Whole-Genome Sequences of *Mycobacterium abscessus* subsp. *massiliense* Isolates from Brazil

**ABSTRACT** The *Mycobacterium abscessus* complex comprises multidrug-resistant, opportunistic, and rapidly growing pathogens responsible for severe infections. Here, we report the genome composition of four *Mycobacterium abscessus* subsp. *massiliense* isolates from three sources: two from the lung of a cystic fibrosis patient, one from a mammary cyst, and one from a gutter system.

The *Mycobacterium abscessus* complex (MABSC) is so far composed of three subspecies: *Mycobacterium abscessus* subsp. *massiliense*, *Mycobacterium abscessus* subsp. *bolletii*, and *Mycobacterium abscessus* subsp. *abscessus* (1). Although common in soil and water, they are frequent (opportunistic) human pathogens associated with a broad spectrum of diseases, ranging from pulmonary to superficial skin and soft tissue infections to severe disseminated infections in immunocompromised patients (2, 3), such as those with cystic fibrosis, among other chronic lung diseases (4).

Infections caused by the MABSC are becoming more prevalent worldwide (5), including in Brazil, which has reported 2,128 skin and soft tissue infections since 2004, with significant outbreaks (6). The effective treatment of infections caused by the MABSC is challenged by the high resistance to antibiotics displayed by these organisms (5, 7).

Here, we announce the genome sequencing of four clinical strains of *M. abscessus* subsp. *massiliense* (MAB1 to MAB4) isolated in Brazil. The samples were processed with N-acetyl-l-cysteine (NALC)-NaOH and 5% oxalic acid decontamination (8) and cultured in Lowenstein-Jensen medium in an incubator at 35°C for 7 days. All isolates were tested for susceptibility against amikacin (AMK), ciprofloxacin (CIP), doxycycline (DOX), tobramycin (TOB), clarithromycin (CLR), cefoxitin (CTX), moxifloxacin (MFX), linezolid (LZD), and trimethoprim-sulfamethoxazole (TMP-SXT) using the MIC protocol according to the Clinical and Laboratory Standards Institute (CLSI) (9) performance standards for susceptibility testing for rapidly growing mycobacteria (RGM).

MAB1 was isolated from a sewer wastewater station in the city of Vitória, Espírito Santo State, in 2009 and demonstrated drug resistance to CIP, DOX, and TOB. MAB2 and MAB4 were isolated from sputum samples from the same patient with cystic fibrosis in November 2009 and May 2010, respectively. MAB2 was resistant to CIP, CLR, DOX, TMP-SXT, and MFX; MAB4 was resistant to the same drugs except that it showed
| Isolate | Source Description | Drug resistance profile | BioSample accession no | GenBank accession no | SRA accession no | No. of reads | Genome size (bp) | Genome completeness (%) | No. of contigs | N50 (bp) | Genome coverage (x) | GC content (%) |
|---------|-------------------|-------------------------|------------------------|----------------------|-----------------|--------------|------------------|------------------------|---------------|----------|----------------------|----------------|
| MAB1    | Sewer water       | CIP (R, 4.0); DOX (R, 32.0); TOB (R, 16.0) | SAMN16557383 JADEYL000000000 | SRS8489819          | 20,332,477     | 5,232,272    | 100.00          | 190                     | 161,580       | 777      | 64.0                 |
| MAB2    | Sputum            | CIP (R, 4.0); DOX (R, >32.0); MFX (R, 4.0); CLR (R, >64.0); TMP-SXT (R, 4.0/76) | SAMN16557407 JADEYM000000000 | SRS8489820          | 8,243,873      | 4,966,199    | 100.00          | 71                      | 191,720       | 332      | 64.2                 |
| MAB3    | Mammary cyst biopsy specimen | Pan-susceptible | SAMN16557408 JADEYN000000000 | SRS8489821          | 1,658,365      | 5,051,696    | 99.05           | 654                     | 14,176        | 66       | 64.1                 |
| MAB4    | Sputum            | CIP (R, 4.0); DOX (R, 32.0); MFX (R, 8.0); TMP-SXT (R, 4.0/76) | SAMN16557409 JADEYO000000000 | SRS8489822          | 3,842,124      | 4,962,832    | 100.00          | 79                      | 155,714       | 155      | 64.2                 |

a) R, drug resistance; CIP, ciprofloxacin; DOX, doxycycline; TOB, tobramycin; CLR, clarithromycin; MFX, moxifloxacin; TMP-SXT, trimethoprim-sulfamethoxazole.
susceptibility to CLR. The exact reason for this was not investigated but might be due to heteroresistance and isolation of a different fraction of the population on both occasions. Isolate MAB3 was from a biopsy specimen from a cyst that developed after implantation of a mammary prosthesis in 2003 and presented no drug resistance (Table 1).

Genomic DNA was obtained from the Lowenstein-Jensen culture using cetyltrimethylammonium bromide (CTAB) phenol-chloroform-based extraction and sequenced using the Nextera XT library preparation kit (Illumina, San Diego, CA, USA) on the Illumina HiSeq 2500 platform with 2 × 150-bp paired-end reads.

The raw reads were evaluated using FastQC v.0.11.9 (10) and filtered and trimmed using Trimmomatic v.0.36 (11) (LEADING:3, TRAILING:3, SLIDINGWINDOW:3:28, HEADCROP:19, and MINLEN:40). The genome sequences were de novo assembled using SPAdes v.3.11.1 (12), and the assembly quality was evaluated using Quast v.5.0.2 (13) and CheckM v.1.1.2 (14), applying the lineage analysis workflow. Annotation was performed using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v.4.3 (15). No plasmids were found in the four samples analyzed, according to the PlasmidSeeker v.1.3 tool (16). Default parameters were applied unless otherwise specified.

In summary, the genome size ranged from 4.96 to 5.23 Mb with samples harboring 4,932 to 5,469 genes, roughly representing a difference of 6% in size and 10% in gene content (Table 1). The environmental isolate displayed the largest genome sequence.

**Data availability.** The genomic sequences are publicly available in NCBI under the BioProject accession number PRJNA672126.

**ACKNOWLEDGMENTS**

This study was approved by the Brazilian Ethics Committee and Regional Committee (213/08-CEP HUCFF UFRJ).

A.C.R.G., M.C., and P.N.S. conceived and designed the experiments. F.C.O. and E.M. performed the in silico experiments. F.C.O., E.M., A.C.R.G., M.C., and P.N.S. analyzed the data. A.C.C., S.V., E.C.C., M.C.L., F.S.C., and P.H.C.C. were responsible for the sample collection, strain isolation, conventional and molecular identification, and drug susceptibility testing. F.C.O., E.M., A.C.R.G., M.C., and P.N.S. contributed to the manuscript writing.

**REFERENCES**

1. Lee M-R, Sheng W-H, Hung C-C, Yu C-J, Lee L-N, Hsueh P-R. 2015. Mycobacterium abscessus complex infections in humans. Emerg Infect Dis 21:1638–1646. https://doi.org/10.3201/2198.141634.
2. Tortoli E. 2003. Impact of genotypic studies on mycobacterial taxonomy: the new mycobacteria of the 1990s. Clin Microbiol Rev 16:319–354. https://doi.org/10.1128/CMR.16.2.319-354.2003.
3. Seemann T, Wirth T, Conlan S, Parkhill J, Harris SR. 2017. Genomic epidemiology of a national epidemic of upward transmission of a single clone of Mycobacterium abscessus in Scotland. J Infect 75:17–24. https://doi.org/10.1016/j.jinf.2017.02.031.
4. PARKHILL J, LEE M-R, HANG J-H, HSUEH P-R. 2015. Comparative genomic epidemiology of Mycobacterium abscessus complex infections in humans. Emerg Infect Dis 21:1638–1646. https://doi.org/10.3201/2198.141634.
5. Everall I, Nogueira CL, Bryant JM, Sánchez-Busó L, Chimara E, Duarte RDS, Darlington K, Ramos JP, Lima KVB, Lopes ML, Palaci M, Kipnis A, Monego F, Floto RA, Parkhill J, Léao SC, Harris SR. 2017. Genomic epidemiology of a national outbreak of post-surgical Mycobacterium abscessus wound infections in Brazil. Mircob Genom 3:e000117. https://doi.org/10.1099/mgen.0.000117.
6. Léao SC, Viana-Niero C, Matsumoto CK, Lima KVB, Lopes ML, Palaci M, Kipnis A, Monego F, Floto RA, Parkhill J, Léao SC, Harris SR. 2017. Genomic epidemiology of a national outbreak of post-surgical Mycobacterium abscessus wound infections in Brazil. Mircob Genom 3:e000117. https://doi.org/10.1099/mgen.0.000117.
7. Wee WY, Dutta A, Choo SW. 2017. Comparative genome analyses of Mycobacterium abscessus complex infections in humans. Emerg Infect Dis 21:1638–1646. https://doi.org/10.3201/2198.141634.
8. Brasil Ministério da Saúde. 2008. Manual nacional de vigilância laboratorial da tuberculose e outras micobactérias, 1st ed. Ministério da Saúde, Secretaria de Vigilância em Saúde, Departamento de Vigilância Epidemiológica, Brasília, Brazil.
9. CLSI. 2018. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes, 3rd ed. CLSI document M24-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
10. Andrews S. 2015. FastQC: a quality control tool for high throughput sequence data. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.
11. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible tracer for illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
12. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
13. Gurevich A, Saveliev V, Vyahhi N, Lesin VM, Nikolenko SI, Pham S, Pevzner PA. 2012. QUAST: quality assessment tool for genome assemblies. Bioinformatics 28:1072–1075. https://doi.org/10.1093/bioinformatics/bts186.
14. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055. https://doi.org/10.1101/gr.160722.114.
15. Tatusova T, DiCuccio M, Badetdin A, Chetvenin V, Nawrocki EP, Zaslavsky L, Lesin VM, Nikolenko SI, Pham S, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
16. Roosare M, Puustusmaa M, Mõls M, Vaheer M, Remm M. 2018. Plasmid Seeker: identification of known plasmids from bacterial whole genome sequencing reads. PeerJ 6:e4588. https://doi.org/10.7717/peerj.4588.