Segniliparus rugosus from the sputum of a child with cystic fibrosis in Ecuador: challenges in bacterial identification

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Abstract

Using sequencing analyses of the 16S rRNA gene, we identified *Segniliparus rugosus* in an 8-year-old child with cystic fibrosis. We describe the difficulties we encountered in identifying this bacterium. To the best of our knowledge, this is the first reported case of *S. rugosus* in Ecuador.

Keywords: 16S rRNA, Cystic fibrosis, Ecuador, *Mycobacterium*, *Segniliparus rugosus*

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Segniliparus rugosus is a rapidly growing acid-fast bacillus (AFB) that was isolated from human samples in 2005 [1]. It has been identified as an emerging human pathogen that has been isolated from patients with and without cystic fibrosis (CF) [2–4].

We describe the case of an 8-year-old child who was diagnosed with CF 2 years ago. The patient had the F508del/H609R mutations. Unlike many CF patients, he did not have a history of chronic *Pseudomonas aeruginosa* infections; however, *Staphylococcus aureus* and *Burkholderia cepacia* had previously been isolated from his sputum. Although AFB cultures yielded *Mycobacterium terrae* growth on one occasion (2018), he did not require treatment. *Aspergillus fumigatus* was isolated from this patient in 2016, 2017 and 2018. He was then diagnosed with allergic bronchopulmonary aspergillosis (ABPA) and was treated with corticosteroids and itraconazole.

On 8 November 2019, we received a sputum sample in our laboratory for bacteriological culture and testing for acid-fast staining. Because the sputum was 1+ AFB smear-positive, the sample was inoculated into Middlebrook 7H9 broth (Becton Dickinson, USA), using the Bactec MGIT 320 system (BD Diagnostics, USA), and Lowenstein–Jensen medium. On the fourth day of incubation, the Middlebrook broth was positive, and a subculture was performed on chocolate agar to test for rapidly growing non-tuberculosis bacteria (RGNTB). The bacteria grew on the chocolate agar at 35°C by the fourth day. Acid-fast staining tests of these colonies on the chocolate agar were positive. Therefore, we proceeded to perform PCR restriction fragment length polymorphism analysis (PRA) using the heat shock protein 65 (*hsp65*) gene [5], and sequencing of the RNA polymerase subunit β (*rpoB)* gene [6]. However, PCR of the *hsp65* gene did not produce amplicons, and *rpoB* gene sequencing identified *S. rotundus* with a low percentage identity (91.92%). We chose to sequence the 16S rRNA gene, which showed *S. rugosus* with 100% identity. Despite performing the recommended laboratory procedures, based on this AFB strain, the first molecular methods chosen were not appropriate in this case.

Antimicrobial susceptibility testing was performed using the Sensititre™ Rapid Myco (Thermo Scientific). The minimal inhibitory concentrations (MICs) were determined after incubation at 35°C for 4 days. The break points used for the RGNTB were determined according to the Clinical and Laboratory Standards Institute (CLSI) [7]. The clinical *S. rugosus* (Z&Z215) isolate was susceptible to all the antibiotics tested (Table 1).

S. rugosus may be confused with RGMTB due to its acid-fast staining properties. In our case, only the sequencing analyses of the 16S rRNA genes allowed for the accurate identification of *S. rugosus*.

TABLE 1. Antimicrobial susceptibility patterns of *Segniliparus rugosus* (Z&Z215)

Antibiotics	MIC (μg/mL)	Interpretation[a]
Trimethoprim/sulfamethoxazole	<0.25/4.75	S
Ciprofloxacin	<0.12	S
Moxifloxacin	<0.25	S
Cefoxitin	<4	S
Amikacin	<1	S
Doxycycline	<0.12	S
Tigecycline	0.06	S
Clarithromycin	<0.06	S
Linezolid	<1	S
Imipenem	<2	S
Cefepime	<1	S
Amoxicillin/clavulanic acid	<2/1	S
Ceftriaxone	<4	S
Minocycline	<1	S
Tobramycin	<1	S

MIC, minimal inhibitory concentration; S, susceptible.
[a]According to the break points for rapid growth mycobacteria. Read on the 4th day of incubation.

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With the presence of AFB in the sputum of CF patients, the *Segniliparus* genus should be considered as a potential pathogen in additional to the classical bacteria such as *Nocardia*, *Actinomyces* and *Mycobacterium*. The identification of this genus continues to be a challenge for microbiology laboratories. We need to learn more about the importance and impact of *Segniliparus* in CF patients. This is the first reported case of *S. rugosus* in a patient with CF in Ecuador.

Conflict of interest

The authors declare no conflict of interest.

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