



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

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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 (CXT), 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 Vitoria, 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

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TABLE 1 Genomic features and accession numbers of the *Mycobacterium abscessus* subsp. *massiliense* isolates sequenced

| Isolate | Source | Drug resistance profile ^a | BioSample accession no. | GenBank accession no. | SRA accession no. | No. of reads | Genome size (bp) | Genome completeness (%) | No. of contigs | N_{50} (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 |

^aR, 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](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA672126).

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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.

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