidic acid-susceptible and -resistant groups, or *M. peregrinum* type I and type II. The pipemidic acid-susceptible group (type I) included the ATCC type strain 14467^T of Bojalil et al. (4), while the pipemidic acid-resistant group (type II) included ATCC 35755.

In 1999, Ringuet et al. compared results of sequencing of the Telenti fragment (40) of the *hsp65* gene among members of the *M. fortuitum* complex (30). Fourteen clinical isolates of *M. peregrinum* were sequenced, of which 11 (79%) were identical or within 2 bp of ATCC 14467^T, while 3 strains (21%) had very distant sequences that were intermediate between *M. peregrinum* and *M. fortuitum*. The relationship of the latter group to pipemidic acid susceptibility or to PRA patterns was not reported, and strain ATCC 35755 was not studied.

While the 16S rRNA gene of the type strain of *M. peregrinum* has been sequenced and is present in GenBank as well as in other database systems such as RIDOM (17), strain ATCC 35755 has not, to our knowledge, been sequenced previously.

Molecular analysis has become an essential component of accurate mycobacterial species identification (34). We have collected a large series of the type II isolates of *M. peregrinum*, and here we present a polyphasic phenotypic and molecular analysis of this group with a proposal for its recognition as human isolates of the bovine species *Mycobacterium senegalense*.

MATERIALS AND METHODS

Isolates. PRA of the *hsp65* gene has been used routinely in the Mycobacteria/Nocardia Laboratory at the University of Texas Health Center for identification of rapidly growing mycobacteria since 1995. For the current study, PRA records were screened for isolates that had the PRA pattern corresponding to the pipemidic acid-resistant group of *M. peregrinum* (type II) described by Steingrube et al. (37). Upon analysis of these isolates, it became apparent that *M. peregrinum* type II isolates had a unique susceptibility pattern that allowed their recognition among isolates of the *M. fortuitum* group which had been submitted specifically for susceptibility testing without species identification. Two hundred fifty-five consecutive isolates of the *M. fortuitum* group that had been submitted for susceptibility testing and not identification (and that therefore had not undergone *hsp65* PRA analysis) between 2001 and 2004 were then screened for the drug susceptibility pattern of the type II isolates. These isolates, with the type II drug pattern, were then taken from stocks frozen at −70°C, subcultured, and subjected to *hsp65* PRA and biochemical analysis. Isolates confirmed by PRA as type II were included in the study. Approximately 10% of the *M. peregrinum* type II strains used in the study were initially recognized at the Mayo Clinic Laboratories or ARUP Laboratories based on partial sequencing of the 16S rRNA gene.

Reference strains used for comparison included the type strains of *M. peregrinum* (ATCC 14467^T), *M. houstonense* (ATCC 49403^T), *M. fortuitum* (ATCC 6841^T), and *M. neworleansense* (ATCC 49404^T), as well as all 10 strains of *M. peregrinum* currently in the ATCC: ATCC 35755 (formerly TMC 1545), ATCC 700686, ATCC 23001, ATCC 23015, ATCC 23017, ATCC 23020, ATCC 23022, ATCC 23041, ATCC 23047, and ATCC 23049. Also evaluated were *M. senegalense* ATCC 13781 and ATCC 35796^T (both bovine strains) and *M. farcinogenes* ATCC 35753^T. Additional bovine strains of *M. senegalense* were obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany): DSM 43658, DSM 43661, DSM 43663, and DSM 43664.

Information including geographic location, source of infection, and type of disease was obtained. For some patients, follow-up from consultation with their physician on the use of drug therapy was also obtained. Chart reviews were not performed. Thus, informed consent was not obtained.

Phenotypic species identification. Organisms were identified as rapidly growing mycobacteria on the basis of growth within 7 days on both Middlebrook 7H10 or 7H11 agar and Trypticase soy agar and on the basis of typical colony morphology. These isolates had also been identified as rapidly growing mycobacteria or as members of *Mycobacterium* species before their submission to our laboratory by the referring laboratory. Isolates were identified as presumptive members of the *Mycobacterium fortuitum* group on the basis of the absence of pigmentation and typical drug susceptibility patterns that included disk diffusion susceptibility to polymyxin B and amikacin (51), susceptible MICs to amikacin (38), ciprofloxacin (43), and sulfamethoxazole, and intermediate or susceptible MICs to cefoxitin (38, 44) and imipenem (see the next section for methods and breakpoints) (39, 44).

Isolates within the *M. fortuitum* group were identified as *M. peregrinum* by the pattern of utilization of carbohydrates as sole carbon sources according to Silcox et al. (32) and Tsukamura (41), which included being positive for D-mannitol and negative for i-*myo*-inositol, D-glucitol (sorbitol), and citrate (14-day incubation). These studies had not included *M. senegalense*, while the study by Chamoiseau showed this rapidly growing mycobacterium to have a similar carbohydrate utilization pattern (12).

Isolates were also identified as *M. peregrinum* by PRA of the 441-bp Telenti fragment of the *hsp65* gene according to Steingrube et al. (37). By using PRA and selected growth and biochemical tests, the isolates were grouped as *M. peregrinum* type I on the basis of pipemidic acid susceptibility (36), failure to grow at 45°C, inability to utilize acetamide as a sole carbon source, and an *hsp65* gene PRA pattern of BstEII fragments of 235 and 210 bp and HaeIII fragments of 145, 140, and 100 bp. The previous study by Steingrube et al. (37) had shown *M. peregrinum* ATCC 14467^T of Bojalil et al. to belong to this group. Isolates were grouped as *M. peregrinum* type II if they were pipemidic acid resistant by the disk diffusion method (36), grew at 45°C, were able to utilize acetamide as a sole carbon source, and had an *hsp65* gene PRA pattern with BstEII fragments of 235, 115, and 80 bp and with HaeIII fragments of 140, 125, and 60 bp or 140, 125, and 100 bp. The reference strain for this group was ATCC 35755.

Susceptibility testing. Broth microdilution MIC testing for susceptibility to 12 antimicrobial agents was performed according to standard methods (9, 27, 38, 39). Drugs tested included amikacin, ciprofloxacin, sulfamethoxazole, imipenem, cefoxitin, tobramycin, and doxycycline (group A). More-recent isolates were also tested for susceptibility to five newer agents: linezolid (47), levofloxacin, gatifloxacin, meropenem, and clarithromycin (group B) (10, 11). Susceptible and resistant breakpoints were those recently recommended by the Clinical Laboratory Standards Institute (CLSI, formerly NCCLS) except for meropenem, levofloxacin, and gatifloxacin, which have not been addressed by the CLSI (27). For these drugs, the breakpoints used were those of bacterial species that grow aerobically (26). Quality control organisms utilized were *Staphylococcus aureus* ATCC 29213 and *M. peregrinum* ATCC 700686, as recommended by CLSI guidelines (27).

Disk diffusion testing for susceptibility to cephalothin (30 μg) (50), polymyxin B (300 U) (51), pipemidic acid (30 μg) (36), and amikacin (30 μg) (51) was also performed using commercial disks as previously described. The pipemidic acid disks are no longer commercially available, and consequently they were tested only against early isolates.

HPLC. High-performance liquid chromatography (HPLC) of cell wall mycolic acids was performed as previously described using fluorescence detection (8, 46, 53). Reference strains used for comparison included *M. peregrinum* ATCC 14467^T of Bojalil, *M. senegalense* ATCC 35796^T, *M. fortuitum* ATCC 6841^T, *M. houstonense* ATCC 49403^T, and *M. farcinogenes* ATCC 35753^T.

MALDI-TOF MS. A previous study suggested that intact cell matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS), which detects ions derived from cell wall components, could be used for mycobacterial species and/or strain identification (M. L. Pignone, K. M. Greth, and J. Tang, Abstr. 103rd Gen. Meet. Am. Soc. Microbiol., abstr. I-77, 2003; K. M. Greth, M. I. Pignone, and J. Tang, Abstr. 103rd Gen. Meet. Am. Soc. Microbiol., abstr. C-237, 2003). Selected mycobacterial strains were chosen for comparison by this method. Details of the method are included in the supplemental material.

Reference strains used included *M. fortuitum* ATCC 6841^T, *M. porcinum* ATCC 33776^T, *M. farcinogenes* ATCC 35753^T, *M. houstonense* ATCC 49403^T, *M. senegalense* ATCC 35796^T and ATCC 13781, *M. chelonae* ATCC 35752^T, *Mycobacterium chelonae* subsp. *niacinogenes* ATCC 19237^T, and *M. abscessus* ATCC 19977^T.

Partial sequencing of the *hsp65* gene. DNA from isolates of *M. peregrinum* type II (23 isolates, including ATCC 35755) and the bovine strains of *M. senegalense* (6 isolates) underwent sequencing of the 441-bp Telenti fragment using primers TB11 and TB12 (40) and an ABI PRISM 310 Genetic Analyzer capillary sequencer (Applied Biosystems). Sequences were compared to those in GenBank or sequenced in-house. Strains for *hsp65* sequencing were the same as those for 16S rRNA gene extended or complete sequencing. Pairwise alignments were performed using ClustalW and phylogenetic analyses using the neighbor-joining method in the MEGA 2.1 software (21).

PRA. PRA was performed on the 441-bp Telenti fragment of the *hsp65* gene as described for mycobacteria (40) and modified by Steingrube et al. for rapidly growing mycobacteria (37). The amplicon was digested with BstEII and HaeIII and separated on 3% metaphor agarose gels. Fragment sizes in base pairs were estimated on a computerized Bio Image System (Millipore, Bedford, Mass.).

Sequencing of the 16S rRNA gene. Genomic DNAs from selected isolates were sequenced using the MicroSeq 500 16S rDNA Bacterial Identification System (Applied Biosystems, Foster City, Calif.), which sequences the first 500 bp at the 5′ end (13, 15, 28) and includes hypervariable regions A and B (19, 33). Data analysis was performed using MicroSeq version 1.4 (Applied Biosystems) at the Mayo Clinic (Rochester, Minn.) or ARUP (Salt Lake City, Utah).

This fragment of the 16S rRNA gene cannot differentiate between several rapidly growing species of mycobacteria, and additional sequencing in the latter part of the 16S rRNA gene was performed as described by Turenne et al. (42) on selected clinical isolates with the type II pattern ($n = 22$), *M. peregrinum* ATCC strains 35755, 23001, and 23015, and the bovine strains of *M. senegalense* from the DSMZ. Analysis spanned at least *Escherichia coli* bp 970 to 1390, a region that includes differentiating bases within species that are identical in the first 500 bp of the gene. To our knowledge, no mycobacterial species that are identical in these two regions present variations outside this range.

Complete 16S rRNA sequencing (*E. coli* bp 8 to 1508) was performed for clinical strains MF-1868, MF-548, and MF-1796 and was previously performed for reference strains *M. senegalense* ATCC 35796^T, *M. farcinogenes* ATCC 35753^T, and *M. peregrinum* ATCC 14467^T, ATCC 23001, and ATCC 23015 (42). Sequence databases used for comparison included an in-house database (42), RIDOM (http://www.ridom-rdna.de) (17, 18), and GenBank. Phylogenetic analyses were performed using MEGA2 as described above.

16S–23S ITS1 region sequencing. The same strains for which *hsp65* sequencing was performed also underwent sequencing of the 16S–23S internal transcribed spacer (ITS1) region using primers 16S-1511f (5′-AAG TCG TAA CAA GGT ARC CG-3′) and 23S-23r (5′-TCG CCA AGG CAT CCA CC-3′) (14). Sequences were compared to available sequences currently in GenBank and RIDOM (17, 18). Phylogenetic analyses were performed using MEGA2 as described above.

Partial sequencing of the *rpoB* gene. Sequence analysis of a 723-bp region of the *rpoB* gene was performed as described by Adékambi et al. (1), using primers MycoF and MycoR for amplification and primers MycoseqF and MycoseqR for sequencing of the PCR product. Sequences were compared with those of Adékambi et al. (1), available in GenBank, and were retrieved for phylogenetic analyses using MEGA2 as described above.

DNA-DNA hybridization. Total-DNA relatedness studies were performed on a set of nine strains. The methods used to cultivate cells and to prepare, isolate, and purify labeled and unlabeled DNA, as well as the methods used for DNA reassociation and the separation of single-stranded and double-stranded DNA on hydroxyapatite, have been described previously (31). DNA relatedness was determined at the optimal (75°C) reassociation temperature. Percent divergence was calculated to the nearest 0.5% (7).

Nucleotide sequence accession numbers and newly submitted strains. Sequences of the partial *hsp65* gene (accession numbers AY684045 to AY684049), the 16S–23S internal transcribed spacer regions (AY684050 to AY684055), and the partial *rpoB* gene (AY684056 to AY684063) recognized in this study have been deposited in GenBank.

Human isolates of *M. senegalense* have been deposited in the American Type Culture Collection as ATCC BAA-849, BAA-850, and BAA-851.

RESULTS

Isolates. By routine PRA of clinically submitted isolates, 24 isolates of *M. peregrinum* type II were identified. Among the 255 consecutive clinical isolates of the *M. fortuitum* group submitted for susceptibility testing between 2001 and 2004, 15 had the susceptibility pattern suggestive of *M. peregrinum* type II and were screened by phenotypic testing and PRA. Of these, 14 (5.5%) were *M. peregrinum* type II. Five additional isolates were recognized based on partial 16S rRNA gene sequences.

Overall, a total of 43 clinical isolates of *M. peregrinum* type II were identified and studied in more detail. The majority of the 42 isolates with available information were from skin and soft-tissue infections (25/42 [60%]), with infections following surgical procedures or accidental trauma. At least five cases were known to be associated with osteomyelitis. Other sources included catheter- or pacemaker-related infections (7/42 [17%]) and respiratory sites (8/42 [19%]) whose clinical significance was generally unknown (see Table S1 in the supplemental material).

TABLE 1. Susceptibility results of *Mycobacterium peregrinum* type II clinical isolates (including two designated reference strains) and two reference strains of *M. senegalense*

Isolates tested and drug	MIC (μg/ml) for the indicated ATCC reference strain				Type II clinical isolates				
	Type II BAA-849	*M. peregrinum* 35755	*M. senegalense*		No. tested	MIC (μg/ml)[a]			
			13781	35796^T		Range	50%	90%	Mode
Group A (all isolates)									
Amikacin	0.5	≤0.25	≤2	≤1	43	≤0.25–4	≤1	≤2	≤1
Cefoxitin	16	8	16	16	43	4–32	16	32	16
Ciprofloxacin	0.25	≤0.063	1	0.5	43	0.25–1	0.5	1	0.5
Doxycycline	1	≤0.25	≤0.12	≤0.12	42	≤0.12–1	≤0.25	0.5	≤0.12
Imipenem	2	≤0.5	4	2	42	0.25–4	1	≤4	1
Sulfamethoxazole	64	≤1	16	16	42	≤1–64	8	32	8
Tobramycin	8	2	4	8	43	1–8	4	8	4
Group B (selected isolates)									
Clarithromycin	0.5	≤0.12	0.5	0.25	30	≤0.12–2	0.25	1	≤0.25
Levofloxacin	0.5	≤0.12	0.5	0.5	23	≤0.12–1	0.5	1	0.5
Linezolid	4	≤2	4	≤2	30	1–8	2	8	≤2
Gatifloxacin	0.25	≤0.06	0.25	0.12	24	≤0.12–0.5	≤0.12	0.25	≤0.12
Meropenem	1	2	8	4	24	≤0.5–4	2	4	1, 2, 4

[a] 50% and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.

The 41 isolates for which geographic sites were known were from 16 states and the Dominican Republic. The majority of the isolates (22/41 [54%]) were from Texas, Florida, and North Carolina, and 26/41 (63%) were from Southern coastal states.

Phenotypic identification. All of the isolates of *M. peregrinum* type II grew in less than 7 days on Trypticase soy agar and Middlebrook 7H10 or 7H11 agar, were nonpigmented, and had morphology typical of the *M. fortuitum* group. On Mueller-Hinton agar, used for disk diffusion susceptibility testing, the isolates tended to show a fine growth and were often unreadable at 72 h, which is relatively unusual for members of the *M. fortuitum* group.

The results of growth and biochemical testing are listed in Table S2 in the supplemental material. The isolates were similar to other members of the *M. fortuitum* group including *M. peregrinum* type I (ATCC 14467^T) and previous groupings that did not recognize the two types. Selected tests were performed against all isolates (group A). These revealed that all isolates were nonpigmented and grew at 30 and 35°C and that 78% grew at 45°C. Like isolates of *M. peregrinum* type I, including ATCC 14467^T, the isolates were able to utilize D-mannitol as a sole carbon source but not citrate, i-*myo*-inositol, or D-sorbitol (20, 32, 37). Isolates were also negative for L-rhamnose and positive (90%) for D-trehalose (but generally positive late [at 2 weeks], unlike the pattern for other carbohydrates, for which isolates were positive after 3 days of incubation). Unlike isolates of *M. peregrinum* type I, the type II isolates were positive for acetamide, and most grew at 45°C, as previously noted (37).

Some traditional tests were performed against a minority of the type II isolates (group B). They showed that the type II isolates were arylsulfatase positive at 3 days, reduced nitrate, grew on MacConkey agar, and were iron uptake positive (20, 32).

The two bovine ATCC reference strains of *M. senegalense* had the same biochemical reactions as the *M. peregrinum* type II isolates, except that both isolates of the former were D-trehalose negative. The single available ATCC strain of *M. peregrinum* type II (ATCC 35755) was also D-trehalose negative (see Table S2 in the supplemental material).

M. farcinogenes ATCC 35753^T was also studied, because it is the closest relative by 16S rRNA gene sequencing to *M. senegalense*, along with *M. houstonense* ATCC 49403^T, with which *M. farcinogenes* shares 100% 16S rRNA gene sequence identity. *M. farcinogenes* grew slowly, had a low semiquantitative catalase reaction (10 mm), was Tween positive, was negative for 3-day arylsulfatase, nitrate, iron uptake, urease, and D-mannitol, and did not grow on 5% NaCl. These results are similar to those listed in the catalogue of the Trudeau Mycobacterial Culture Collection (TMC) (25a), where the isolate was stored prior to being transferred to the ATCC.

Susceptibility testing. Susceptibility testing was performed using the CLSI-recommended broth microdilution method for rapidly growing mycobacteria (27). The type II isolates gave a strikingly uniform pattern of susceptibility that was readily separable from those of other clinical species within the *M. fortuitum* group, including the *M. peregrinum* type I isolates. In general they were highly drug susceptible (Table 1). For all the type II isolates, clarithromycin MICs were ≤1 μg/ml, much lower than those for most other recognized species (10, 46, 48). In keeping with the resistance of type II isolates to pipemidic acid (an early quinolone) (36), ciprofloxacin MICs were in the upper limits of the CLSI susceptible category (27). The type II isolates were all doxycycline susceptible, with MICs of ≤0.5 μg/ml, which makes this the first *M. fortuitum* group taxon whose members are uniformly susceptible to the tetracyclines (27, 38, 39, 45, 46). The type II isolates also had low susceptible MICs to amikacin, were uniformly susceptible to linezolid, imipenem, and sulfamethoxazole, and were susceptible or intermediate to cefoxitin (27).

The two ATCC bovine strains of *M. senegalense* (ATCC 35796^T and ATCC 13781) gave the same susceptibility pattern as did the type II clinical isolates (see Table 1).

HPLC. By fluorescence HPLC, *M. peregrinum* type II isolates gave mycolic acid patterns that were generally indistinguishable from those of other members of the *M. fortuitum-M. smegmatis* group, including *M. fortuitum*, *M. porcinum*, the bovine strain of *M. senegalense*, *M. houstonense*, and *M. pere-*

TABLE 2. Sequevar designations of 8 reference strains and 19 clinical strains for which sequencing of the partial *hsp65* and *rpoB* genes and the complete 16S–23S ITS1 region was performed

Strain(s)	Sequevar by sequencing of:		
	hsp65	16S–23S rRNA ITS1	*rpoB*
M. senegalense ATCC 35796^T, DSM 43661, DSM 43663, DSM 43664	I	I	I
M. peregrinum type II			
MF-278, MF-417, MF-421, MF-528, MF-532, MF-548, MF-593, MF-620, MF-1756, MF-1787, MF-1788, MF-1796, MF-1868, MF-2132	II	II[b]	II[c]
MF-263 (ATCC BAA-849)	II	VI	VI
ATCC 35755	III	IIIa	IIIa
MF-378	III	IIIa	IIIb
MF-495	III	IIIa	II
MF-386 (ATCC BAA-851)	III	No data[d]	IIIa
MF-125[a]	III	IIIb	IIIa
MF-1738 (ATCC BAA-850)	IV	IV	IVa
MF-1816	IV	IV	IVb
MF-1925	V	No data	V

[a] Strain MF-125 presented with a single-base-pair variation in the 16S rRNA gene from the sequence of *M. senegalense* at bp 1246 in the *E. coli* sequence.
[b] Strains MF-593, MF-1788, and MF-1796 were not tested.
[c] Strains MF-1788 and MF-1796 were not tested.
[d] No data, evidence of the presence of two different ribosomal operons having different 16S–23S sequences; analysis could not be performed.

grinum ATCC 14467^T of Bojalil (type I). The reference strain of *M. farcinogenes*, ATCC 35753^T, had a late major peak that differed from the peaks of these isolates (see Fig. S3 in the supplemental material).

Mass spectroscopy. The 22 isolates tested were grouped into four clusters that showed intracluster variations of 0.12 or less (see Fig. S4 in the supplemental material). Six of the seven ATCC strains identified as *M. peregrinum* type I formed a cluster along with the *M. peregrinum* type strain, ATCC 14467^T. The three isolates of *M. chelonae*/*abscessus* formed the second cluster. The third cluster consisted of both isolates of *M. porcinum* and three out of four strains of *M. peregrinum* type II, while the fourth cluster consisted of one strain each of *M. peregrinum* type I and type II, the two bovine strains of *M. senegalense*, *M. fortuitum*, *M. houstonense*, and *M. farcinogenes*. Overall, the clustering of strains by MALDI-TOF MS was similar to that by molecular methods.

Partial sequencing of the *hsp65* gene. Four sequevars (sqv), which diverged from each other by 1 to 7 bp, or 0.2 to 1.6%, were identified among the type II isolates. These were referred to as sqv II through V (Table 2). These sequences, along with the sequences for the bovine strains of *M. senegalense*, were submitted to GenBank and were assigned accession numbers AY684045 to AY684049.

The two ATCC bovine strains of *M. senegalense*, ATCC 13781 and ATCC 35796^T, and the three DSM bovine strains of *M. senegalense* had the same *hsp65* sequence, sqv I, and exhibited 2-, 6-, 4-, and 5-bp differences (0.5 to 1.4% divergence) from sequevars II, III, IV, and V of the *M. peregrinum* type II group, respectively (Table 2). The most prevalent sequevar, sqv II, was seen in 15 of 23 (65%) type II isolates and differed by only 2 bp from sqv I at positions 168 (T to C) and 342 (A to G) of the 441-bp fragment. Interestingly, these two variations are present in all sequevars analyzed against the animal strains of *M. senegalense* and also in closely related taxa such as *M. farcinogenes*, *M. houstonense*, *M. neworleansense*, *M. fortuitum*, and *M. peregrinum* (type I) (see Table S5 in the supplemental material). The largest divergence (1.6%) was seen between sqv III and V, with 7 variations. The intraspecies variation exhibited by the different type II sequevars was comparable to the variation seen with the bovine reference strains of *M. senegalense* (≤1.4%), and all clustered together by phylogenetic analyses (Fig. 1b).

These sequences were then compared to the closest relatives based on the 16S rRNA gene sequence, mostly strains within the *M. fortuitum* group and *M. farcinogenes*, and against *hsp65* sequences submitted to GenBank. The species established as closest by 16S rRNA analysis exhibited a 6- to 10-bp *hsp65* sequence divergence from the four type II sequevars and *M. senegalense*. These species (strains) included the two species with identical 16S rRNA gene sequences, *M. houstonense* (ATCC 49403^T) and *M. farcinogenes* (ATCC 35753^T), and *M. fortuitum* (ATCC 6841^T) (see Table S5 in the supplemental material).

The remaining species within the *M. fortuitum* group exhibited a higher degree of *hsp65* sequence divergence, with differences of ≥12 bp (≥2.7%) from the type II gene sequences. This included all isolates of the *M. fortuitum* third-biovariant sorbitol-negative group (*M. neworleansense* ATCC 49404^T, *M. porcinum* ATCC 33776^T, *M. boenickei* ATCC 49935^T, *M. septicum* ATCC 700731^T), as well as *M. peregrinum* type I (ATCC 14467^T). The latter differed from the four type II sequevars by 13, 13, 14, and 15 bp, respectively, or ≥3.2%. All nucleotide variations seen among the type II sequevars, the bovine reference strains of *M. senegalense*, and their closest relatives were synonymous.

PRA of the *hsp65* partial gene sequence. The four *hsp65* gene sequevars among the clinical isolates of *M. peregrinum* type II gave one major and two minor *hsp65* gene PRA patterns (PRA types II to IV). The majority of isolates (40/43 [93%]) gave fragments of 235, 115, and 85 bp with BstEII and 140, 125, and 60 bp with HaeIII (PRA type II). These selected isolates (including ATCC 35755) were subsequently identified as having *hsp65* gene sequevars II and III as described above. A second group (2/43 [5%]) (PRA type III) had the same BstEII restriction fragments but gave HaeIII restriction fragments of 140, 125, and 100 bp. Sequencing showed that these isolates lacked one of the six HaeIII restriction sites present in the 441-bp sequence of sequevars II and III due to a C→T transition at base 402 (bp 546 of the complete gene sequence). This pattern identified *M. peregrinum* type II *hsp65* sequevar IV. A third group (1/43 [2%]) had the same HaeIII restriction fragments as PRA type III but gave a BstEII fragment of 125 (PRA type IV) instead of 115 bp, resulting from a T→C transition at base 327 (bp 471 of the complete *hsp65* gene), and identified *hsp65* sequevar V.

The 10 reference strains of *M. peregrinum* in the ATCC were subjected to PRA. Of these 10 isolates, 1 produced the common PRA pattern of *M. senegalense* (ATCC 35755), 1 exhibited the pattern of *M. porcinum* (ATCC 23041), and 5 showed patterns of *M. peregrinum* type I (ATCC 14467^T, 23001, 23020,

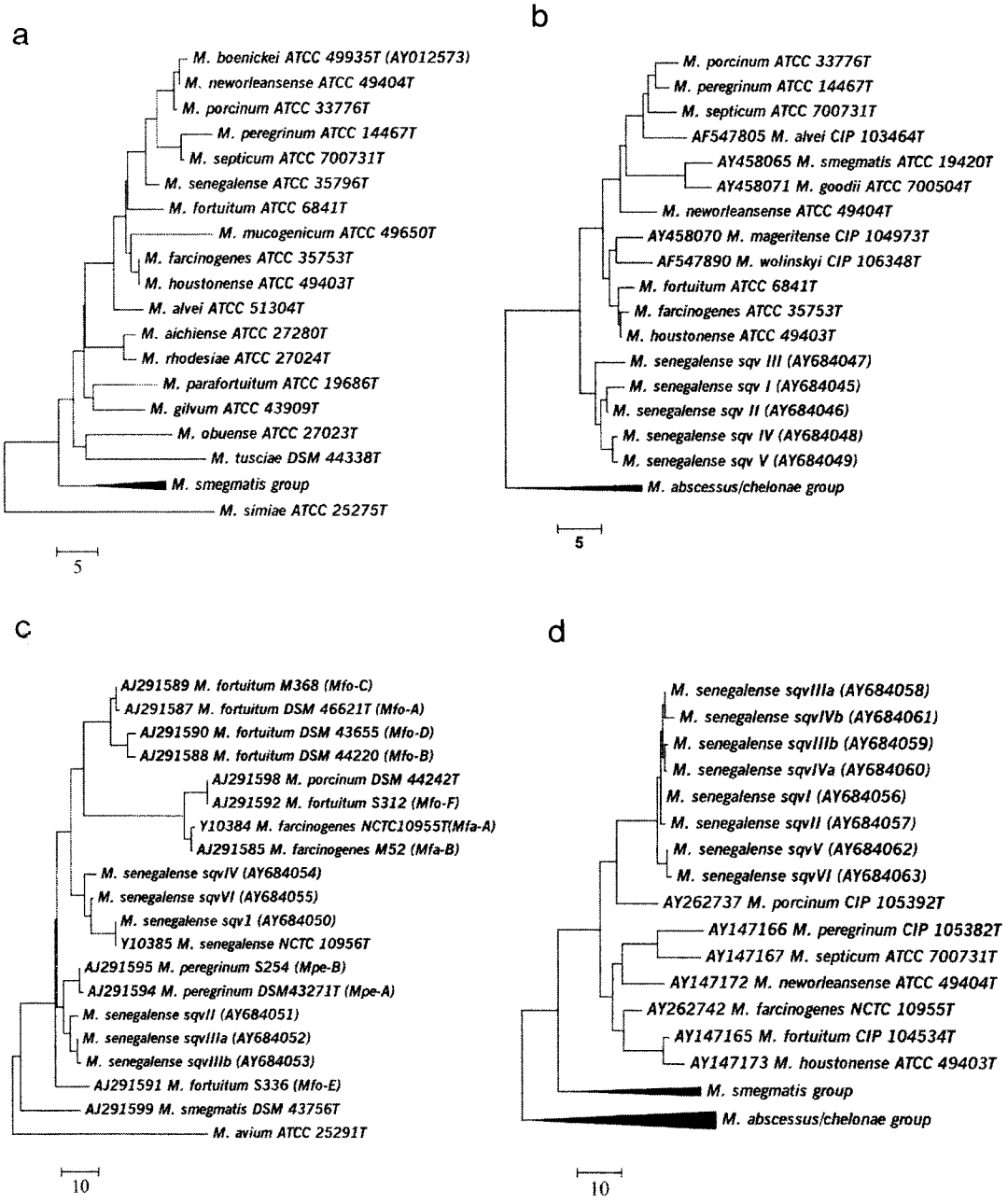

FIG. 1. Phylogenetic analyses of *M. senegalense* by 16S rRNA gene sequencing (1,427 bp) (a) and of its various sequevars by sequencing of the partial *hsp65* gene (441 bp) (b), the 16S–23S ITS1 region (c), and the partial *rpoB* gene (687 bp) (d). Analyses were determined using the neighbor-joining method. Bars, numbers of base pair differences.

23047, and 23049). Three isolates (ATCC 23015, 23017, and 23022) yielded unrecognized PRA patterns.

A restriction digest pattern identical to that of the *hsp65* PRA type II was observed with three other species: the *M. fortuitum* third-biovariant D-sorbitol-negative species *M. neworleansense* (ATCC 49404^T), the *M. fortuitum* third-biovariant D-sorbitol-positive species *M. houstonense* (ATCC 49403^T), and *M. farcinogenes* ATCC 35753^T. However, these species were readily separated from the type II isolates by drug susceptibilities, carbohydrate utilization, and growth rates.

The two bovine reference strains of *M. senegalense* (ATCC 13781 and ATCC 35796^T) gave a different and unique PRA pattern (PRA type I) that consisted of the same BstEII fragments but only two HaeIII fragments, of 185 and 140 bp. Sequencing of the *hsp65* gene fragment showed that the bovine strains also lack a single HaeIII cutting site present in the other closely related taxa, a result of a C→T substitution at position 168 of the 441-bp fragment. This sequence was designated sequevar I. This profile was also observed for the three bovine strains of *M. senegalense* from the German culture collection:

TABLE 3. Hamming distances[a] among *M. senegalense rpoB* sequevar I (region V), the seven sequevars of *M. peregrinum* type II, and the type strains of the closest species[b]

Sequevar or strain[c]	No. of base pair differences between the indicated sequevars or strains													
	I	II	IIIa	IIIb	IVa	IVb	V	VI	*M. porcinum*	*M. farcinogenes*	*M. neworleansense*	*M. fortuitum*	*M. houstonense*	*M. septicum*
II	1													
IIIa	1	2												
IIIb	2	3	1											
IVa	1	2	2	1										
IVb	3	4	2	3	4									
V	3	4	4	5	4	6								
VI	4	5	5	6	5	7	1							
M. porcinum	17	18	18	19	18	20	20	19						
M. farcinogenes	21	22	22	23	22	24	24	23	24					
M. neworleansense	26	27	27	28	27	29	28	27	24	19				
M. fortuitum	27	26	28	29	28	30	30	29	26	12	23			
M. houstonense	31	32	32	33	32	34	34	33	30	16	27	6		
M. septicum	35	36	36	37	36	38	38	37	35	24	25	25	28	
M. peregrinum type I	37	38	38	39	38	40	40	39	31	24	25	30	34	19

[a] Numbers of base pair differences.
[b] The closest species are *M. porcinum*, *M. farcinogenes*, *M. neworleansense*, *M. fortuitum*, *M. houstonense*, *M. septicum*, and *M. peregrinum* type I.
[c] Sqv II through VI are sequevars of *M. peregrinum* type II.

DSM 43661, DSM 43663, and DSM 43664. Thus, despite the fact that for *M. peregrinum* type II sequevar II, there was only a 2-bp difference from the bovine *M. senegalense* sequevar I, the two sequevars gave different PRA patterns by use of HaeIII.

Partial 16S rRNA gene sequencing. A total of 34 *M. peregrinum* type II isolates were subjected to 500-bp 16S rRNA gene sequencing using MicroSeq (16, 28). All resulted in 100% sequence identity to the type strains of *M. senegalense*, *M. farcinogenes*, and *M. houstonense*.

A total of 25 of the type II isolates and representatives from each *hsp65* PRA type were subjected to more-complete 16S rRNA gene sequencing. Two belonged to other species and were excluded. Of the remaining 23 isolates (including ATCC 35755), 22 had 100% sequence identity with *M. senegalense*, while isolate MF-125 showed a single-base-pair difference from *M. senegalense* at *E. coli* position 1246. Overall, all 43 clinical isolates underwent partial (500-bp) and/or more-complete (903-bp) 16S rRNA gene sequencing.

Of the animal reference strains from the DSMZ, three were identified as *M. senegalense* with 100% identity (DSM 43661, DSM 43663, and DSM 43664) while DSM 43658 was identified as *M. farcinogenes*/*M. houstonense* (species which cannot be distinguished from each other by 16S rRNA genes) and was therefore excluded from the study.

By using the complete 16S rRNA gene sequence, the closest species to *M. senegalense* are *M. farcinogenes*, *M. houstonense*, and *M. porcinum*, each with 4-bp differences, followed by *M. neworleansense* (5 bp), *M. boenickei* (6 bp), *M. septicum* (7 bp), *M. fortuitum* (8 bp), and both *M. alvei* and *M. peregrinum* with 11-bp differences. The taxonomic placement of *M. senegalense* among its closest mycobacterial relatives can be seen in Fig. 1a.

Two additional ATCC strains of *M. peregrinum* (type I) were available for testing, and 16S rRNA gene analysis revealed 100% sequence identity with *M. peregrinum* ATCC 14467^T of Bojalil.

16S–23S ITS1 region. Rapidly growing mycobacteria, with the exception of *M. abscessus* and *M. chelonae*, carry two ribosomal operons in which interoperon variability in spacer regions can render sequence analysis unsuccessful if the PCR product is used directly for sequencing. However, since ITS1 sequence analysis was previously successful for bovine strains of *M. senegalense* (16), 20 type II clinical isolates were subjected to such analysis, along with *M. senegalense* ATCC 35796^T and the 3 DSM animal strains identified as *M. senegalense* by 16S rRNA gene analysis. Of these isolates, two (MF-386 and MF-1925) showed two different sequences and could not be analyzed. The remaining 18 type II strains and the bovine *M. senegalense* strains revealed six sequevars: sqv I, II, IIIa, IIIb, IV, and VI. As much as possible, these were in correlation with the sequevar numbers assigned to *hsp65* gene sequevars to avoid confusion. No sequevar V was assigned, since the one isolate in this group, strain MF-1925, could not be analyzed by the current PCR-based sequencing method. All four bovine strains shared 100% sequence identity, as previously reported (16) (sqv I). Sqv IIIa and IIIb differed from each other by 1 bp. While our analysis of the type strain *M. senegalense* ATCC 35796^T revealed 100% identity with type strains DSM 43656^T in RIDOM and NCTC 10956^T in GenBank, none of the five *M. peregrinum* type II ITS1 sequevars corresponded with any close similarity with other sequences in public databases (Fig. 1c). However, organisms with top scores included members of the *M. fortuitum* group, including *M. senegalense*. As with the *hsp65* gene, the ITS1 sequences of the bovine *M. senegalense* strains had a similar but unique sequence compared with the clinical *M. peregrinum* type II isolates. Six examples of these type II sequences were submitted to GenBank as sequences AY684050 to AY684055.

Partial sequencing of the *rpoB* gene. Partial *rpoB* gene sequencing (687 bp) was performed for 21 clinical strains of *M. peregrinum* type II, *M. senegalense* bovine strains ATCC 35796^T, DSM 43661, and DSM 43663, and *M. farcinogenes* TMC 805^T (ATCC 35753^T). Eight closely related sequevars were identified. These were designated sqv I, II, IIIa, IIIb, IVa, IVb, V, and VI. As with ITS1 sequences, these were in correlation with the sequevar numbers assigned to *hsp65* gene se-

TABLE 4. DNA relatedness of representative strains labeled with *Mycobacterium senegalense* ATCC 35796^T

Source of unlabeled DNA	*hsp65* sequevar	DNA-DNA binding[a] (divergence)[b]
M. senegalense ATCC 35796^T	I	100
M. peregrinum type II MF-263 (ATCC BAA-849)	II	99 (1.5)
M. peregrinum type II MF-125	III	96 (1.5)
M. peregrinum type II MF-417	II	90 (1.5)
M. peregrinum type II MF-1738 (ATCC BAA-850)	IV	89 (2.0)
M. peregrinum type II MF-386 (ATCC BAA-851)	III	83 (1.5)
M. peregrinum type II (ATCC 35755)	III	76 (1.0)
M. peregrinum type I ATCC 14467^T		47 (8.0)

[a] Relative binding ratio [(percentage of heterologous DNA bound to hydroxyapatite/percentage of homologous DNA bound by hydroxyapatite) × 100] at 75°C. Values were means for at least two hybridization reactions.

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

quevars to avoid confusion (Table 2). The seven type II sequevars differed from each other by 1 to 7 bases, with 99.0 to 99.9% identity. The type II sequevars differed by only 1 to 4 bases from the bovine sequence (sqv I) of *M. senegalense* (99.4 to 99.9% identity). These sequences were submitted to GenBank and were assigned accession numbers (AY684056 to AY684063). As with the sequevars of *hsp65*, the base pair changes with *rpoB* were all synonymous. However, this was also true when the *rpoB* sequences were compared to that of *M. porcinum* CIP 105392^T (AY262737). *rpoB* sequences of *Mycobacterium* species are limited in GenBank, yet sequences of most members of the non- or late-pigmenting rapidly growing species are available for comparison (1). The closest match for the eight sequevars was the *rpoB* sequence of *M. porcinum* CIP 105392^T (AY262737), with 96.8 to 97.2% identities. While partial *rpoB* gene sequence determination revealed a larger number of sequevars among the type II group, there is a significant jump in the number of base pair differences when the type II isolates are compared by *rpoB* sequencing to their closest relative, *M. porcinum* (Table 3).

DNA-DNA hybridization. *Mycobacterium senegalense* ATCC 35796^T was found to be related at the species level to the human *M. peregrinum* type II strains MF-263 (ATCC BAA-849), MF-125, MF-417, MF-1738 (ATCC BAA-850), MF-386 (ATCC BAA-851), MF-1925, and ATCC 35755 (76 to 99% relatedness, with divergence ranging from 1 to 2%). DNA hybridization data indicated that *M. senegalense* ATCC 35796^T was unrelated to *M. peregrinum* ATCC 14467^T (47% relatedness at 75°C) (Table 4).

DISCUSSION

In this study, the bovine strains of *M. senegalense* and the human strains of *M. peregrinum* type II exhibited minimal differences and were closely clustered by polyphasic molecular and phenotypic properties. Their 16S rRNA genes exhibited 100% sequence identity, and the most common sequevar of the *hsp65* gene found among the type II clinical isolates (sequevar II) differed by only 2 bp from the sequence for the bovine strains of *M. senegalense* (99.55% identity), although this difference affected a HaeIII cutting site, resulting in different PRA patterns. The most common sequevar of the *rpoB* gene among the type II isolates differed by only 1 bp from *M. senegalense* (99.9% identity). Similarly minimal differences were seen with the sequevar groupings of the ITS1 sequence, with a closely clustered relationship for all three sequences that, for other rapidly growing species, has been seen only among strains of the same species (1, 48). The degree of relatedness or difference for housekeeping genes that define the same or different species has not been established, however, and may vary depending on the species or the gene (sequence). Some guidelines for the *rpoB* sequence have been suggested, however. Adekambi et al. studied 20 type strains and 59 clinical strains of rapidly growing mycobacteria and observed an interspecies variability for the 723-bp *rpoB* fragment of >3%, with an intraspecies variability of <1.7% (1). The type II *M. peregrinum* strains and the bovine *M. senegalense* strains exhibited a variability of 0.1% to 1.0% among eight sequevars.

The bovine and human strains had identical drug susceptibility patterns that were unique relative to those of other members of the *M. fortuitum* complex in that all isolates had the combination of doxycycline susceptibility, higher ciprofloxacin MICs, and clarithromycin MICs of ≤1 μg/ml. They also had the same biochemical pattern except that they may differ in their abilities to utilize D-trehalose as a sole carbon source. Finally, DNA-DNA hybridization showed the bovine and human strains to have >70% homology, with 5/7 human strains exhibiting ≥89% homology with the bovine *M. senegalense* type strain. The obvious conclusion drawn from the highly similar or identical phenotypic and molecular profiles of the bovine strains of *M. senegalense* from Senegal and the human "*M. peregrinum*" type II isolates from the United States is that such similarity is possible only if they belong to the same species. DNA-DNA hybridization studies confirmed this hypothesis (35).

The fact that the sequevar of the *hsp65* gene, the ITS1 region, and the *rpoB* gene found in the bovine strains of *M. senegalense* was not found among clinical isolates shows that the two groups are not identical. Interestingly, similar differences (i.e., 100% identical complete 16S rRNA gene sequence but minor *hsp65* gene sequence variance) was demonstrated between porcine strains of *M. porcinum* from Japan and human strains of *M. porcinum* from the United States (48), and we have observed similar differences in the simian type strain of *M. simiae* (from India or Africa) and human clinical isolates (R. Wallace, Y. Zhang, and C. Turenne, unpublished data). As with the current *M. senegalense* study, however, DNA-DNA relatedness studies comparing the porcine and human *M. porcinum* strains proved them to be one species (31). In the situations involving *M. porcinum* and *M. simiae*, as well as *M. senegalense*, there is also a major geographic separation between the origins of the animal and human strains, and this may also be a factor in the differences observed.

A curious observation was presented here with regard to *M. farcinogenes.* Interestingly, both *M. senegalense* and *M. farcinogenes* were originally described in the same paper by Chamoiseau as causes of bovine lymphadenitis ("farcy") in Senegal; hence the names of the two species (12). Although there is 100% 16S rRNA gene sequence identity between *M. farcinogenes* and the nonpigmented, rapidly growing third-biovariant

complex species *M. houstonense* (31), *M. farcinogenes* was originally described as a yellow-pigmented, slow-growing species (12). In our hands, *M. farcinogenes* was demonstrated to be a nonpigmented slow grower, growing best on Middlebrook 7H11 agar and Löwenstein-Jensen agar, not at all on Trypticase soy agar, unlike other rapidly growing species. In a 1983 study of 15 strains by Ridell and Goodfellow (29), the species was not pigmented and required more than 5 days to grow. It was negative for 3-day arylsulfatase and catalase and generally clustered with slow growers. It should be noted that characterization of the type strain (ATCC 35753^T) while it was housed in the Trudeau Mycobacterial Culture Collection (TMC 805) showed it to be negative for arylsulfatase at 3 days, negative for nitrate, and negative for iron uptake; *M. peregrinum* type II, *M. senegalense*, and other members of the *M. fortuitum* group are positive by all of these tests (25a). We obtained similar results with our testing. Given that the species grows and behaves biochemically as a slow grower, it is remarkable that it has a 16S rRNA gene sequence identical to that of another, rapidly growing species, that its *hsp65* sequence shows >99% identity with that of a rapidly growing species, and that the HPLC pattern of its mycolic acids and the mass spectroscopy pattern of cell wall proteins are indistinguishable from those of members of the *M. fortuitum* group; thus, it behaves like a chimera that is part rapid grower and part slow grower.

In this study, we have evaluated the ITS1 regions and the partial *hsp65* and *rpoB* genes of 23 strains of *M. peregrinum* type II. These sequences were heterogeneous, exhibiting several closely related sequevars. This should not be unexpected, since protein-coding genes are anticipated to exhibit more base pair divergence than the 16S rRNA gene. In a study by Ringuet et al. (30), the 441-bp Telenti fragment was sequenced for multiple species of rapidly growing mycobacteria, all of which exhibited some degree of intraspecies DNA polymorphism. As previously noted, these investigators studied 14 clinical isolates of *M. peregrinum*, 11 of which exhibited three sequences which were identical to or closely aligned with those of ATCC 14467^T. A recent study of *M. porcinum* showed it to consist of five closely related *hsp65* gene sequevars, one of which was unique to animals (pigs) (48).

Description of human isolates of *M. senegalense*. The organism is acid fast and grows readily in 3 to 4 days on Middlebrook 7H10 or 7H11 agar as well as on Trypticase soy agar. It is nonpigmented and produces smooth to rough colonies. It is positive for 3-day arylsulfatase, acetamide, iron uptake, nitrate reduction, D-mannitol, and D-trehalose as a sole carbon source (14 days) and generally grows at 45°C. It is negative for L-rhamnose, D-sorbitol, i-*myo*-inositol, and citrate. It contains typical mycobacterial mycolic acids, and by HPLC its pattern is not distinguishable from that of other members of the *M. fortuitum*/*smegmatis* group. The isolates are uniformly susceptible to doxycycline, clarithromycin, amikacin, imipenem, and sulfamethoxazole by use of current CLSI guidelines (27). They have relatively high MICs for ciprofloxacin (0.5 to 1.0 μg/ml) compared to *M. fortuitum* or *M. peregrinum* type I and are susceptible or intermediate to cefoxitin. The organism is a recognized cause of human disease, including skin and soft-tissue infections, posttraumatic or postsurgical osteomyelitis, catheter-related infections, and possibly pulmonary infections. Its complete 16S rRNA gene sequence (bp 8 to 1500) shows 100% identity with that of the bovine strain of *M. senegalense* ATCC 35796^T. Its *hsp65* gene exhibits four closely related sequence variants and gives three PRA patterns with the 441-bp Telenti fragment and restriction enzymes BstEII and HaeIII. The most common PRA pattern is not distinguishable from the pattern seen with *M. farcinogenes*, *M. houstonense*, and *M. neworleansense* but is distinguishable from the bovine sequevar of *M. senegalense* and from *M. peregrinum* type I (ATCC 14467^T). The *hsp65* sequence observed for the bovine reference strains of *M. senegalense* (ATCC 35796^T and ATCC 13781) and its associated PRA pattern with HaeIII of fragments of 185 and 140 bp were not observed among the human isolates. The human strains exhibited five closely related ITS1 sequevars, while a few strains showed evidence of two different spacer sequences. They also exhibited seven closely related sequevars of the *rpoB* gene partial sequence. By DNA-DNA reassociation, they exhibited >70% relatedness with the bovine strain from Senegal ATCC 35796^T. The strains chosen as species reference strains are ATCC 35755 (a sputum isolate from Texas, formerly TMC 1545); ATCC BAA-849, which was recovered from a case of foot osteomyelitis in Florida; ATCC BAA-850, which was recovered from an abdominal wound in Florida; and ATCC BAA-851, which was recovered from a thigh infection in Texas. Examples of the different sequevars have been deposited in GenBank as sequences AY684045 to AY684049 (*hsp65*), AY684056 to AY684063 (*rpoB*), and AY684050 to AY684055 (16S–23S ITS1 region).

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