Draft Genome Sequences of Azole-Resistant and Azole-Susceptible *Aspergillus turcosus* Clinical Isolates Recovered from Bronchoalveolar Lavage Fluid Samples

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ABSTRACT We present the draft genome sequences of two clinical strains of *Aspergillus turcosus*, one azole-susceptible (strain HMR-AF-23/LSPQ-01275) and the other azole-resistant (strain HMR-AF-1038/LSPQ-01280), isolated from bronchoalveolar lavage fluid of two adult patients. These two strains are the first reported clinical isolates of *A. turcosus*.

Invasive aspergillosis is a serious fungal infection of major concern in immunocompromised patients and is associated with a high mortality rate (1–4). *Aspergillus fumigatus* is the most frequent species causing invasive aspergillosis. Its genome was completely sequenced in 2005, along with *A. nidulans* and *A. oryzae* (5–7). *Aspergillus* section *Fumigati* comprises about 60 species (8), often misidentified as *A. fumigatus* by morphotyping (9–12). First described in 2008, *Aspergillus turcosus* is a rare species belonging to this section (13). The two isolates whose complete genomes are presented herein are the first reported clinical isolates and the first occurrence of this species outside South Korea.

Four *A. turcosus* were identified among a collection of 1,186 presumed *A. fumigatus* clinical isolates collected at Maisonneuve-Rosemont Hospital in Montréal, Québec, Canada. Identification was based on phenotypic characteristics and confirmed by sequencing the following three relevant loci: beta-tubulin, calmodulin, and internal transcribed spacer (13, 14). Antifungal susceptibility testing, performed according to the Clinical and Laboratory Standards Institute (CLSI) broth microdilution procedure (15), revealed reduced susceptibility of strain HMR-AF-1038 to voriconazole, itraconazole, and posaconazole, compared to wild-type *A. fumigatus* (16). We sequenced its whole genome, along with that of a randomly selected azole-susceptible *A. turcosus* isolate (HMR-AF-23), for comparison. HMR-AF-23 and HMR-AF-1038 were isolated from bronchoalveolar lavage fluid samples in 2000 and 2012, respectively.

Whole-genome sequencing was performed at the provincial public health laboratory. Isolates were grown on potato dextrose agar at 30°C for 4 to 7 days, then DNA was extracted from conidia with the ZR fungal/bacterial DNA miniprep kit (Zymo Research Corp.). The Nextera XT library preparation kit (Illumina, Inc.) was used to prepare sequencing libraries for both strains. They were then sequenced with a MiSeq 600-cycle sequencing kit v3 as paired-end reads (2×300 bp) on a MiSeq sequencing platform (Illumina). HMR-AF-23 generated 25,658,892 raw reads, and the mean per-base sequence depth reached $85\times$. HMR-AF-1038 generated 25,586,278 raw reads for a $42\times$ per-base mean coverage. Reads were quality and quantity controlled with FastQC version 0.11.5 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and then

cleaned with Trimmomatic v0.36 (parameters, LEADING:20 TRAILING:20 SLIDINGWINDOW: 4:20 MINLEN:50 ILLUMINACLIP:NexteraPE-PE.fa:2:30:10) (17). Mitochondrial sequences were discarded by mapping the cleaned reads onto the *A. fumigatus* mitochondrial genome (reference genome JQ346807) with SMALT v0.7.6. Remaining nuclear reads were assembled with SPAdes v3.7.0 (18) on Calcul Québec servers (http://www .calculquebec.ca/). An in-house Python script (https://github.com/EricFournier3/Python GenomicTools) was used to eliminate contigs with coverage lower than 5$\times$ and length less than 2 kb. The quality of the assembly was further assessed with QUAST 4.1 (19). The absence of duplicated contigs was ensured by running dedup.sh (https://jgi.doe .gov/data-and-tools/bbtools/), and the absence of Illumina adapters was checked with the Linux grep command. A total of 939 contigs for HMR-AF-23 (N_{50} = 110,871 bp) and 1,043 contigs for HMR-AF-1038 (N_{50} = 95,868 bp) were found. The overall genome size and G+C content for HMR-AF-23 and HMR-AF-1038 are 32,352,689 bp and 48% and 34,076,230 bp and 46%, respectively.

Genome annotation was performed with funannotate v1.4.2 (https://github.com/ nextgenusfs/funannotate). We used an Augustus (20) pretrained gene model of *A. fumigatus* to predict 9,223 genes for HMR-AF-23, including 833 with functional annotation. For HMR-AF-1038, 9,174 genes were predicted, from which 830 have functional annotation. The completeness of these annotations was assessed by comparing the number of conserved orthologs to those of *A. fumigatus*, the closest reference species, using the tool BUSCO v2.0 (21) run with the Dikarya data set. Approximately 94% orthologs were found for both isolates, which is nearly as many as for *A. fumigatus* (96%). We found 1,232 and 36 complete and fragmented orthologs, respectively, for HMR-AF-23, compared to 1,227 and 39, respectively, for HMR-AF-1038.

These first draft genome sequences of *A. turcosus* may be useful in deciphering the genetic differences which result in elevated azole resistance for this species and allow a better understanding of virulence through genomic comparison with other related clinically relevant *Aspergillus* species.

Data availability. These whole-genome shotgun projects have been deposited in GenBank under the accession numbers NKHV00000000 for strain HMR-AF-23 and NIDN00000000 for strain HMR-AF-1038. The versions described in this paper are the second versions, NKHV02000000 and NIDN02000000, respectively. Raw sequence data can be accessed using the following run numbers: SRR8165489 (HMR-AF-23) and SRR8165475 (HMR-AF-1038).

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