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Letter to the editor

Pulmonary infection caused by *Segniliparus rugosus* in a patient without systemic immunodeficiency but with bronchiectasis: first identification by matrix-assisted laser desorption ionization time-of-flight mass spectrometry with whole-genome confirmation

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Dear Editor,

Segniliparus spp. are acid-fast, rapidly growing bacteria associated with pulmonary infections, particularly in patients with underlying lung disease. Two species, *Segniliparus rugosus* and *Segniliparus rotundus*, were first reported in 2005 from unidentifiable mycobacterial samples suspected of being involved in respiratory mycobacteriosis [1]. *Segniliparus* spp. infection in humans is exceedingly rare. To date, eight cases have been reported worldwide, with most having been caused by *S. rugosus* in patients with cystic fibrosis or bronchiectasis [2–7]. In previous reports, species identification relied primarily on 16S rRNA gene sequencing and less frequently on *rpoB* gene sequencing. Here, we present a case of pulmonary *S. rugosus* infection in a patient without evidence of systemic immunodeficiency but with bronchiectasis and prior nontuberculous mycobacterial pulmonary disease (NTM-PD), identified for the first time by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and confirmed by whole-genome sequencing (WGS). This case highlights that MALDI-TOF MS can identify rare acid-fast organisms within routine clinical workflows, with WGS providing species-level confirmation.

A 66-year-old woman presented with worsening cough and purulent sputum for 6 months. She had no evidence of systemic immunodeficiency (HIV-negative, no history of malignancy or immunosuppressive therapy). She had been diagnosed with NTM-PD caused by *Mycobacterium avium* 2 years earlier. For suspected *M. avium* complex pulmonary disease, empirical treatment comprising clarithromycin, ethambutol, and rifampin was initiated at another institution following positive acid-fast bacillus (AFB) staining. When the patient failed to respond to the treatment after 10 weeks and was referred to our institution, a new regimen

consisting of azithromycin, ethambutol, and clofazimine was initiated. After 5 months without clinical improvement, all antibiotics were discontinued because she developed azotemia and IgA nephropathy.

After antibiotic discontinuation, serial sputum samples were strongly AFB-positive, and occasional growth was observed in BD BACTEC MGIT broth (Becton, Dickinson and Company) and on Ogawa agar (Fig. 1A). Isolates from these cultures were also strongly AFB-positive (Fig. 1B). However, because repeated Allplex TB/NTM real-time PCR results (Seegene) were negative, the isolates were initially regarded as contaminants. On a colony smear, the organism showed strongly acid-fast, short, nonbranching rods.

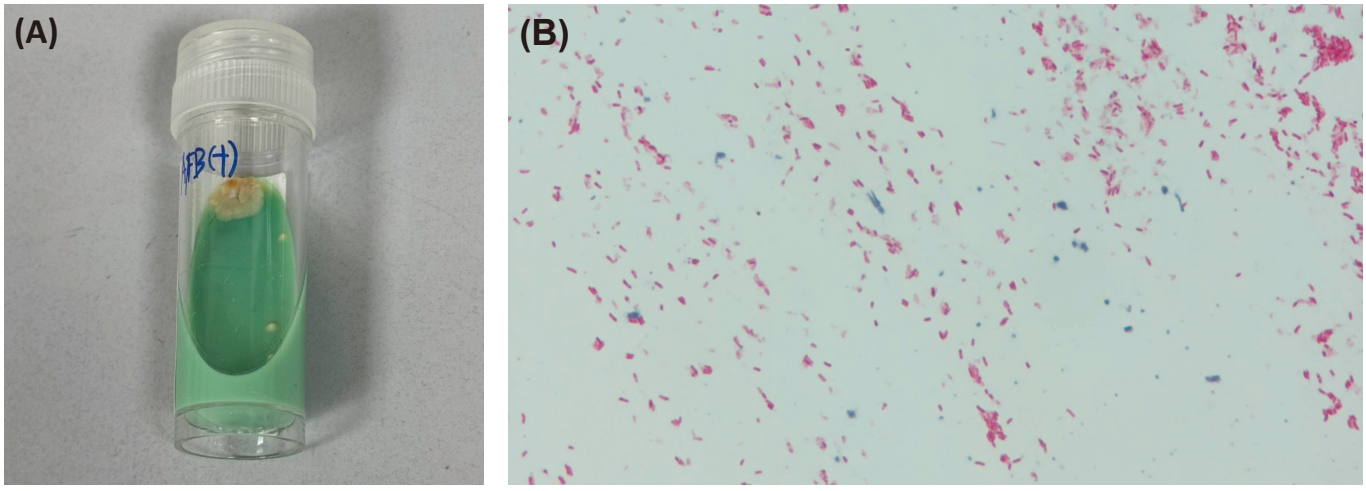

Fig. 1. Colony morphology and acid-fast staining of *Segniliparus rugosus*. (A) Colonies on Ogawa medium. Rough, dry, buff-to-orange colonies. (B) Acid-fast bacillus stain (colony smear). Strongly acid-fast short rods were observed, unlike *Nocardia* spp., which are typically weakly/partially acid-fast and show branching filaments.

When the patient's renal function improved but respiratory symptoms and CT findings worsened, antibiotic therapy consisting of azithromycin, ethambutol, and clofazimine was resumed after a 10-month hiatus. After 3 months of minimal symptomatic improvement, colonies previously presumed to be contaminants were subcultured on blood agar and analyzed using MALDI-TOF MS to identify the causative organism. The colonies were processed using a direct transfer method and identified as *S. rugosus* (score: 2.09) using a Bruker Biotyper and MALDI Biotyper Compass Library (DB12348, version 12.0; Bruker Daltonics).

The patient was admitted, and empirical antibiotic therapy—aligned with regimens for rapidly growing mycobacteria—was initiated, comprising azithromycin, clofazimine, amikacin, and imipenem. The symptoms improved dramatically within a few days of therapy initiation, and management was subsequently continued on an outpatient basis. Drug susceptibility testing (DST) was initially attempted with the Sensititre RAPMYCOI plate (Thermo Fisher Scientific) in cation-adjusted Mueller–Hinton broth, but the isolate failed to grow. In line with prior reports on *Segniliparus* spp., DST was repeated in Middlebrook 7H9 broth (Korean Institute of Tuberculosis, Cheongju, Korea; Table 1). Amikacin was replaced with ciprofloxacin 3 months later, in light of the DST results and the patient's renal function.

Table 1. Antimicrobial susceptibility of the *S. rugosus* clinical isolate

Antimicrobial agent	MIC (μg/mL)	Interpretation [a)]
Clarithromycin	> 32	R
Amikacin	> 128	R
Moxifloxacin	2	I
Linezolid	16	I
Streptomycin	64	N/A
Ciprofloxacin	≤ 0.25	S
Doxycycline	> 16	R
Clofazimine	≤ 0.25	N/A
Trimethoprim/Sulfamethoxazole	≤ 0.5/9.5	S
Cefoxitin	128	R
Imipenem	4	S
Meropenem	8	I
Tobramycin	> 8	R
Tigecycline	4	N/A

[a)]Interpretations were made according to CLSI M62 for rapidly growing mycobacteria and are in line with those reported in previous case studies.
Abbreviations: MIC, minimum inhibitory concentration; R, resistant; I, intermediate; S, susceptible; N/A, no available breakpoint.

WGS was performed using the NovaSeq 6000Dx platform (Illumina). Raw Illumina paired-end reads were adapter- and quality-trimmed with fastp and assembled using SPAdes (isolate mode), and contigs < 200 bp were excluded prior to calculating assembly metrics. Contigs flagged as contaminants by the National Center for Biotechnology Information (NCBI) contamination screen (primate/fungal) were removed prior to public deposition, and all assembly metrics and downstream analyses reported herein were calculated for the decontaminated assembly. The assembled genome measured 3,872,893 bp across 153 contigs (N50: 217,954 bp; largest contig: 410,624 bp; GC content: 68.16%). BUSCO v6.0.0 using the Actinomycetes odb12 lineage (n = 355) showed 95.8% completeness (340/355 complete single-copy), indicating a high-quality assembly. The average nucleotide identity (ANI) was calculated using FastANI v1.34 against the NCBI RefSeq reference genomes of representative *Segniliparus* strains, yielding 99.61% for *S. rugosus* (GCF_000185725.2; 1144/1265 fragments mapped, 90.4% coverage) and 81.69% for *S. rotundus* (GCF_000092825.1; 719/1265 fragments mapped, 56.8% coverage). As these values were above and well below the ~95%–96% species threshold [8], respectively, the isolate was assigned to *S. rugosus*, consistent with the MALDI-TOF MS results. Beyond the ANI calculated from the assembled genome, phylogenomic analysis using the Type Strain Genome Server (accessed September 5, 2025), based on Genome BLAST Distance Phylogeny, placed the isolate within the *S. rugosus* clade, clustered with the type strain, and independently corroborated species assignment (Fig. 2).

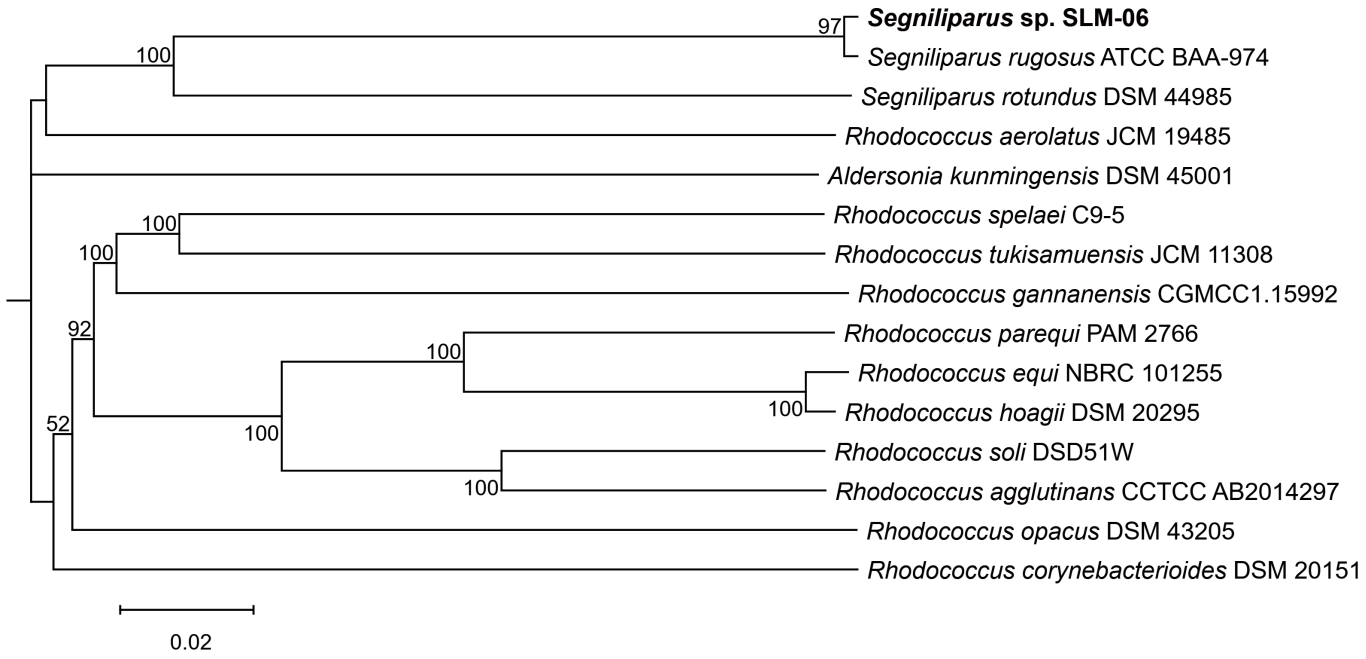

Fig. 2. Phylogenomic placement of the clinical *Segniliparus rugosus* isolate by Type Strain Genome Server (TYGS). A genome-based tree was generated with the TYGS (accessed September 5, 2025) using Genome BLAST Distance Phylogeny (GBDP) distances and FastME. The isolate clustered within the *S. rugosus* clade together with the type strain, supporting the species assignment. The tree was visualized using iTOL v7. Node labels indicate GBDP pseudo-bootstrap support values (only ≥ 50% are shown), and the scale bar indicates GBDP distance.

Here, we describe a case of pulmonary disease caused by *S. rugosus* infection in a patient without evidence of systemic immunodeficiency but with bronchiectasis and prior NTM-PD. All previous reports of *Segniliparus* infections involved patients with either cystic fibrosis or bronchiectasis, both of which predispose patients to nontuberculous mycobacterial disease [9]. Although further investigation is required, we believe that such structural lung damage and impaired mucociliary clearance can predispose patients to infection by rare pathogens, such as *Segniliparus* spp. As our patient had a history of NTM-PD and radiologically identifiable bronchiectasis, she may have shared these risk factors.

As summarized in Table 2, most reported *S. rugosus* infections occurred in patients with cystic fibrosis or bronchiectasis, and the diagnosis relied primarily on 16S rRNA or, in one case, *rpoB* sequencing. To the best of our knowledge, no previous case has identified *S. rugosus* by MALDI-TOF MS or confirmed its identification by WGS, expanding the spectrum of susceptible hosts to patients with prior NTM-PD. Despite limited reference spectra in the Bruker MALDI Biotyper Compass library (DB12348, version 12.0), the isolate was identified as *S. rugosus* with a high-confidence score (2.09; ≥ 2.0 generally supports species-level identification).

Table 2. Human infections due to *Segniliparus* spp., including the present case, with identification method, risk factors, DST, and outcomes

Author (year)	Species	Identification method	Risk factor	DST	Outcome
Butler et al. (2007) [2]	*S. rugosus* (3 cases)	16S rRNA sequencing	CF	BMD in Middlebrook 7H9 broth	Treated
Hansen et al. (2009) [3]	*S. rugosus*	16S rRNA sequencing	CF	Sensititre RAPMYCOI in Middlebrook 7H9 broth	Treated
Koh et al. (2011) [4]	*S. rotundus*	16S rRNA and *rpoB* sequencing	Non-CF bronchiectasis	Done	Treated
Choi et al. (2012) [5]	*S. rugosus*	16S rRNA sequencing	Non-CF bronchiectasis	Done	Not treated
Lee et al. (2014) [6]	*S. rugosus*	16S rRNA sequencing	Non-CF bronchiectasis	Failed	Treated
Zurita et al. (2020) [7]	*S. rugosus*	16S rRNA sequencing	CF	Sensititre RAPMYCOI	Not reported
Present case	*S. rugosus*	MALDI-TOF MS and WGS	Previous NTM-PD	BMD in Middlebrook 7H9 broth	Treated

Abbreviations: DST, drug susceptibility testing; CF, cystic fibrosis; BMD, broth microdilution; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; NTM-PD, nontuberculous mycobacterial pulmonary disease; WGS, whole-genome sequencing.

AFB-positive but TB/NTM PCR-negative isolates are a recurring diagnostic pitfall and may be dismissed as contaminants. Therefore, we suggest a pragmatic workflow: retain AFB-positive, PCR-negative cultures, perform routine subculture, and apply MALDI-TOF MS to any growth before discarding it as contamination. WGS-based confirmation (e.g., ANI and phylogenomics) can be reserved for selected cases with therapeutic implications or clinic–laboratory discordance. This blind spot is not unique to *Segniliparus*, as other non-mycobacterial acid-fast taxa can also yield culture-positive, PCR-negative results, underscoring the need for culture-based identification along with molecular testing [10].

In terms of antimicrobial therapy, previous reports described variable responses to imipenem, rifabutin, moxifloxacin, and trimethoprim-sulfamethoxazole [2,3]. More recent cases have adopted regimens aligned with treatment guidelines for rapidly growing mycobacteria, typically combining a macrolide with a fluoroquinolone and a parenteral agent, such as amikacin or imipenem [6]. In the present case, dramatic clinical improvement was achieved with azithromycin, clofazimine, amikacin, and imipenem, although amikacin was later replaced with ciprofloxacin owing to high minimum inhibitory concentrations and renal dysfunction. As reflected in Table 2, treatment outcomes in earlier cases have been heterogeneous, likely owing to the rarity of the organism and the absence of standardized regimens. Our findings emphasize the importance of performing DST under appropriate culture conditions and tailoring therapy according to both microbiological results and patient comorbidities. Such individualized management is particularly relevant for rare organisms, such as *S. rugosus*, for which evidence-based treatment guidance remains limited.

This case highlights that MALDI-TOF MS can rapidly identify *S. rugosus* from AFB-positive TB/NTM PCR-negative isolates that might otherwise be dismissed as contaminants, while WGS provides species-level confirmation when clinically indicated. Adopting this stepwise approach may shorten identification time and support individualized therapies for rare acid-fast infections.

Ethics statement

Written informed consent was obtained from the patient for the publication of this case report and the accompanying images.

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

Funding

None.

Data availability

The whole-genome shotgun (WGS) project has been deposited at GenBank under the WGS master accession JBQWBN000000000.1. Raw sequencing reads are available in the NCBI Sequence Read Archive (SRA) under BioProject PRJNA1313124 (BioSample SAMN50884781).

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