First Isolations of *Segniliparus rugosus* from Patients with Cystic Fibrosis[▽]

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We report three cases of the new genus *Segniliparus* isolated from patients with cystic fibrosis. All isolates were unambiguously identified by 16S rRNA gene sequencing as *Segniliparus rugosus* (GenBank accession no. AY 60892). Drug susceptibility results that may enhance treatment for cystic fibrosis patients with this opportunistic pathogen are presented.

CASE REPORTS

Cases 1 and 2. In 2003, acid-fast bacteria (AFB) were isolated from a male sibling pair (sib-pair) with cystic fibrosis (CF). The sib-pair exhibited rapidly progressive lung disease not considered typical of CF. Physicians suspected the abrupt decline in health was secondary to the presence of AFB infection. The sib-pair both carried the delta F508/G542X mutation in the CF transmembrane conductance regulator gene. The history for the older sibling was notable for first evidence of acquisition of *Pseudomonas aeruginosa* at 5 years old. However, he was not chronically colonized and was not treated with intravenous (i.v.) antibiotics for respiratory decline until he was 9 years old. The other sib-pair patient was four years younger and had been diagnosed in utero with CF. When this sibling was 22 months old, *P. aeruginosa* was detected and he was treated with i.v. anti-pseudomonal antibiotics. Baseline chest computed tomography performed in 2001 revealed mild bronchiectasis in the sib-pair, then ages 6 and 10 years. In 2003 the health of the sib-pair, now ages 8 and 12, declined, as was noted by a marked deterioration in lung function and significant weight loss, especially in the younger sibling. Aggressive anti-pseudomonal i.v. antibiotic therapies were not effective. In August 2003, the younger sibling underwent endoscopic sinus surgery and a flexible bronchoscopy. The bronchoalveolar lavage revealed the presence of AFB, 1 to 10/field (9), subsequently identified by biochemical testing as *Mycobacterium abscessus* by an outside laboratory. A few months later, the older sibling showed an acute worsening of lung function and induced sputum was positive for AFB, 1 to 10/field, which was identified by using the same method as *M. abscessus* at an outside laboratory. Cultures from each patient were sent to the

Mycobacteria/Nocardia Laboratory at the University of Texas Health Center (UTHCT) for drug susceptibility testing and confirmation of identification. PCR restriction endonuclease analysis of the 65-kDa heat shock protein gene used for identification of clinically significant aerobic actinomycetes failed to produce an amplicon, a result inconsistent with the previous identification of *M. abscessus* (10, 11). The sib-pair isolates were referred to the Canadian Service Center for Human and Animal Health in Winnipeg, Canada, where the first 500-bp region from the 5' end of the 16S rRNA gene sequence (bp 54 to 510, *Escherichia coli* numbering) matched 100% the recently deposited sequence in GenBank (accession no. AY60892) for *Segniliparus rugosus* (1, 8). Two representative isolates from the siblings, designated MO 1714#3 and MB 549, were compared in this study.

Drug susceptibility testing was prolonged and problematic due to inadequate growth and fungal contamination of the organisms. Awaiting susceptibility results, the health of the sib-pair declined. Supplemental oxygen was started, and gastrostomy tubes were used for nutritional failure. An initial prolonged regimen of gatifloxacin (GAT), linezolid (LZD) and clofazimine (CLO) resulted in slight stabilization. The younger sibling had a positive AFB smear and was subsequently culture positive for *S. rugosus* after 7 months of treatment. The older sibling had a flexible bronchoscopy 3 months into treatment, with samples analyzed from two separate lung segments. One of the samples was AFB negative by both smear and culture. The other sample was AFB smear negative but was culture positive for *Segniliparus*. Complications arose during treatment; LZD was discontinued due to peripheral neuropathy; and GAT was discontinued due to development of diabetes. CLO was continued, and nebulized amikacin (AMK) (twice daily) was added. However, the sib-pair continued a slow decline with this regimen.

The older sibling had high fevers persisting for weeks in April 2006. Blood cultures were negative for all organisms including AFB. Previous sputum samples from February were smear and culture positive for *S. rugosus*. In April, sputum

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TABLE 1. Antimicrobial susceptibility patterns for *Segniliparus* strains

Strain	State ^a ; source	MIC of drug ^b (μg/ml)																			
		AMK	AMC	CEF	CIP	CLO	CLR	EMB	FOX	GAT	IMP	LZD	MIN	MOX	RFB	RIF	STR	SMX	TIG	SXT	TOB
<i>S. rugosus</i> ATCC BAA-974 ^T	AL; sputum	>128	64/32	64	16	2	32	>16	(64)	8	2	64	>32	<u>0.5</u>	<u>0.5</u>	>16	>128	<u>4</u>	>2	<u>≤0.25/4.8</u>	>64
<i>S. rugosus</i> ATCC BAA-975	PA; bronch	>128	>64/32	>64	4	2	64	>16	256	8	<u>4</u>	>64	>32	8	<u>1</u>	>16	>128	<u>8</u>	>2	<u>0.5/9.5</u>	>64
<i>S. rugosus</i> MO 1714#3	MA; sputum	>128	64/32	>64	<u>8</u>	>2	>64	>16	>256	<u>2</u>	<u>2</u>	64	>32	8	<u>2</u>	>16	128	<u>8</u>	>2	<u>0.5/9.5</u>	>64
<i>S. rugosus</i> MB 549	MA; BAL	>128	64/32	>64	8	>2	>64	>16	>256	8	<u>2</u>	32	>32	8	<u>2</u>	>16	>128	<u>32</u>	>2	<u>2/38</u>	>64
<i>S. rugosus</i> AS 513	DE; BAL	>128	32/16	64	(2)	>2	>64	>16	128	<u>1</u>	<u>1</u>	32	>32	(2)	<u>1</u>	>16	>128	<u>8</u>	>2	<u>≤0.25/4.8</u>	>64
<i>S. rotundus</i> ATCC BAA-972 ^T	TN; sputum	>128	<u>2/1</u>	<u>4</u>	<u>≤0.25</u>	0.5	(4)	>16	<u>16</u>	<u>2</u>	<u>2</u>	<u>2</u>	<u>≤0.5</u>	<u>≤0.12</u>	<u>0.25</u>	<u>≤0.25</u>	8	<u>4</u>	ND	<u>≤0.25/4.8</u>	>64
<i>S. rotundus</i> ATCC BAA-973	MO; nasal	>128	<u>4/2</u>	(16)	<u>≤0.12</u>	1	8	>16	(32)	<u>0.5</u>	<u>2</u>	<u>2</u>	<u>≤0.5</u>	<u>≤0.5</u>	<u>1</u>	<u>0.5</u>	16	<u>4</u>	1	<u>≤0.25/4.8</u>	64

^a States are designated by official U.S. postal abbreviations.^b MICs were interpreted using criteria for mycobacteria and aerobic actinomycetes (5). MICs underlined in bold type were susceptible, those in parentheses were intermediate, and others were resistant, with the exception of CLO, STR, and TIG, which do not have established nontuberculous mycobacterial breakpoints and whose MICs were not interpreted. For drug abbreviations, see the text. ND, not determined.

samples were smear and culture positive for *S. rugosus*. Overall, *S. rugosus* was isolated multiple times from bronchial washings and sputum of these patients. Susceptibility testing showed susceptibility to imipenem (IMP) (4 μg/ml), rifabutin (RFB) (2 μg/ml), and trimethoprim-sulfamethoxazole (SXT) (2/38 μg/ml), and a new aggressive treatment plan was initiated, including the following: IMP (1 g i.v. every 6 to 8 h for 6 months), in addition to oral RFB (150 mg once daily) and oral sulfamethoxazole (400 mg)-trimethoprim (80 mg), which have been continued as suppressive therapy. After 6 months, sputum from the sib-pair was negative by AFB smear and culture. Clinically the sib-pair have improved in quality of life, lung function, and radiologic findings.

Case 3. The third case was a 28-year-old male CF patient with the CF transmembrane conductance regulator genotype delta F508/R560T. His medical history was significant for recurrent infections with *Pseudomonas* and *Mycobacterium abscessus*. The patient was unable to produce sputum, and all isolates were obtained from bronchial washings. An initial treatment in May 2004 consisted of 3 weeks of i.v. cefoxitin (FOX) and AMK, followed by 3 months of oral doxycycline and clarithromycin (CLR). In September 2004 he was hospitalized and placed on i.v. piperacillin-tazobactam and tobramycin (TOB) for 3 weeks for concurrent *Pseudomonas* infection. In November 2004, he was given i.v. AMK and FOX for 2 months. AMK was discontinued due to ototoxicity. Aztreonam was added, and piperacillin-tazobactam was continued for *Pseudomonas* infection. In early 2005, due to a recurrence of symptoms, a bronchoalveolar lavage was performed and samples sent to a local regional laboratory for AFB culture. The AFB smear was 4+ (>9 AFB per field) (9). During 2004 and early 2005, several isolates submitted to different outside laboratories were identified as *Mycobacterium chelonae* and *Mycobacterium abscessus*. Ultimately, in 2005, six isolates were identified as *S. rugosus* by UTHCT. A representative isolate, designated AS 513, was used in this study. In the latter half of 2005, medications were regularly altered, approximately every 2 weeks, and by early March of 2006, the patient had improved and antibiotics were discontinued. The patient continued on meropenem for treatment of *Pseudomonas*.

In 2005, a group of rapidly growing AFB isolated from human sources was characterized as belonging to a novel genus, *Segniliparus*, with species *Segniliparus rotundus* and *S. rugosus* (1). The presence of a long-carbon-chain-length alpha mycolate segregated the isolates from known mycolic acid-containing bacteria and made detection possible with the high-performance liquid chromatography (HPLC) method routinely used in our laboratory (Mycobacteriology Laboratory Branch, CDC, Atlanta, GA) for identification of the genus *Mycobacterium* (2). A retrospective study of mycolic acid patterns revealed 13 strains in the past 14 years to be similar as determined by HPLC. Sequence comparison of the 16S rRNA gene revealed a 78- to 79-bp difference from any other known mycolic acid-containing bacteria. These strains were submitted to our laboratory as uncharacterized nontuberculous mycobacteria causing respiratory disease. A characterization study demonstrating a novel group of organisms, phenotypically

FIG. 1. Dendrogram of electrophoretic types showing relationships among the three clinical CF isolates and the four control strains of *Segniliparus*. See the text for an explanation of assigned numbers.

matching some characteristics of rapidly growing *Mycobacterium* (RGM) and *Tsukamurella* species, was described previously (1). The characterization study did not determine the environmental niche, mode of transmission, or clinical association. Further information is provided in this report on the association of the genus with the inherited disease CF. This study was initiated when the Mycobacteria/Nocardia Laboratory at UTHCT detected three *S. rugosus* isolates (MO 1714#3, MB 549, and AS 513). The strains were isolated from patients being treated with CF disease (6, 7).

The CF clinical isolates were compared to previously identified isolates of *Segniliparus*, including *S. rugosus* ATCC BAA-974^T and 975 and *S. rotundus* ATCC BAA-972^T and 973 (1). Strains were stored at -70°C at the CDC in Middlebrook 7H9 broth and at UTHCT in trypticase soy broth with 15% glycerol. Strains were recovered from storage and grown in Middlebrook 7H9 broth or on Middlebrook 7H11 medium incubated at 30 to 37°C .

HPLC was used to verify the presence of characteristic late-emerging mycolic acids in the three CF clinical isolates by comparison to the library of mycolic acid control patterns for this genus as previously described (1).

Bacterial strains were tested for drug susceptibility by a broth microdilution method in Middlebrook 7H9 broth multiple times with comparable results. MICs were determined after incubation for 3 days at 30°C for selected drugs, including AMK, amoxicillin clavulanic acid (AMC), ceftriaxone (CEF), ciprofloxacin (CIP), CLR, ethambutol (EMB), FOX, IMP, LZD, minocycline (MIN), RFB, rifampin (RIF), TOB, sulfamethoxazole (SMX), and SXT using CLSI MIC breakpoints for nontuberculous mycobacteria and *Nocardia* (3, 5). Because there are no CLSI-approved breakpoints for streptomycin (STR), tigecycline (TIG), and CLO, MICs for these agents were not interpreted as susceptible or resistant. CLSI bacterial breakpoints were used for GAT and moxifloxacin (MOX) (3,

5). Due to the similarity of *Segniliparus* to RGM, it was reasonable to assume that susceptibility testing procedures used for RGM could be employed to determine a potential treatment regimen (3, 4, 5). However, drug susceptibility testing was prolonged and problematic due to inadequate growth of the organisms with the approved CLSI standard susceptibility testing media for mycobacteria. It was necessary to substitute Middlebrook 7H9 broth in place of the cation-adjusted Mueller-Hinton broth to accommodate the inadequate growth of *Segniliparus* strains in Mueller-Hinton broth. Despite these limitations, MICs were determined for antibacterial and anti-tuberculosis drugs (Table 1). Isolates of *S. rugosus* were generally intermediate or resistant to AMC, CEF, CIP, CLR, FOX, LZD, MIN, MOX, RIF, and STR. Generally, both of the *Segniliparus* species were resistant to AMK, EMB, and TOB and susceptible to IMP, RFB, SMX, and SXT (Table 1).

The genetic relationships among the clinical isolates were determined by comparing the mobilities of 16 cellular enzymes from bacterial lysates using multilocus enzyme electrophoresis according to methods previously described for typing nontuberculous mycobacteria (12). Enzymes analyzed were phosphogluconate dehydrogenase using NAD as a cofactor, phosphogluconate dehydrogenase acid using NADP as a cofactor, isocitrate dehydrogenase using NAD as a cofactor, isocitrate dehydrogenase using NADP as a cofactor, esterase, benzyl alcohol dehydrogenase, adenylate kinase, phosphoglucosmutase, glutamate oxalacetic transaminase, leucine aminopeptidase, indophenol oxidase, phosphoglucose isomerase, aconitase, glucose-6-phosphate dehydrogenase, nucleoside phosphorylase, and fumarase. None of the CF clinical isolates had the same electrophoretic types (ET), although MB 549, a sib-pair isolate, did match ATCC BAA-975, a control strain for *S. rugosus*. The close physical relationship of the sib-pair suggested the possibility of human transmission for these strains. However, comparison of the strains with genetic cluster analysis by multilocus enzyme

electrophoresis demonstrated a genetic divergence of 0.07 for the sib-pair strains MB 549 and MO 1714#3, an indication they were closely related but not the same ET (Fig. 1). The different ETs do not support person-to-person transmission but would suggest acquisition from an environmental source. It was also observed that there have not been any other family members without CF displaying any symptoms.

An unexpected ET result revealed separation of the control strains for *S. rugosus* BAA-974^T and BAA-975 by a genetic distance of 0.85. This distant genetic relationship was reflective of different species and was an indication of an inconsistency in the *Segniliparus* nomenclature. This nomenclature discrepancy demonstrates the incomplete taxonomy of *Segniliparus* as well as the problems with identification, issues that will not be resolved until more strains of the genus become available for comparison.

Laboratories attempting species identification encountered a diagnostic problem with *Segniliparus* strains because of phenotypic profiles comparable to those of rapidly growing members of the genus *Mycobacterium*. Because of rapid growth on culture media designed for *Mycobacterium*, strains of *Segniliparus* were confused with species of nonchromogenic RGM. Also, the *Segniliparus* bacilli stain strongly acid fast. Adding to the confusion, strains of *S. rugosus* maintained for several weeks demonstrated colony conversions from the rough form to smooth (R. Butler, unpublished observation). Admittedly, until publication of the species characterization in 2005, a source for interlaboratory comparisons did not exist, thus making accurate identification impossible.

These patients represent the first reported cases of *S. rugosus* as an opportunistic pathogen in CF. Clinically, the cases exhibited a marked and rapid decline in lung function and radiologic studies over a short period of time which was not characteristic of CF or infections usually associated with this disease. However, the complete clinical picture of *Segniliparus* involved with CF cannot be determined from the small number of cases studied. In fact, until further information is available, the efficacy of drugs based upon in vitro susceptibility testing may be ambiguous. Numerous questions involving the public health significance of this new genus require further evaluation. A comprehensive medical presentation of the sib-pair cases, including radiologic and immunological assessment, will be presented elsewhere. However, laboratorians and physicians should be aware that the presence of AFB in respiratory infections with CF patients may represent this newly described genus and not the genus *Mycobacterium*. More needs to be

learned about the significance and impact of *Segniliparus* on patients with CF.

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