Genomic Characterizations of Clade III Lineage of Candida auris, California, USA

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Candida auris is an emerging multidrug-resistant yeast. We describe an ongoing *C. auris* outbreak that began in October 2019 in Los Angeles, California, USA. We used genomic analysis to determine that isolates from 5 of 6 patients belonged to clade III; 4 isolates were closely related.

Candida auris was isolated from a patient in Tokyo, Japan in 2009 (1), although clinical isolates have been retrospectively identified from as early as 1996 (2). Since then, bloodstream and other invasive infections caused by *C. auris* have been reported worldwide (3-5). Many strains of *C. auris* are multidrug-resistant; some strains require elevated MICs to azoles, echinocandins, and polyenes. In 2019, the US Centers for Disease Control and Prevention (CDC) listed *C. auris* as an urgent threat to public health (6), highlighting the need for active surveillance and appropriate infection prevention.

Whole-genome sequencing (WGS) and phylogenetic analyses have revealed ≥ 4 major clades of *C. auris*; each clade covers a distinct geographic area, giving *C. auris* a global distribution (7,8). Researchers have documented several *C. auris* outbreaks in the United States, mostly caused by strains belonging to clades I and IV (9). We describe several cases of *C. auris* colonization and infection in patients of long-term acute-care (LTAC) facilities in and around Los Angeles, California, USA.

The Study

We screened patients at high risk for drug-resistant infections who were transferred to University of California, Los Angeles (UCLA)–affiliated hospitals from LTAC and skilled nursing facilities (SNFs). We analyzed swab samples of patients' axilla and groin and

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yeast isolates from positive fungal culture of clinical specimens using PCR selective for the ITS2 region of the *C. auris* genome. We conducted antifungal susceptibility testing using broth microdilution; WGS using Illumina MiSeq (Illumina, https://www.illumina.com); and k-mer and single-nucleotide polymorphism (SNP) analyses using CLC Genomics Workbench (QIAGEN, https://www.qiagen.com) and Geneious Prime (Geneious, https://www.geneious.com) (Appendix 1, https://wwwnc.cdc.gov/EID/article/27/5/20-4361-App1.pdf).

During September 2019–September 2020, we screened 113 patients using in-house PCR selective for *C. auris* according to Los Angeles County Public Health and CDC guidelines (Appendix 1). Six patients tested positive for *C. auris* with cycle threshold (C_t) values of 22.6–39.7 (Table 1). Patient A tested positive in October 2019; patients B–F tested positive during July–September 2020.

The 6 patients were residents of 4 LTAC facilities in Los Angeles County. All 6 had a history of tracheostomy. Patients A and F had prior history of *C. auris* colonization; patient F had active infection of a bronchopulmonary fistula. Patient D had *C. auris* and severe acute respiratory syndrome coronavirus 2 co-infection (Table 1). We cultured *C. auris* isolates from inguinal and axillary swab samples of patients A, C, D, and E; pleural fluid of patient F; and tracheal aspirate of patient A. The sample from patient A produced few colonies; we treated the patient for bacterial pneumonia. We were not able to isolate *C. auris* from patient B (C_t = 39.5).

All *C. auris* isolates were resistant to amphotericin B (MIC = $2 \mu g/mL$) and fluconazole (MIC >64 $\mu g/mL$) but susceptible to echinocandins (Table 2). We conducted k-mer analysis using 261 *C. auris* sequences available on GenBank, most of which were described previously (*10*) (Appendix 2 Table 1, https://wwwnc. cdc.gov/EID/article/27/5/20-4361-App2.xlsx). All 6

UCLA isolates belonged to clade III (Appendix 2 Table 1). We conducted a phylogenetic analysis of clade III isolates using k-mers (Appendix 1 Figure).

In the United States, researchers have identified isolates belonging to all 4 clades; although these isolates show geographic relationships (9), clade I is predominant across the country. Clade III isolates have been identified in Indiana, Texas (11), and Florida. We conducted a k-mer-based phylogenetic analysis of *C. auris* isolates in the United States (Figure). SNP analysis showed that 5 of the UCLA isolates were closely related (3–12 SNPs); isolate F1 was genetically distinct (77–79 SNPs). All 6 isolates were distinct from isolates from Indiana (65–139 SNPs) and Florida (47–117 SNPs) (Appendix 1 Table 1).

We also analyzed the sequences of 2 genes associated with antifungal resistance: *erg11* (lanosterol 14- α demethylase) and *fks1* (subunit of 1,3- β -D-glucan synthase). Sequences of *erg11* were identical among all isolates, with 99.6% pairwise nucleotide identity to the reference (GenBank accession no. CP043531) and 2 amino acid substitutions: V125A and F126L

Table 1.	able 1. Characteristics of patients with Candida auris infection, Los Angeles, California, USA, 2019–2020*							
	Date of	Cycle	C. auris isolate	Approximate		Current signs, symptoms, and		
Patient	positive PCR	threshold	(specimen type)	age, y	Clinical history	diagnosis		
A	2019 Oct 8	22.6	UCLA_A1	65	Coronary artery disease, stroke,	Septic shock caused by		
			(inguinal–		chronic respiratory fracture,	methicillin-resistant		
			axillary);		tracheostomy and ventilator	Staphylococcus aureus		
			UCLA_A2		dependence, gastrostomy tube	bacteremia and multifocal		
			(tracheal,		dependence, urinary	pneumonia. C. auris, Candida		
			deemed		incontinence, multiple ulcers,	albicans, and Candida		
			colonization)		heart failure, atrial fibrillation,	parapsilosis were isolated from		
					and previous carbapenem-	tracheal suction culture.		
					heateremia. This petient had a			
					prior biotony of C ourio			
					colonization at the long-term			
					acute-care facility			
В	2020 July 28	39.5	Not isolated ⁺	45	Anoxic brain injury caused by	Hemoptysis upper		
2	2020 0019 20	00.0	Horibolatou	10	MRSA endocarditis and	gastrointestinal bleeding.		
					pulseless electrical activity	hypotension, and tachycardia.		
					arrest, stroke, and gastrostomy	MRSA, Escherichia coli,		
					tube dependence.	Providencia stuartii, Proteus		
						mirabilis, and Acinetobacter		
						<i>baumanii</i> grew on blood		
						cultures. Candida glabrata		
						grew on lower respiratory		
						culture.		
С	2020 Aug 12	22.6	UCLA_C1	65	Hypertension, hyperlipidemia,	Respiratory failure caused by		
			(Inguinal-		Intracranial nemorrnage and	pulmonary edema. P. mirabilis		
			axiliary)		trachoostomy and gastrostomy	grew on unne cultures.		
					tube dependence			
D	2020 Aug 19	39.7	UCLA D1	55	Hypertension, hyperlipidemia.	Elevated liver enzymes and		
_			(inguinal-		type 2 diabetes, aplastic	gastrointestinal bleeding		
			axillary)		anemia, stroke, pulmonary	complicated by Enterococcus		
			• /		embolism, pneumothorax, and	bacteremia and E. coli urinary		
					coronavirus disease-related	tract infection.		
					pneumonia causing respiratory			
					failure, tracheostomy, and			
					gastrostomy tube dependence.			
E	2020 Aug 31	28.3	UCLA_E1	65	Hypertension, hyperlipidemia,	Worsening generalized		
			(inguinal-		tracheostomy, and gastrostomy	weakness possibly caused by		
			axillary)		tube dependence.	chronic intermittent		
F	2020 Sen 2	30 A		85	Subarachnoid hemorrhage	Bronchopulmonary fistula		
1	2020 064 0	50.0	(pleural fluid	00	tracheostomy gastrostomy tube	auris Pseudomonas		
			active infection)		dependence, stage IV sacral	aeruginosa, and Enterococcus		
					decubitus ulcer, and chronic	faecalis grew on pleural fluid		
					kidney disease. This patient had	cultures.		
					a prior history of C. auris			
					colonization at the long-term			
					acuto caro facility			

*MRSA, methicillin-resistant Staphylococcus aureus.

+C. auris was not isolated from the inguinal-axillary surveillance swab of patient B.

	MIC, μg/mL (interpretation)†					
Antifungal	UCLA_A1	UCLA_A2	UCLA_C1	UCLA_D1	UCLA_E1	UCLA_F1
Amphotericin B	2 (R)	2 (R)	2 (R)	2 (R)	2 (R)	2 (R)
Fluconazole	>64 (R)	>64 (R)	>64 (R)	>64 (R)	>64 (R)	>64 (R)
Voriconazole	2	1	1	0.5	0.5	2
Itraconazole	1	1	0.5	0.25	0.25	1
Posaconazole	0.06	0.12	≤0.03	≤0.03	0.06	0.06
Anidulafungin	0.5 (S)	0.12 (S)	0.25 (S)	0.12 (S)	0.06 (S)	1 (S)
Caspofungin	0.5 (S)	0.12 (S)	0.5 (S)	0.5 (S)	0.12 (S)	0.5 (S)
Micafungin	0.25 (S)	0.25 (S)	0.25 (S)	0.12 (S)	0.25 (S)	0.25 (S)

Table 2. Antifungal susceptibility results for Candida auris isolates, Los Angeles, California, USA, 2019–2020*

*I, intermediate; R, resistant; S, susceptible.

†MIC testing was conducted on panels prepared in-house in accordance with Clinical and Laboratory Standards Institute guidelines (https://standards.globalspec.com/std/10266416/CLSI%20M27). Interpretive breakpoints were defined by the CDC Antifungal Susceptibility Testing and Interpretation guidelines for *C. auris* (https://www.cdc.gov/fungal/candida-auris/c-auris-antifungal.html), which were adapted from interpretive criteria for closely related *Candida* spp. Tentative breakpoints for the following antifungal drugs were: amphotericin B ($\geq 2 \mu g/mL$), fluconazole ($\geq 32 \mu g/mL$), and micafungin ($\geq 4 \mu g/mL$).



Figure. K-mer analysis of *Candida auris* isolates, United States, 2009–2020. K-mer analysis was conducted with CLC Genomics Workbench (QIAGEN, https://www.qiagen.com) using genome sequences from patients in Los Angeles, California, USA during 2019–2020 (i.e., UCLA_A1, UCLA_A2, UCLA_C1, UCLA_D1, UCLA_E1, and UCLA_F1) and 55 publicly available *C. auris* strains in GenBank (Appendix 2 Table 2, https://www.c.cdc.gov/EID/article/27/5/20-4361-App2.xlsx). Each node represents a unique isolate. Node color indicates clade. The color of the isolate name (i.e., label text color) indicates state of origin. The metadata shows the susceptibility of each isolate (if available) to fluconazole (FCZ), amphotericin B (AMB), and micafungin (MCF); red indicates resistant, green indicates susceptible. Asterisk indicates that branches shorter than 0.0050 are shown as 0.0050.

DISPATCHES

(Appendix 1 Table 2). Mutations at aa 126 are associated with increased azole resistance in C. auris (7) and are a common feature of clade III isolates (12). The F126L mutation appears to be exclusive to clade III (10). These findings are consistent with results of antifungal susceptibility testing, which showed that all isolates were resistant to fluconazole (Table 2). Sequences of fks1 were identical in 5 isolates (A1, A2, C1, D1, E1), with 99.9% pairwise nucleotide identity to the reference (GenBank accession no. CP043531); these isolates had 1 amino acid substitution: I1572L (Appendix 1 Table 2). Isolate F1 had the same substitution in addition to I1095L. All isolates had a wildtype serine at aa 639; mutations at this location are linked to echinocandin resistance in C. auris (13). All isolates were susceptible to caspofungin, micafungin, and anidulafungin.

Conclusions

To identify and prevent the spread of *C. auris* in this hospital system, we used an in-house PCR to screen patients for this pathogen. WGS of isolates from patients transferred from LTAC facilities revealed that these isolates are closely related, suggesting an ongoing outbreak with community spread in the Los Angeles area.

The isolates described here were all resistant to fluconazole and amphotericin B but susceptible to echinocandins according to the CDC tentative breakpoints (https://www.cdc.gov/fungal/candida-auris/c-aurisantifungal.html). In addition, all isolates had an F126L mutation in the *erg11* gene, which is unique to clade III strains and associated with fluconazole resistance (10).

Patient D was admitted to an SNF after complications from pneumonia caused by coronavirus disease (COVID-19). Few cases of C. auris and COV-ID-19 co-infection have been reported (14,15). After COVID-19 infection, patient D had multiple complications requiring a tracheostomy and enteral feeding tube; the patient was subsequently transferred to an LTAC for rehabilitation. A substantial portion of adult patients who recover from severe COVID-19 have long-term sequelae and might require admission to SNFs or LTACs. Therefore, the COVID-19 pandemic might lead to increased transmission of C. auris in SNFs because of increased admissions and shortages of personal protective equipment. During critical shortages, CDC guidelines permit extended use of isolation gowns for patients who are known to be infected with the same infectious disease if there are no additional known coinfections transmitted through contact (https://www.cdc. gov/coronavirus/2019-ncov/hcp/non-us-settings/

emergency-considerations-ppe.html#ppe-specificstrategies). To encourage appropriate use of personal protective equipment and prevent transmission, it is essential that facilities screen patients for *C. auris*.

One limitation of this study is the lack of additional epidemiologic history of the patients, especially in the context of travel-related exposures. The ability to track cases to a location with known outbreaks of clade III *C. auris* strains is essential to determining the origin of the current outbreak. Further investigation is needed to explain why patient F had a genetically distinct isolate, suggesting a separate introduction.

In conclusion, we identified a unique clade III *C. auris* strain in an ongoing outbreak in LTAC facilities since 2019. These findings indicate active community spread of multidrug-resistant *C. auris* in the Los Angeles area.

About the Author

Dr. Price is a clinical microbiology fellow at the David Geffen School of Medicine at University of California, Los Angeles in Los Angeles, California, USA. His research interests include microbial genomics, fungal species identification, and antimicrobial resistance mechanisms.

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Genomic Characterizations of Clade III Lineage of *Candida auris*, California, USA

Appendix 1

Appendix Methods

Patient Screening Criteria

Patients were screened for *Candida auris* based on Los Angeles County Department of Public Health and U.S. Centers for Disease Control and Prevention guidance for patients at high risk of colonization. Screening recommendations were based on prevalence of *C. auris*; however, at the time of publication patients were screened if: they were a resident of a long-term acute-care or skilled nursing facility that had an ongoing *C. auris* outbreak; if they had an overnight stay in a hospital outside of the United States or in New York, New Jersey, or Illinois (i.e., states where *C. auris* outbreaks have been reported) in the past 12 months; if they had a history of carbapenem-resistant *Enterobacteraciae* infection; or if they had a known history of *C. auris* or had been in contact with a person with *C. auris*. Patients with a positive *C. auris* test were reported back to the facility and to public health.

Candida auris PCR

Inguinal/axillary surveillance swab samples or pure isolates of suspected *C. auris* growth were extracted using the EZ1 Tissue Extraction Kit (QIAGEN, https://www.qiagen.com). Specimens were pelleted and resuspended in 500µL G2 lysis buffer in a tube with 0.5 mm glass beads (Bertin Technologies, https://bertin-technologies.com); specimens were then heated at 95°C for 10 min and placed in a Precellys 24 homogenizer (Bertin Techologies) using the following settings: 6000 rotations per min, 60 s for 3 cycles with 5 s rest. After bead beating, specimens were centrifuged at 5000 rotations per min for 1 minute and 200µL of supernatant was transferred to the EZ1 DNA Tissue Kit Sample Tube. 5µL of the Simplexa Extraction and Amplification Control DNA (DiaSorin Molecular LLC, https://molecular.diasorin.com) was then

added to each sample and extracted using the EZ1 Advanced XL (QIAGEN) using the following settings: 200µL loading volume and 100µL elution volume.

The PCR reaction was conducted using the DiaSorin Molecular LIAISON MDX (previously known as the 3M Integrated Cycler). 5μ L of eluted DNA was added to 5μ L reaction mix consisting of the following: 2.5X TA Master Mix, SEAC DNA Primer Pair, *C. auris* Primer Pair (DiaSorin Molecular LLC). The PCR was selective for the ITS2 region of *C. auris*. The cyclin parameters were kept consistent with the manufacturer's instructions. A cycle threshold cutoff of 40 was used. An internal laboratory validation showed 100% sensitivity and specificity with a limit of detection of 10^2 CFU/mL (manuscript under review).

Antifungal Susceptibility Testing

Antifungal susceptibility testing by broth microdilution was conducted on panels prepared in-house according to Clinical and Laboratory Standards Institute standards (https://standards.globalspec.com/std/10266416/CLSI%20M27). The U.S. Centers for Disease Control and Prevention tentative MIC breakpoints were used for interpretation of the following drugs: amphotericin B ($\geq 2\mu g/mL$), fluconazole ($\geq 32\mu g/mL$), anidulafungin ($\geq 4\mu g/mL$), caspofungin ($\geq 2\mu g/mL$), and micafungin ($\geq 4\mu g/mL$). Voriconazole, itraconazole, and posaconazole were also tested but no interpretative criteria for *C. auris* are available.

Whole Genome Sequencing

Specimens from patients with positive *C. auris* surveillance PCR results were subcultured on inhibitory mold agar and CHROMagar Candida (Becton, Dickinson and Company, https://www.bd.com); yeast isolates were identified using the VITEK MS matrix-assisted laser desorption/ionization time-of-flight system (bioMérieux Inc, https://www.biomerieux-usa.com). Pure cultures of *C. auris* isolates were stored at -80° C.

The Qiagen EZ1 Blood and Tissue Kit and the EZ1 Advanced XL instrument were used to extract genomic DNA from pure isolates of *C. auris*. Extracted DNA was quantified with the Qubit 1X dsDNA HS assay using the Qubit 3.0 Fluorometer (ThermoFisher Scientific, https://www.thermofisher.com). Acceptable quantities of DNA were ≥ 0.04 ng/µL.

DNA was diluted in water to obtain concentrations within the range of 100–500 ng in 30µL. Library preparation was performed using the Nextera DNA Flex Library Prep Kit (Illumina, https://www.illumina.com) according to manufacturer's instructions. Transposome

mediated fragmentation DNA was amplified using a limited-cycle PCR set according to the input DNA concentrations. A library cleanup step was performed using a 2-step bead purification procedure. Libraries were quantified, as described above, using Qubit; acceptable concentrations were $\geq 0.1 \text{ ng/}\mu\text{L}$. Following quantification, the Agilent DNA 1000 Kit and Agilent 2100 Bioanalyzer instrument (Agilent, https://www.agilent.com) were used to analyze the average band size of the libraries with an acceptable range of 300–900 bp.

Acceptable libraries were normalized to 2nM or 4nM concentrations for the Illumina MiSeq version 2 or version 3 reagent kit, respectively. Five microliters of each sample were pooled, denatured, and diluted; 600µL were loaded into the reagent cartridge. The Illumina MiSeq System was used to produce 250 bp paired-end reads. Data were uploaded to the Illumina BaseSpace cloud and de-multiplexed.

Six *C. auris* isolates from five patients were sequenced; sequences were uploaded to GenBank under the BioProject: PRJNA672695. UCLA_A1 was isolated from an inguinal/axillary swab of patient A on October 8, 2019 (GenBank accession no. SRR12916694). UCLA_A2 was isolated from a tracheal suction specimen of patient A on October 8, 2019 (GenBank accession no. SRR12916693). UCLA_C1 was isolated from an inguinal/axillary swab of patient C on August 12, 2020 (GenBank accession no. SRR12916692). UCLA_D1 was isolated from an inguinal/axillary swab of patient D on August 19, 2020 (GenBank accession no. SRR12916691). UCLA_E1 was isolated from an inguinal/axillary swab of patient E on August 31, 2020 (GenBank accession no. SRR12916690). UCLA_F1 was isolated from a right pleural fluid specimen of patient F on September 3, 2020 (GenBank accession no. SRR12916689).

Bioinformatics and Phylogenetic Analyses

CLC Genomics Workbench version 12.0.3 was used to pair, trim, and map the sequence reads. Reads were mapped to a reference *C. auris* complete genome (ATCC MYA-5022, GenBank accession no. CP043531); this strain is part of Clade III. The number of sequence reads ranged from 1,056,808–6,212,112. The percent of reads mapped to the reference genome ranged from 93.82%–96.33%. The percent of the reference genome with at least $10 \times$ coverage of mapped reads ranged from 91.90%–97.74%.

K-mer analyses were done using the Create K-mer Tree tool in CLC Genomics Workbench. K-mer's of 16 nt lengths were used; Jensen-Shannon Divergence was used to construct the phylogenetic trees. The K-mer trees are displayed as circular phylograms. Single nucleotide polymorphism (SNP) analyses were done using the Basic Variant Detection tool in CLC Genomics Workbench. This tool was used to create a variant track following read mapping to the reference genome. The variant tracks for each isolate were then compared using the Create SNP Tree tool. SNP trees were created using maximum-likelihood algorithms.

Sequence reads were also mapped to the *erg11* and *fks1* genes of the reference genome. For *erg11*, the percent of the reference genome with $\geq 10 \times$ coverage of mapped reads ranged from 84.83%–100%. For *fks1*, the percent of the reference genome with $\geq 10 \times$ coverage of mapped reads ranged from 86.71%–100%. Using the Extract Consensus Sequence tool, the consensus sequences for both genes were determined. Consensus sequences were then exported from CLC Genomics Workbench and imported into Geneious Prime (Geneious, https://www.geneious.com) version 2020.0.3 for further analysis. The consensus sequence and the translated amino acid sequence of both genes for each isolate were compared to the reference sequence using the Geneious Alignment tool.

Additional Genomes Used

An additional 251 previously analyzed genomes of *C. auris* were used in the k-mer and SNP analyses (Appendix 2 Table, https://wwwnc.cdc.gov/EID/article/27/5/20-4361-App2.xlsx). Collection dates range from 2009–2020 from a variety of specimen sources and geographic locations (*1–8*).

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	UČLA_	UCLA_	UCLA_	UCLÁ_	UCLA_	UCLA_	,	,			Clade
Isolate†	A1	A2	C1	D1	E1	F1	Clade III	Clade III	Clade I	Clade II	IV
UCLA_A1	-	3	12	10	7	77	47	66	24,374	35,804	95,891
UCLA_A2	3	-	11	9	6	78	46	65	24,373	35,803	95,890
UCLA_C1	12	11	-	4	7	77	51	74	24,382	35,808	95,893
UCLA_D1	10	9	4	-	5	79	53	72	24,380	35,810	95,894
UCLA_E1	7	6	7	5	-	76	50	69	24,377	35,807	95,894
UCLA_F1	77	78	77	79	76	-	117	139	24,404	35,797	95,918
Clade III	47	46	51	53	50	117	-	61	24,370	35,792	95,884
Clade III	66	65	74	72	69	139	61	-	24,385	35,814	95,906
Clade I	24,374	24,373	24,382	24,380	24,377	24,404	24,370	24,385	-	36,606	95,700
Clade II	35,804	35,803	35,808	35,810	35,807	35,797	35,792	35,814	36,606	-	97,644
Clade IV	95,891	95,890	95,893	95,894	95,894	95,918	95,884	95,906	95,700	97,644	-

Appendix Table 1. Single nucleotide polymorphism analysis of Candida auris isolates, United States, 2013–2020*

*Reads were mapped to the *C. auris* reference genome (GenBank accession no. CP043531); consensus sequences were extracted from the alignments. GenBank accession no. SRR12073484 was isolated from Florida; GenBank accession no. SRR7909359 was isolated from Indiana. †Clade III represented by GenBank accession no. SRR12073484 and SRR7909359; clade I represented by GenBank accession no. SRR7909162; clade II represented by GenBank accession no. SRR7909356; clade IV represented by GenBank accession no. SRR7909221.

		erg11	tK\$1		
C. auris	Nucleotide pairwise		Nucleotide pairwise		
Isolate	identity, %	Protein sequence variants	identity, %	Protein sequence variants	
UCLA_A1	99.6	p.V125A	99.9	p.I1572L	
		p.F126L			
UCLA_A2	99.6	p.V125A	99.9	p.I1572L	
		p.F126L			
UCLA_C1	99.6	p.V125A	99.9	p.I1572L	
		p.F126L			
UCLA_D1	99.6	p.V125A	99.9	p.I1572L	
		p.F126L			
UCLA_E1	99.6	p.V125A	99.9	p.I1572L	
		p.F126L			
UCLA_F1	99.6	p.V125A	99.8	p.I1095L	
		p.F126l		p.115721	

p.F126L p.I1572L *Nucleotide pairwise identity to *C. auris* strain ATCC MYA-5002 (GenBank accession no. CP043531). Protein translation and variant analysis conducted using Geneious Prime (Geneious, https://www.geneious.com).



Appendix Figure. Global phylogenetic analysis of *Candida auris* clade III isolates. Analysis based on kmers of 261 publicly available *C. auris* sequences (Appendix 2 Table 1, https://wwwnc.cdc.gov/EID/article/27/5/20-4361-App2.xlsx). Colors indicate country of origin. Scale indicates branch lengths within the tree.