High-Quality Draft Genome Sequences of Rare Nontuberculous Mycobacteria Isolated from Surfaces of a Hospital

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ABSTRACT Nontuberculous mycobacteria (NTM), some of which had multidrug-resistant profiles, were isolated from a tertiary care hospital setting. Although most NTM are nonpathogenic, contamination of hospital surfaces by these opportunistic pathogens poses a health risk to vulnerable inpatients. These high-quality NTM draft genomes are fundamental for future genetic and epidemiological studies.

Nontuberculous mycobacteria (NTM) are ubiquitous in nature and in human-engineered environments. Their classification and clinical relevance have been considered since the 1950s, owing to the efforts of Ernest Runyon, in understanding the role of these organisms in atypical human infections acquired from the environment (1, 2). Despite their opportunism and high resistance to standard therapeutic antibiotics and disinfectants, which have been widely acknowledged, their prevalence in anthropogenic environments and real impact on human health are still neglected (3).

Recent phylogenomic analyses reclassified the genus Mycobacterium into five distinct genera, namely, Mycobacterium, Mycolicibacterium, Mycolicibacter, Mycolicibacillus, and Mycobacteroides (4, 5).

We have performed a microbiological survey aimed at the investigation of the presence of NTM populations in a tertiary care hospital (6). Here, we present the high-quality draft genome sequences of the five NTM strains isolated from surfaces of different wards in that hospital. Samples were recovered using swabs to sample each surface and transported in tubes containing peptone water and after 3 h of shaking were used to inoculate Middlebrook 7H10-PANTA medium (PANTA medium contains an antibiotic mixture of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC) (6). The phylogenetic classification of the isolates was performed by concatenation of partial sequences of the 16S rRNA, rpoB, and hsp65 genes. Isolates 10AIII, 29AIII, and 35AIII were classified as closely related to strains of Mycobacterium paragordonae, while isolate 22DIII was considered to be related to strains of Mycolicibacterium obuense and isolate 24AIII to strains of Mycolicibacterium mucogenicum. Remarkably, the three M. paragordonae strains displayed differences in their antibiotic susceptibility profiles, while the M. obuense and M. mucogenicum strains were found to be resistant to several CLSI-recommended drugs (6).

The release of the draft genome sequences of these NTM strains recovered from small areas of hospital surfaces is indicative of a potentially significant ward contamination and is relevant for future population epidemiologic and genetic studies since they pose a potential threat to vulnerable inpatients.

The genomic DNA of the five NTM pure cultures grown in the medium used for isolation as described above was extracted using a protocol adapted from Nielsen et al.,...
### TABLE 1 Data regarding phylogenetic assignment, raw data, and de novo assembly results for the five strains in this study

| Strain | Species                     | No. of high-quality raw sequences | No. of contigs | \( N_{50} \) (bp) | Draft genome size (bp) | No. of coding sequences | G+C content (%) | Coverage (X) | DDH (%) | Model CI (%) | GenBank accession no. |
|--------|-----------------------------|----------------------------------|----------------|-------------------|------------------------|------------------------|-----------------|-------------|---------|-------------|-----------------------|
| 10AIII | *Mycobacterium paragordonae* | 37,870,761                       | 60             | 210,802           | 7,046,008              | 6,372                  | 66.9            | 331         | 83.4    | 80.5–85.8    | SDLQ00000000           |
| 29AIII | *Mycobacterium paragordonae* | 38,831,078                       | 49             | 1,195,780         | 6,697,711              | 6,015                  | 67.1            | 308         | 83.7    | 80.9–86.2    | SDLN00000000           |
| 35AIII | *Mycobacterium paragordonae* | 44,929,625                       | 48             | 286,333           | 7,049,864              | 6,335                  | 66.9            | 317         | 84.6    | 81.8–87     | SDLM00000000           |
| 22DIII | *Mycolicibacterium obuense*  | 9,985,256                        | 16             | 467,358           | 5,599,206              | 5,314                  | 68              | 92          | 90.5    | 87.3–92.9    | SDLP00000000           |
| 24AIII | *Mycolicibacterium mucogenicum* | 10,783,531                      | 47             | 239,262           | 5,494,553              | 5,331                  | 67.4            | 113         | 72.1    | 69.1–74.9    | SDLO00000000           |

\( a \)Values presented refer to those calculated by formula 2 as recommended at https://ggdc.dsmz.de, where the calculations were made. CI, confidence interval.
with initial incubation for 2 h at 37°C in glucose Tris-EDTA (GTE) buffer (50 mM glucose, 25 mM Tris-HCl at pH 8.0, and 10 mM EDTA) containing lysozyme (20 mg/ml) (7, 8). Libraries were prepared using the Nextera XT library prep workflow (Illumina), and 2 × 150-nucleotide (nt) paired-end reads were generated on an Illumina MiSeq instrument. Quality trimming was executed using the sliding-window operation in TrimGalore (9) with default parameters. The final assembly was performed using the SPAdes (10) assembler (version 3.50) using kmers of 33, 55, and 77 nt. The assembly was subjected to binning with MetaBAT (11), and a quality check was performed on the final resulting file with CheckM (12). The high-quality-draft genome sequences were used to determine DNA-DNA hybridization (DDH) values (13) against the type strain genomes presented at NCBI GenBank and corroborate the phylogenetic classification described above. DDH values, metadata, and de novo assembly values are shown in Table 1.

The assembled genomes were annotated using the NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) and have been deposited at DDBJ/EMBL/GenBank.

**Data availability.** These whole-genome shotgun projects have been deposited at DDBJ/ENA/GenBank under the accession numbers SDLQ00000000, SDLP00000000, SDL000000000, SDLN00000000, and SDLM00000000. Strains are available from the authors upon request. Raw sequencing reads for the strains are available in the NCBI Sequence Read Archive under accession numbers SRR8483011 to SRR8483015.

**ACKNOWLEDGMENTS**

We acknowledge Fundação para a Ciência e a Tecnologia (FCT) and COMPETE 2020, Operational Programme for Competitiveness and Internationalization (POCI) for grants UID/NEU/04539/2019 (POCI-01-0145-FEDER-007440) and POCI-01-0145-FEDER-029221; for FCT investigator contract (IF/01061/2014) to Igor Tiago; Msc fellowship (project IN075614-00003/2019 (POCI-01-0145-FEDER-007440) and POCI-01-0145-FEDER-029221; for Operational Programme for Competitiveness and Internationalization (POCI) for grants INFARMED, Fundo para a Investigação em Saúde, FIS-FIS-2015-01_DIA_20150630-144. We acknowledge Fundação para a Ciência e a Tecnologia (FCT) and COMPETE 2020, Operational Programme for Competitiveness and Internationalization (POCI) for grants UID/NEU/04539/2019 (POCI-01-0145-FEDER-007440) and POCI-01-0145-FEDER-029221; for FCT investigator contract (IF/01061/2014) to Igor Tiago; Msc fellowship (project IN075614-00003/2019 (POCI-01-0145-FEDER-007440) and POCI-01-0145-FEDER-029221; for Operational Programme for Competitiveness and Internationalization (POCI) for grants INFARMED, Fundo para a Investigação em Saúde, FIS-FIS-2015-01_DIA_20150630-144.

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