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Human Infections Caused by Clonally Related African Clade (Clade III) Strains of *Candida auris* in the Greater Houston Region

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ABSTRACT *Candida auris* is a pathogen of considerable public health importance. It was first reported in 2009. Five clades, determined by genomic analysis and named by the distinct regions where they were initially identified, have been defined. We previously completed a draft genome sequence of an African clade (clade III) strain cultured from the urine of a patient hospitalized in the greater Houston metropolitan region (strain LOM). Although initially uncommon, reports of the African clade in the United States have grown to include a recent cluster in California. Here, we describe a second human *C. auris* infection in the Houston area. Whole-genome sequence analysis demonstrated the Houston patient isolates to be clonally related to one another but distantly related to other African clade organisms recovered in the United States or elsewhere. Infections in these patients were present on admission to the hospital and occurred several months apart. Taken together, the data demonstrate the emergence and persistence of a clonal *C. auris* population and highlights the importance of routine high-resolution genomic surveillance of emerging human pathogens in the clinical laboratory.

KEYWORDS *Candida*, *Candida auris*, Houston, whole-genome sequencing, infection control, phylogenetic analysis

Candida auris was first reported in 2009 and has caused several outbreaks in health care facilities (1, 2). Two notable features of *C. auris* are its ability to persist in the hospital environment, particularly on surfaces, and its ability to resist killing by common antiseptic cleaning agents (1, 3). While not necessarily more virulent or more transmissible than other *Candida* spp., *C. auris* is a cause of serious public health concern due to its tendency to acquire resistance to multiple antifungals, including azoles, echinocandins, and amphotericin B, and to resist environmental decontamination (1–4).

We previously sequenced the genome of *C. auris* strain LOM that was cultured from the urine of a patient hospitalized in the Houston metropolitan region in April 2019 (5). The patient had multiple comorbidities and was transferred to our hospital from a long-term acute care facility. The patient's *C. auris* urinary tract infection was present on admission (5). The antimicrobial susceptibility phenotype of strain LOM was determined following CDC guidance, as CLSI breakpoints do not yet exist for *C. auris* (https://www.cdc.gov/fungal/candida-auris/c-auris-antifungal.html). Strain LOM is fluconazole resistant (256 μg/ml), amphotericin B susceptible (1 μg/ml), and micafungin susceptible (0.12 μg/ml). Due to concerns about possible intrahospital transmission, the patient was placed into enhanced-barrier contact isolation. The urinary tract infection cleared after a full course of micafungin therapy. After discharge in May 2019, the patient's room and attached restroom underwent a terminal cleaning using a sporicidal disinfectant and ultraviolet light total room disinfection (Tru-D SmartUVC) (6). All fabrics in

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the room, including the curtains, were laundered. As of 3 February 2020, no further hospital-acquired *C. auris* infections have been identified in our hospital system.

In August of 2019, our diagnostic laboratory recovered *C. auris* from a second patient in the Houston metropolitan region. We were alerted to the infection by an automated script, which reviews new microbiology culture results and emails key stakeholders when a *C. auris* infection is identified. The infection was determined to be present on admission from a long-term acute care facility. *C. auris* was recovered from multiple specimens, including one blood culture, a peripherally inserted central catheter (PICC)-line catheter tip culture, a second blood culture, and a urine culture. The strains were named LOM-2, LOM-3, LOM-4, and LOM-5, respectively. The antimicrobial susceptibility phenotype of each strain was identical to strain LOM. Given the public health concern of community-acquired *C. auris* infections and our recent experience with strain LOM, we rapidly sequenced the genome of the four strains recovered from the second patient to determine their genetic relationship to each other and to strain LOM.

MATERIALS AND METHODS

Yeast cultures and identification. Strains were cultured in the Houston Methodist Hospital Diagnostic Microbiology Laboratory on tryptic soy agar supplemented with 5% sheep blood. Taxonomic classification was performed using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (MALDI Biotyper; Bruker Daltonics) using the research use only database (RUO) v4.1.80. Antimicrobial susceptibility was determined using standard methods (Sensititre Yeast One; Trek Diagnostics). This work was approved by our Institutional Review Board (IRB1010-0199).

Automated *C. auris* alert. After identifying the *C. auris* strain in April 2019, we created an automated lab information system (LIS) database search script to find new *C. auris* isolate results. We scheduled the search to run every hour and chose minute 40 of the hour to avoid interfering with other scheduled scripts. Any new isolate triggered an email to the microbiology medical directors, laboratory manager, and department chair that included the patient's name and location, medical record number, laboratory order number, sample source and site, sample collection date, and culture type. The script was executed on a Linux server in our institution's data center as a cron job and connected to the LIS database using the Java Database Connectivity (JDBC) application programming interface (API) (Oracle, Redwood Shores, CA).

Genome sequencing. DNA extraction from overnight growth on solid agar used ballistic lysis with FastPrep Matrix Y (MP Biomedicals) and the MasterPure yeast DNA purification kit (Lucigen). We first rapidly confirmed the MALDI-TOF mass spectrometry identification of *C. auris* from blood culture isolate LOM-2 using the Oxford Nanopore field sequencing kit (catalog no. SQK-LRK001). We then sequenced the genomes of strains LOM-2, LOM-3, LOM-4, and LOM-5 using the Oxford Nanopore PCR barcoding kit (catalog no. LSK-109) and a GridION instrument with FLO-MIN106 flow cells and Guppy v2.0.10 basecaller. Reads were filtered using FiltLong v0.1.1 with 5-kb read cutoff and 100-fold coverage target (https://github.com/rrwick/Filtlong). The genomes also were sequenced using a Nextera XT library preparation kit (Illumina) and NextSeq instrument (Illumina). Illumina reads were trimmed using Trimmomatic and error corrected using Musket v1.1 (7, 8). Unicycler v0.4.3 was used to assemble hybrid genomes using both Illumina and Oxford Nanopore data in the normal mode (bridge cutoff, 10.0), using SPAdes v3.10.1, Bowtie v2.2.3, SAMtools v1.9, Pilon v1.22, and Miniasm with Racon polishing (9). Genomes were compared using ProgressiveMauve v2.4.0 and MUMmer v4.0 (10). Single nucleotide polymorphisms (SNPs) were called against the strain LOM reference genome (GenBank assembly accession no. GCA_005234155.1) and the B11221 reference genome (GenBank assembly accession no. GCA_002775015.1) using SMALT v0.7.6, SAMtools v1.9, and FreeBayes v0.9.20-16-g3e35e72 with minimum alternate fraction of 0.7 (11). SNPs were further filtered by vcffilter using DP $>$ 9 and QUAL $>$ 30. SNPs prephix and phrecon (https://github.com/codinghedgehog/) were used to generate SNP alignments to create a neighbor-joining tree using FastTree2 (12). The trees were visualized using CLC Genomics Workbench v12 (Qiagen Bioinformatics). We used snp-dist v0.2.6 to create distance matrices (https://github.com/tseemann/snp-dists).

RESULTS

The four *C. auris* strains (LOM-2, LOM-3, LOM-4, and LOM-5) recovered from the second Houston-area patient were sequenced with an Oxford Nanopore GridION to develop draft genome sequences. Within 2 h, the field sequencing kit yielded more than 50,000 reads from LOM-2, which was sufficient to confirm the isolate's taxonomic classification as *C. auris*. The strains were sequenced by Illumina and Oxford Nanopore sequencing technologies to generate short and long reads for hybrid assemblies. The run statistics and assembly statistics are summarized in Table 1.

We compared the assembly of each strain recovered from the second Houston-area patient to the *C. auris* strain LOM reference genome (GenBank assembly accession no.

TABLE 1 Sequencing and assembly statistics[a]

Characteristic	LOM-2	LOM-3	LOM-4	LOM-5
Source	Blood	PICC tip	Blood	Urine
No. of ONT reads	1,300,000	1,200,000	122,000	123,000
ONT yield (Gb)	3.9	2.9	1.6	2.0
ONT coverage depth (×)	317	236	129	163
No. of Illumina reads	167,000	16,000	163,000	164,000
Illumina yield (bp)	100,000,000	96,600,000	97,800,000	98,700,000
Illumina coverage depth (×)	8	8	8	8
Assembly size (bp)	12,309,234	12,284,797	12,392,210	12,288,268
No. of contigs	60	36	75	35
N_{50} (bp)	918,543	1,552,302	1,942,869	2,271,542
Largest contig (bp)	2,655,495	3,445,501	2,367,777	4,280,604

[a]All specimens were collected over 2 days in August 2019. Assembly information (size, number of contigs, N_{50}, largest contig) is presented for the final polished Unicycler hybrid assembly as described in Methods and Materials. Yield, assembly size, N_{50}, and largest contig are measured in base pairs (bp). ONT, Oxford Nanopore Technologies GridION long-read data. Illumina reads represent paired-end 2×300-bp reads.

GCA_005234155.1). We discovered that for strain LOM-5, the 7 longest contigs correspond to the 7 chromosomes characteristic of *C. auris*. Similarly, the longest contigs generated from strains LOM-2, LOM-3, and LOM-4 also corresponded to the 7 reference genome chromosomes, except, for each of these three, chromosome 1 was broken into two or more fragments. Some variation in hybrid assembly contigs may be due to the selection of longer ONT (Oxford Nanopore Technologies GridION long-read data) reads during preparation of the ONT libraries for LOM-4 and LOM-5.

Phylogenetic analysis was performed to compare the four *C. auris* strains recovered from the second Houston patient to the reference genome assembly of the African clade (clade III) strain B11221. We also compared two additional African clade isolates recovered in the United States (LOM and B12631) and one representative strain from each of the other *C. auris* clades (IFRC2087, B11843, B12043, and B8441) (Fig. 1).

We discovered that strain LOM, which was isolated from the first Houston patient in April 2019, and the 4 strains recovered from the second Houston patient in August 2019, are both most closely related to African clade (clade III) strain B11221 (13, 14) (Fig. 1). Strains from the other clades are 48,354 to 288,886 SNPs distant to the Houston isolates. We found that strains LOM-2, LOM-3, LOM-4, and LOM-5 differ from the African clade (clade III) B11221 genome by 5 to 14 single nucleotide polymorphisms. In comparison, the five Houston strains differ from the African clade (clade III) strain B12631 by 251 to 257 SNPs (Table 2). The pairwise distances among these strains varied from 8 to 21 SNPs (Table 2). The very short pairwise distances are within the range reported for strains implicated in an intensive care unit (ICU) outbreak in the United Kingdom (15). The original African clade (clade III) isolate from Houston, strain LOM, is 25 SNPs distant to B11221.

Of note, strains LOM-2, LOM-3, LOM-4, and LOM-5 have the same *ERG11* gene allele as strain LOM, which includes an F126L amino acid replacement that is common to clade III strains and is associated with fluconazole resistance (14).

DISCUSSION

Herein, we demonstrate the clinical utility and public health relevance of real-time long-read whole-genome sequencing to facilitate rapid high-resolution data for pathogen identification, antimicrobial susceptibility prediction, and phylogenic analysis (16, 17). As predicted in our earlier publication of the draft genome of *C. auris* strain LOM (5), the availability of a high-quality reference genome served as a crucial resource when additional isolates were recovered from a second infected patient in the greater Houston metropolitan area (or elsewhere). The data also demonstrate the ability of hospital-based laboratories to use whole-genome sequencing to effectively contribute to outbreak investigations and guide patient care decisions and public health maneuvers.

Our first goal was to apply whole-genome sequencing to the strains isolated from

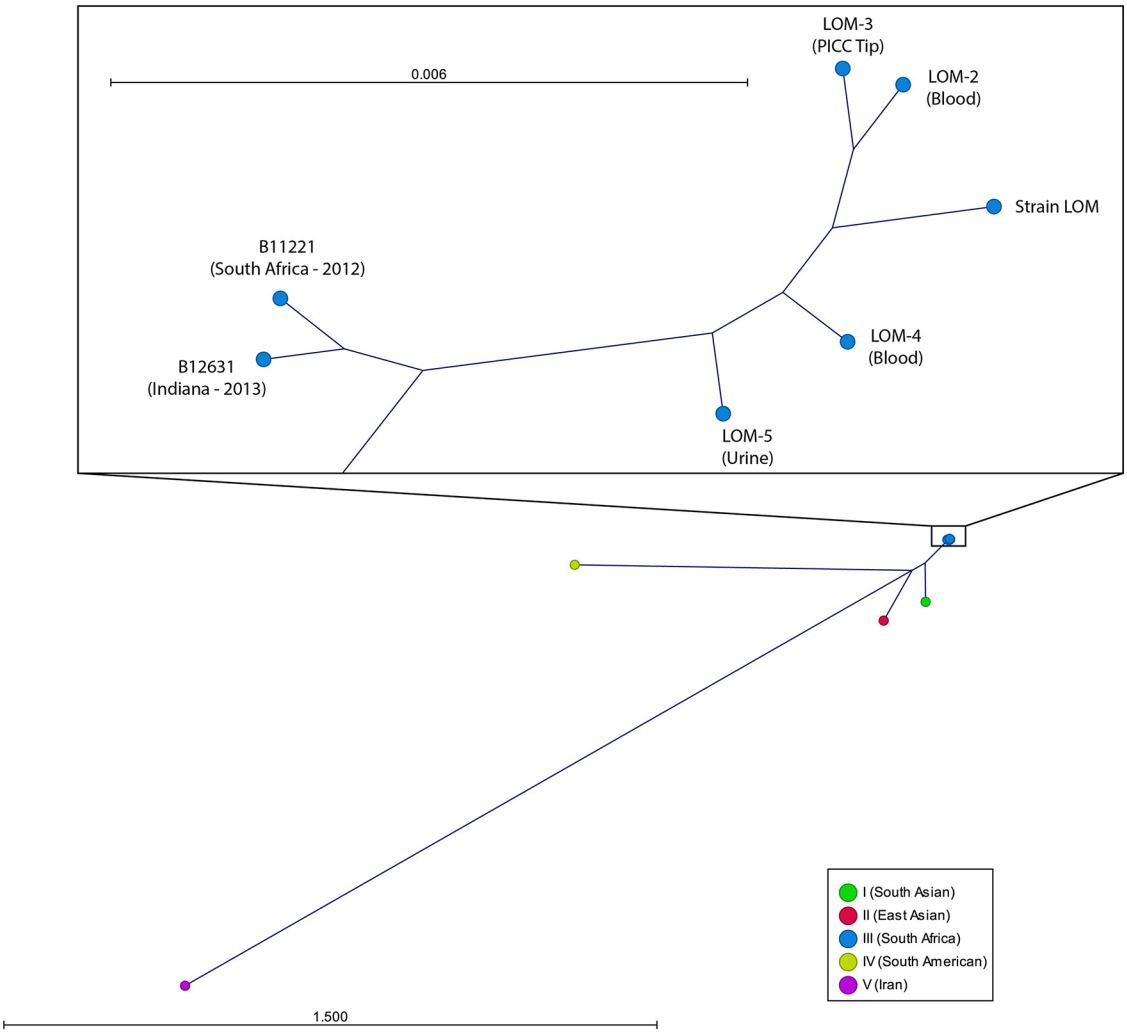

FIG 1 Neighbor-joining radial phylogenetic tree showing the relationship of the second patient's *C. auris* strains (LOM-2 through LOM-5) and the first patient's isolate, strain LOM (GenBank assembly accession no. GCA_005234155.1), to distantly related reference strains from the five clades of *C. auris* (B11220 [SRA accession no. SRR3883442], B11221 [SRA accession no. SRR3883453], B11843 [SRA accession no. SRR7909220], B12043 [SRA accession no. SRR7909185], B12631 [SRA accession no. SRR7909359], and IFRC2087 [SRA accession no. SRR9007776]). Phylogenetic relationships are determined relative to reference strain LOM. Geographic association of the clades is included for reference.

the second Houston-area patient to confirm the taxonomic classification of *C. auris*. Remarkably, the pathogen's identity was confirmed within 2 h of retrieving the strain from the microbiology laboratory. This rapid retrieval and investigation were enabled in part by our use of an automated alert. The alert consists of a script which scans new microbiology results every hour to look for *C. auris* identifications. The interval of 60 min was chosen because our lab performs culture identifications 24 h a day, yet frequent database queries can slow LIS performance. The new isolate was entered in our LIS at 12:38 p.m., and the email notified key stakeholders just 2 min later at 12:40 p.m. Second, we sought to determine if the strains from the two patients were closely related or represented distinct introductions of clade III strains to the Houston metropolitan area. Identification of two human infections caused by the African clade (clade III) was unexpected, since at the time the second patient's isolates were identified, most reported *C. auris* isolates recovered in the United States belonged to the South Asian (clade I), East Asian (clade II), or South American clades (clade IV) (2, 18). Since that time, we have learned of a reported outbreak of African clade (clade III) strains in a long-term acute care (LTAC) facility in California (19). This discovery raises the question if African

TABLE 2 Pairwise distances between the closed genome of strain B11221, representative strains from the other clades of *C. auris*, strain LOM, and the four new isolates, LOM-2 through LOM-5

	Pairwise distance (SNPs) from strain:										
Strain or isolate	B11221.V1	IFRC2087[a]	LOM-5	LOM-4	LOM-3	LOM-2	LOM	B8441[b]	B12631[c]	B12043[d]	B11843[e]
B11221.V1	0	288,869	14	7	9	5	25	48,355	252	68,583	181,849
IFRC2087[a]	288,869	0	288,873	288,866	288,872	288,868	288,886	291,362	289,014	295,204	303,779
LOM-5	14	288,873	0	17	21	15	23	48,365	266	68,591	181,857
LOM-4	7	288,866	17	0	12	8	30	48,354	251	68,580	181,846
LOM-3	9	288,872	21	12	0	12	32	48,358	255	68,586	181,852
LOM-2	5	288,868	15	8	12	0	26	48,358	255	68,582	181,850
LOM	25	288,886	23	30	32	26	0	48,374	277	68,598	181,868
B8441[b]	48,355	291,362	48,365	48,354	48,358	48,358	48,374	0	48,537	72,515	183,109
B12631[c]	252	289,014	266	251	255	255	277	48,537	0	68,707	181,969
B12043[d]	68,583	295,204	68,591	68,580	68,586	68,582	68,598	72,515	68,707	0	187,035
B11843[e]	181,849	303,779	181,857	181,846	181,852	181,850	181,868	183,109	181,969	187,035	0

[a]IFRC2087 represents the Iranian clade (clade V).
[b]B8441 is a strain from the South Asian clade (clade I).
[c]B12631 is an African clade (clade III) strain collected in the United States.
[d]B12043 is a strain from the East Asian clade (clade II).
[e]B11843 is a strain from the South American clade (clade IV).

clade (clade III) isolates have a propensity to populate a niche in long-term care settings. Recently, a fifth clade associated with infections in Iran has been described, illustrating that additional clonal groups may be discovered as global surveillance efforts expand (20). Of note, the African clade III strain B11221 is a blood culture isolate recovered from a patient in South Africa in 2012, and the clade III strain B12631 is an arm wound isolate recovered from a patient in Indiana in 2013 (18). Our whole-genome sequence data confirm a clonal relationship between the *C. auris* strains recovered from two patients in Houston. However, these strains are genomically distinct compared to the African clade isolate recovered in Indiana (B12643) and more similar to the strain recovered in South Africa (B11221). Our Houston African clade (clade III) isolates likely represent an introduction of African clade strains closely related to the South African isolate B11221 into the United States, rather than spread of the B12643 isolate from Indiana to Houston. A distance matrix comparing the same strains against the closed reference for strain LOM is included as Table S1 in the supplemental material.

Houston is the most ethnically diverse city in the United States (21). Moreover, two international airports and a major international shipping port make the possibility for global import of highly pathogenic organisms a great concern. For this reason, in 201,3 we implemented whole-genome sequencing of microbes as a routine test in our clinical laboratory (22, 23). In this case, we were able to very rapidly confirm the taxonomic classification of African clade (clade III) *C. auris* isolates recovered from two Houston patients and determine their clonal relationship. These data had important patient care and public health implications. Also, the discovery of clonally related strains of *C. auris* in two patients stresses the importance of the ability of the clinical laboratory to perform high-resolution genomic investigations and effectively partner with hospital infection prevention and control teams (24).

Once we confirmed that the *C. auris* strains recovered from the two Houston-area patients were clonally related, we performed an extensive chart review to identify possible commonalities. Both patients presented in the same region of the Houston metropolitan area. They had multiple comorbidities and spent extensive time in long-term acute care facilities and other health care facilities in the community. The infections occurred several months apart and were present on admission to our hospital. The second patient had multiple urine and blood cultures negative for *C. auris* in the months between the first patient's infection and their infection. Taken together, these data suggest that a common source of *C. auris* exists somewhere outside our hospital and emphasizes the well-documented ability of *C. auris* to persist in environments and resist killing by disinfectants. Importantly, to date, no secondary cases have

occurred in our hospital. That is, our infection prevention and control practices, which were informed by the whole-genome sequence data and guided by a close partnership with the laboratory, were highly effective.

Data availability. The BioProject accession no. for *C. auris* strains is PRJNA540998. The genome short reads and assemblies can be found as follows: LOM (GenBank assembly accession no. GCA_005234155.1), B8441 (SRA accession no. SRR10851769), B11221 (SRA accession no. SRR3883453; GenBank assembly accession no. GCA_002775015.1), B11843 (SRA accession no. SRR7909220), B12043 (SRA accession no. SRR7909185), B12631 (SRA accession no. SRR7909359), and IFRC2087 (SRA accession no. SRR9007776).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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