# Candida auris Clinical Isolates Associated with Outbreak in Neonatal Unit of Tertiary Academic Hospital, South Africa

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Candida auris was first detected at a university-affiliated hospital in Johannesburg, South Africa, in 2009. We used whole-genome sequencing to describe the molecular epidemiology of C. auris in the same hospital during 2016–2020; the neonatal unit had a persistent outbreak beginning in June 2019. Of 287 cases with culture-confirmed C. auris infection identified through laboratory surveillance, 207 (72%) had viable isolates and 188 (66%) were processed for whole-genome sequencing. Clade III (118/188, 63%) and IV (70/188, 37%) isolates co-circulated in the hospital. All 181/188 isolates that had a fluconazole MIC ≥32 µg/mL had ERG11 mutations; clade III isolates had VF125AL substitutions, and clade IV isolates had K177R/N335S/E343D substitutions. Dominated by clade III, the neonatal unit outbreak accounted for 32% (91/287) of all cases during the study period. The outbreak may have originated through transmission from infected or colonized patients, colonized healthcare workers, or contaminated equipment/environment.

Candida auris is a multidrug-resistant yeast that causes invasive infections with high associated mortality in acute and long-term healthcare facilities worldwide (1,2). Although *C. auris* was initially described in 2009 in Japan, the earliest cases of infection in South Africa were reported in 2014 (3). Those cases prompted a retrospective review of

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Neonatal outbreaks caused by *C. auris* are occasionally documented, although early outbreaks in South Africa occurred mainly among critically ill adults (7). In more recent years, *C. auris* has caused outbreaks involving neonates and has subsequently become endemic in many hospitals in South Africa (7–10). *C. parapsilosis* has been a major pathogen causing late-onset sepsis among hospitalized neonates in low- and middle-income countries because of its ability to colonize the skin of hospitalized patients and healthcare workers, persist on hospital surfaces, and adhere to indwelling medical devices, characteristics that are also applicable to *C. auris* (11,12).

*C. auris* is classified into several genetically distinct clades. Clade I originated in South Asia, clade II in East Asia, clade III in Africa, clade IV in South America, and clade V in the Middle East (Iran) (13). All clades exhibit minimal intraclade genetic diversity but are separated from each other by tens to hundreds of thousands of single nucleotide polymorphisms (13,14). A possible sixth clade was reported from Singapore and Bangladesh in August 2023 (C. Suphavilai et al., unpub. data, https://www.medrxiv. org/content/10.1101/2023.08.01.23293435v1). Most clade isolates from Africa are resistant to fluconazole; some are resistant to other antifungal classes (9,15).

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<sup>1</sup>Members of GERMS-SA are listed at the end of this article.

Globally, echinocandin-resistant isolates are not as prevalent as azole- and polyene-resistant isolates, which is partly why echinocandins are recommended as first-line treatment (5).

We aimed to describe the molecular epidemiology of *C. auris* at a large tertiary academic hospital in Johannesburg, South Africa by using whole-genome sequencing (WGS), focusing on a persistent outbreak in the hospital's neonatal unit. For GERMS-SA, an ongoing surveillance program, we sought and obtained annual ethics approvals from several university ethics committees in South Africa. A detailed description of GERMS-SA surveillance methods has been published previously (7).

#### **Materials and Methods**

#### **Study Setting and Definitions**

We conducted the cross-sectional study at the 3,200bed Chris Hani Baragwanath Academic Hospital (CHBAH) in Soweto, Johannesburg, South Africa. Affiliated with the University of the Witwatersrand, CHBAH provides a wide range of subspecialist inpatient and outpatient surgical, medical, pediatric and obstetrics/gynecology services. The hospital serves 1.5 million persons around Soweto and surrounding areas as a tertiary referral center. The hospital's neonatal unit caters to neonates requiring admission from  $\approx$ 20,000 annual births within the hospital; 3,000 births from local district hospitals and 10,000 births from the local clinics annually; and those requiring surgical services from the southern Gauteng and North West Provinces (*16*). All *C. auris* isolates in this study were received from the onsite microbiology laboratory at CHBAH.

We included C. auris isolates from all patients admitted to the neonatal unit from June 12, 2019, through May 30, 2020 (overlapping with the neonatal unit outbreak, which began with an index case diagnosed on June 12, 2019), as well as isolates from patients admitted across the hospital from March 1, 2016, through July 31, 2020 (Figure 1). Specimens that were processed for routine microbiology culture and yielded C. auris were from blood, central venous catheter (CVC) tips, urine, and wound and burn swabs. We also included *C. auris* isolates that were cultured from swabs collected by targeted screening of highrisk contact patients in the neonatal unit (e.g., adjacent incubator, same cubicle, or both). For all case-patients with multiple isolates within a 30-day period, we used the first isolate for analysis. We obtained demographic and clinical data such as age, sex, ward location, and site of Candida infection from the GERMS-SA surveillance database. We defined neonates as <28 days of age, infants as 28 days to <12 months of age, children as 1-12 years of age, adolescents as 13-17 years of age, and adults as  $\geq$ 18 years of age.

## Culture, Identification, and Antifungal Susceptibility Testing

All isolates from invasive and noninvasive cases were first processed at the CHBAH onsite laboratory. If



Figure 1. Epidemic curve by ward location for 287 laboratory-confirmed cases of *Candida auris* infection or colonization at an academic tertiary hospital, South Africa, March 2016–July 2020. ICU, intensive care unit.

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yeasts were observed on microscopic direct examination or if fungal culture was specifically requested, we inoculated specimens onto Sabouraud agar (Diagnostic Media Products [DMP], https://www.nhls. ac.za). We used Vitek-2 (bioMérieux, https://www. biomerieux.com) and Microscan Walkaway (Beckman Coulter, www.beckmancoulter.com) for initial identification. We shipped cultures to the National Institute for Communicable Diseases (NICD) on Dorset transport medium or agar plates (DMP); we confirmed identification and performed antifungal susceptibility testing before storage (16,17). For this study, we retrieved C. auris isolates from -70°C freezer storage at the NICD and subcultured on Sabouraud agar to confirm viability, then cultured them onto chromogenic agar (DMP) to confirm purity (CHROMagar Candida powder was sourced from Mast Diagnostics, https://www.mastgroup.com). We conducted matrix-assisted laser desorption/ionization-time of flight mass spectrometry (Bruker Daltonics, https://www.bruker.com) to identify all C. auris isolates again before further testing. We performed antifungal susceptibility testing using commercial gradient diffusion (Etest, bioMérieux) or broth microdilution assays (Sensititre; Thermo Fisher, https://www.thermofisher.com). We used the Etest method to generate amphotericin B MICs because the gradient diffusion strip provides a wider range of MIC values than are generated by broth-microdilution testing; the wider range may help discriminate between susceptible and resistant isolates (17). We interpreted MICs using tentative breakpoints proposed by the US Centers for Disease Control and Prevention (1). All experiments included the quality control strains C. parapsilosis (ATCC 22019) and C. krusei (ATCC 6258), following M27-A3 (18) and M60 recommendations of the Clinical and Laboratory Standards Institute (19).

#### Whole-Genome Sequencing

We extracted DNA using the Zymo Research Quick-DNA Fungal/Bacterial miniprep kit (Zymo Research Corporation, https://www.zymoresearch.com) according to the manufacturer's instructions, with some exceptions. For example, we extracted DNA from yeast colonies directly instead of from a resuspension of yeast cells in water; we used 40  $\mu$ L of elution buffer instead of the 100  $\mu$ L recommended volume. We sent genomic DNA to the NICD Sequencing Core Facility for WGS. We performed sequencing of the 12.6 Mbsized genome on 188 samples. We prepared genomic libraries using Nextera DNA Library Preparation (IIlumina, https://www.illumina.com), followed by 2 × 300 bp sequencing on an Illumina NextSeq 500 platform to produce paired end reads.

## Single-Nucleotide Polymorphism Calling and Phylogenetic Analysis

We assessed the quality of read data using FastQC version 0.11.9 (https://www.bioinformatics.babraham. ac.uk) and performed read filtering and trimming using PRINSEQ version 0.20.4 (http://prinseq. sourceforge.net) (20). We mapped the read data to a reference C. auris genome (clade III, B11221; National Center for Biotechnology Information [NCBI] BioProject accession no. PRJNA535510) using the Burrows-Wheeler data transformation algorithm version 0.7.17 (21). We performed variant calling (i.e., singlenucleotide polymorphism [SNP] analysis) using SAMtools version 1.11 (21) through the Northern Arizona SNP Pipeline (NASP) (http://tgennorth. github.io/NASP/install.html) (22). Filtering parameters involved removing positions that had <10× coverage, <90% variant allele calls and those within duplicated regions in the reference (14). We estimated a maximum parsimony phylogeny tree using MEGA software version 10.0.5 (https://www. megasoftware.net) and bootstrap analysis based on 1,000 replicates using the bestSNP alignment produced by the NASP pipeline (23). We included external sequences representing each clade (I-V) in the analysis to confirm the clade assignments of the isolates: clade I, PEKT02 (B8441, NCBI BioSample no. SAMN05379624) and SRR3883445 (B11214, NCBI BioSample no. SAMN05379602); clade II, PYFR01 (B11220, NCBI BioSample no. SAMN05379608); clade IV, PYGM01 (B11243, NCBI BioSample no. SAMN05379619); clade V, SRR9007776 (NCBI BioSample no. SAMN11570381). Also included in the analysis was a genome of the first South Africa isolate to be reported and sequenced from CHBAH in 2009 (9).

#### Phylodynamic Analysis of Clade III Neonatal Isolates

We created a root-to-tip regression plot using TempEst version 1.5.3 (24) to quantify the mutation rate and to assess the temporal signal of the outbreak strains. We inferred Bayesian phylogenies using BEAST version 1.8.4 (25) by applying a general time-reversible nucleotide substitution model under a strict molecular clock with the mutational rate obtained from the root-to-tip regression. We used the SNP alignment of the clade III isolates for both TempEst and BEAST. We chose a general time-reversible model as a nonspecific model for phylogenetic inference. The model assumes different rates of substitution for each nucleotide and different frequencies of nucleotide occurrence (26). Furthermore, we applied a coalescent exponential population prior distribution and used specimen collection dates (day, month, year) as tip dates. We set the length of the Markov Chain Monte Carlo (MCMC) at 50,000,000 steps and logged parameters every 1,000 steps. We used Tracer version 1.7.1 (https://beast.community/tracer) to visualize and analyze the posterior MCMC samples. The effective sample size was >700, indicating that the MCMC runs had converged. We generated a maximum clade credibility tree with TreeAnnotator version 1.8.4 (https:// beast.community/treeannotator) after discarding the first 10% as burn-in and visualized the tree using FigTree version 1.4.4 (http://tree.bio.ed.ac.uk/ software/figtree). We also performed an exploratory analysis to reconstruct potential transmission routes in the hospital (Appendix, https://wwwnc.cdc.gov/ EID/article/29/10/23-0181-App1.pdf).

#### **Resistance Mutation Identification**

We used CLC Genomics Workbench version 10 (QIA-GEN, https://www.qiagen.com) to assess and identify mutations in resistant *C. auris* isolates. We extracted 2 genes from each resistant *C. auris* genome and assessed for any point mutations (i.e., substitutions, duplications): *ERG11*, which is transcribed into an azole target; and *FKS1*, an echinocandin target gene (27). We used a wild-type strain with no known mutations as a reference in the analysis to determine the presence of mutations (*C. auris* B8441, NCBI accession no. PEKT00000000). Susceptible isolates were also assessed for mutations in the same genes.

#### Results

#### **Patient Characteristics**

During the surveillance period, 287 culture-confirmed cases (invasive and noninvasive) were reported from the hospital from different wards; of those, 207 (72%) had viable isolates available for analysis. Most of the cases were from the neonatal unit (91/287, 32%), pediatric burn unit (66/287, 23%), and adult burn unit (57/287, 20%). The median age was 1.4 years (interquartile range [IQR] 22 days to 21 years; range 0 days to 85 years). Adults accounted for the highest proportion of cases (74/287, 26%) followed by neonates (62/287, 22%). More patients were male (54%, 155/287) than female (42%, 121/287; sex was unknown for 4% (11/287). Most isolates were from blood cultures (161/287, 56%), followed by skin swabs (33/287, 12%) (Table 1).

#### Antifungal Susceptibility of Isolates

Most (199/207; 96%) of the isolates were resistant to fluconazole (MIC  $\geq$  32 µg/mL); 13 (6%) were considered resistant to amphotericin B (MIC  $\geq$  2 µg/

**Table 1.** Characteristics of 287 patients with *Candida auris*infection or colonization admitted to a large academic hospital inSouth Africa, 2016–2020

30util Allica, 2010–2020	
Characteristic	No. (%) isolates
Sex	
M	155 (54)
F	121 (42.2)
Unknown	11 (3.8)
Age group	
Neonates, <u>&lt;</u> 28 d	62 (21.6)
Infants, 28 d–12 mo	56 (19.5)
Children, 1–12 y	54 (18.8)
Adolescents, 13–17 y	11 (3.8)
Adults, >18 y	74 (25.8)
Unknown	30 (10.5)
Specimen type	
Blood	161 (56.1)
Skin swab	33 (11.5)
Arterial catheter tip	28 (9.8)
Central venous catheter tip	24 (8.4)
Urine	11 (3.8)
Tracheal aspirate	8 (2.8)
Tissue, not specified	6 (2.1)
Fluid aspirate, not specified	3`(1)
Skin scraping	1 (0.3)
Burn/wound swab	1 (0.3)
Unknown	11 (3.5)
Ward location	
Neonatal unit	91 (31.7)
Pediatric burn unit	66 (23)
Adult burn unit	57 (19.9)
General adult/pediatric intensive care unit	30 (10.5)
Adult surgical	12 (4.2)
Adult medical	11 (3.8)
Pediatric medical	9 (3.1)
Pediatric surgical	7 (2.4)
Unknown	4 (1.4)́

mL). Multidrug-resistant isolates were also present; 2 (0.9%) isolates were resistant to both caspofungin and fluconazole and 3 (1.4%) were resistant to both fluconazole and amphotericin B. No isolates in this study were resistant to  $\geq$ 3 antifungal classes (panresistant) (Table 2).

#### **Phylogenetic and Resistant Mutation Analysis**

Of 207 viable C. auris isolates, 200 were available for WGS. We excluded 12 sequences of poor quality from the analysis; WGS data of 188 isolates were available for bioinformatics analysis. Overall, 118 (63%) clustered with the Africa clade III reference and the remaining 70 (37%) with the South America clade IV reference (Figure 2). Isolates in this study did not cluster in any other clade. A total of 181 (96%) isolates had a fluconazole MIC value  $\geq 32 \ \mu g/mL$ , and 186 (99%) had known resistance mutations in the ERG11 gene; thus, 5 sequences from phenotypically susceptible isolates also housed resistance mutations (Table 3). Isolates for clade III had the VF125AL substitution that is specific to the clade. Isolates from clade IV had the substitutions K177R/N335S/E343D, which had been documented in this clade previously (28). Some

							110.150	ates w		⊃, μg/	IIIL								
	Breakpoint,	0.0	0.01	0.0														-	%
Drug	µg/mL	08	5	3	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	Unk†	Resistant
AFG	≥4		1		28	129	42	7											0
MFG	≥4			2	96	94	12	1	2										0
5FC	≥128	3			67	125	10	2											0
POS	NA	1	1	15	73	80	31	4	2										NA
VRC	NA				2	8	21	58	103	13	2								NA
ITC	NA			2	10	84	84	25	2										NA
FLC	≥32								1				7	13	34	73	79		96
AMB	≥2						1	9	135	61								1	42
AMB	≥2		1	1	2	9	22	76	79	11	2							4	6.2
Ftest†																			

 Table 2. MIC distribution of Candida auris isolates (n = 207) from patients admitted to a tertiary academic hospital, South Africa, 2016–2020\*

 No. isolates with MIC. ug/mL

\*AMB, amphotericin B; AFG, anidulafungin; FLC, fluconazole; ITC, itraconazole; MFG, micafungin; NA, not available; POS, posaconazole; unk, unknown; VRC, voriconazole; 5FC, flucytosine.

†Isolates missing data or not tested

#MICs were determined using both broth microdilution and E-test method for amphotericin B.

isolates with the clade IV substitutions (27/70, 38%) had an additional E102K substitution.

#### Discussion

The neonatal unit, the location of the outbreak starting in June 2019, was dominated by clade III isolates, whereas clade IV strains dominated the adult and pediatric burn units. We observed clade mixing in both burn units (Figure 3). Isolates from the neonatal unit constituted 37% (68/188) of the isolates in our phylogenetic tree.

#### Phylodynamic Analysis

We included only isolates from clade III, which was the clade responsible for the neonatal unit outbreak, in the following analysis. We created a root-to-tip regression plot to determine whether there was a positive correlation between the time the isolates were sampled and the number of substitutions along the tree topology. Linear regression estimates of the evolutionary rate equated to  $1.3471 \times 10^5$  nt substitutions/site/year (correlation coefficient = 0.5). The rate was similar to that of an outbreak in Kenya caused by clade III isolates in which the calculated mutation of the outbreak strains was 1.8695 e-5 substitutions/site/year (29). The rate supported a strong temporal signal and was sufficient for further Bayesian molecular dating. The root-to-tip regression plot predicted the time to the most recent common ancestor (tMRCA) in 2018 based on the x intercept (Figure 4). The tMRCA for clade III in this hospital dated back to early 2014 (95% highest posterior density 2013 to mid-2015), whereas the tMRCA for the neonatal unit outbreak dated to 2018 (95% highest posterior density, mid-2017 to mid-2018), corresponding to the estimation from the root-to-tip regression analysis (Figure 5, https:// wwwnc.cdc.gov/EID/article/29/10/23-0181-F5.htm). The exploratory analysis of the outbreak reconstruction of clade III revealed that infections were introduced into the neonatal unit from the adult burn unit, adult medical department, and an unknown ward (Appendix).

Fluconazole-resistant clade III *C. auris* isolates caused a large persistent outbreak in the neonatal unit of a tertiary academic South Africa hospital beginning in June 2019 and continuing into 2023. WGS analysis of all viable surveillance isolates from this hospital during 2016–2020 revealed that clade IV *C. auris* isolates dominated in the adult and pediatric burn units with clade III and IV co-circulation. Phylodynamic analysis of the clade III neonatal outbreak isolates did not point to a specific source.

By 2016, C. auris was reported in >100 South Africa acute-care hospitals and had caused large outbreaks in some of these hospitals. C. auris had replaced other pathogens to become the third most common cause of candidemia in South Africa (7). Over our entire study period (i.e., March 2016-July 2020), the neonatal unit outbreak contributed to one third of all the cases at the hospital. Smaller C. auris outbreaks involving neonates have been reported previously in other hospitals in South Africa (7,30). Neonatal units in national central hospitals, such as CHBAH, have become fungal outbreak epicenters, driven by the innate vulnerability of very high-risk patients admitted to these units, occupancy routinely exceeding the approved bed capacity of the unit, and limited resources available for infection prevention and control or antimicrobial stewardship (31). Although the exact manner of C. auris introduction for this outbreak remains undetermined, temporal analysis of the outbreak strains placed tMRCA in early 2018, suggesting a relatively recent introduction (10,32). Continued surveillance until June 2022 has confirmed persistence of C. auris in the unit with sporadic cases of invasive infections.

Most isolates in our study belonged to Africa clade III (118/188, 63%) or the South America clade IV (70/188, 37%). The overall molecular epidemiology of *C. auris* in South Africa was determined in a



**Figure 2.** Phylogenetic tree depicting clade distribution and fluconazole resistance mutations of 188 invasive or colonizing South African *Candida auris* strains isolated from patients admitted to a large metropolitan hospital in South Africa, 2016–2020. The unrooted maximum-parsimony tree was created using MEGA software (https://www.megasoftware.net) using 287,338 single-nucleotide polymorphisms based on 1,000 bootstrap replicates. Scale bar indicates number of pairwise differences. FCZ, fluconazole.

WGS study which reported that 85% of isolates from 2009–2018 belonged to clade III, 12% belonged to clade I, and 3% to clade IV (9). An earlier global molecular epidemiology study reported only clade III isolates (n = 51) from South Africa (*33*). However, clade IV contributed to a proportionally larger case load in this hospital. Because the first reported isolate in South Africa in 2009 belonged to clade IV and was from CHBAH (9), we hypothesize that a clonal expansion of clade IV

occurred in this hospital and those strains continued to circulate during our study period. Circulation of multiple *C. auris* clades in South Africa may be explained by global travel and trade (34–36). Furthermore, phylogeographic mixing of *C. auris* clades has been observed more often in recent years. Countries including Canada (clades I, II, and III), Kenya (clades I and III) and the United States (clades I, II, and IV) also reported cases caused by multiple *C. auris* clades (10).

 Table 3. Clade proportions and frequency of antifungal drug resistance within the study population among 188 Candida auris isolates,

 South Africa

	No. (%) isolates										
Clade	All	Fluconazole resistant	Point mutation in ERG11	Point mutation in FKS1	Amphotericin B resistant						
111	118 (62.5)	117 (99.1)	118 (100)	0	2 (2.8)						
IV	70 (37.4)	66 (91.4)	70 (100)	0	1 (1.42)						

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Most isolates in our study were resistant to fluconazole. Fluconazole-susceptible isolates of *C. auris* are rare worldwide, especially within Africa clade III; this resistance limits the treatment options (9). Fluconazole-resistant *C. auris*, as a cause of healthcare-associated infections, adds costs to the health system since fluconazole is the least expensive and most accessible systemic antifungal agent (8,37). Amphotericin B resistance has been reported among *C. auris* isolates but remains rare while echinocandin resistance has also been reported, especially in clade I isolates (37). Echinocandins are recommended as first-line treatment



**Figure 4.** Root-to-tip regression analysis of 67 South African *Candida auris* outbreak isolates from the neonatal ward of a large metropolitan hospital in South Africa. Genetic distance is plotted against sampling time. Every data point represents a tip on the phylogeny. Black line indicates correlation coefficient for the regression. Green lines represent the evolutionary rate in substitutions/site/day.

for candidemia in adults, although amphotericin B deoxycholate, with better central nervous system penetration, is recommended for neonates who may develop *Candida* meningoencephalitis ( $\delta$ ). We did not observe isolates resistant to all classes of antifungals in this study; however, 2 panresistant isolates were reported in South Africa in a previous study (16).

Most of the fluconazole-resistant isolates in our study had clade-specific mutations in the ERG11 gene that are known to contribute to resistance. The clade III ERG11 mutation (VF125AL) seems to be universal across resistant isolates within the clade and has been seen in other clade III isolates from previous studies, even those isolated outside Africa (33); T.K. Price et al., unpub. data, https://doi.org/10.1101/2020.10.26 .20214908). The ERG11 gene-mediated mechanism of resistance to fluconazole for clade IV isolates varies, and isolates from different geographic areas have different ERG11 mutations (28). Isolates in our study had the same substitutions as isolates originating from Colombia, which contained all 3 Erg11p substitutions (K177R, N335S, E343D), whereas azole resistance in clade I India/Pakistan isolates and some clade IV Colombia isolates was caused by Y132F or K143R substitutions (28). Of interest, the K177R, N335S, and E343D clade IV substitutions have not been shown to contribute to increased azole resistance even in Colombian isolates. Instead, other Colombian isolates had the substitutions I466M or Y501H that have been shown to contribute to resistance, which we did not see in the isolates in our study (28). Some sequences with the described clade IV substitutions had an additional E102K substitution in our study; this substitution has not been documented or proven to contribute to decreased susceptibility in *C. auris* strains. There were cases in our study where susceptible isolates possessed resistant mutations especially in clade IV. It appears that clade IV isolates might have a more complex mechanism of resistance.

A limitation of our study was that we only included cultured isolates from laboratory-based surveillance from patients with routinely diagnosed invasive infections and a few with colonization. Thus, we sampled only a subset of all infections that would have occurred at this hospital. An extensive collection of isolates using systematic sampling in a prospective study, including invasive, colonizing, and environmental strains, would be needed to determine the full extent of transmission routes with a higher resolution and to avoid missing links. In addition, detailed clinical information and travel and hospital transfer data were not available for all cases; this information would have enabled us to potentially determine how the pathogen was introduced into the hospital. Another limitation in our study is that we did not account for recombination analysis, which might have introduced bias in the phylogenetic analysis.

In summary, we characterized *C. auris* isolates circulating in a major metropolitan public hospital in South Africa. Our data showed that clades III and IV co-circulated, and clade III was responsible for a large outbreak in the neonatal unit. Most isolates were resistant to fluconazole and carried previously published clade-specific *ERG11* mutations. We speculate that the neonatal unit outbreak may have originated from cross-unit transmission by infected or colonized patients, colonized healthcare workers, or contaminated equipment. Patient environments may have also served as reservoirs of infection.

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#### References

- Spivak ES, Hanson KE. Candida auris: an emerging fungal pathogen. J Clin Microbiol. 2018;56: e01588-17. https://doi.org/10.1128/JCM.01588-17
- Sardi JCO, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJS. *Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol. 2013;62:10–24. https://doi.org/10.1099/ jmm.0.045054-0
- Satoh K, Makimura K, Hasumi Y, Nishiyama Y, Uchida K, Yamaguchi H. *Candida auris* sp. nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital. Microbiol Immunol. 2009;53:41–4. https://doi.org/10.1111/j.1348-0421.2008.00083.x
- Govender NP, Patel J, Magobo RE, Naicker S, Wadula J, Whitelaw A, et al.; TRAC-South Africa group. Emergence of azole-resistant *Candida parapsilosis* causing bloodstream infection: results from laboratory-based sentinel surveillance in South Africa. J Antimicrob Chemother. 2016;71:1994–2004. https://doi.org/10.1093/jac/dkw091
- Lockhart SR, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, Govender NP, et al. Simultaneous emergence of multidrug-resistant *Candida auris* on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. Clin Infect Dis. 2017;64:134–40. https://doi.org/ 10.1093/cid/ciw691
- World Health Organization. WHO fungal priority pathogens list to guide research, development and public health action. 2022 Oct 25 [cited 2023 Jan 15]. https://www.who.int/ publications/i/item/9789240060241
- van Schalkwyk E, Mpembe RS, Thomas J, Shuping L, Ismail H, Lowman W, et al.; GERMS-SA. Epidemiologic shift in candidemia driven by *Candida auris*, South Africa, 2016–2017. Emerg Infect Dis. 2019;25:1698–707. https://doi.org/10.3201/eid2509.190040
- Govender NP, Avenant T, Brink A, Chibabhai V, Cleghorn J, du Toit B, et al. Federation of Infectious Diseases Societies of Southern Africa guideline: recommendations for the detection, management and prevention of healthcareassociated *Candida auris* colonisation and disease in South Africa. S Afr J Infect Dis. 2019;34:163. https://doi. org/10.4102/sajid.v34i1.163
- Naicker SD, Maphanga TG, Chow NA, Allam M, Kwenda S, Ismail A, et al. Clade distribution of *Candida auris* in South Africa using whole genome sequencing of clinical and environmental isolates. Emerg Microbes Infect. 2021;10:1300– 8. https://doi.org/10.1080/22221751.2021.1944323
- Mashau RC, Meiring ST, Dramowski A, Magobo RE, Quan VC, Perovic O, et al.; Baby GERMS-SA. Cultureconfirmed neonatal bloodstream infections and meningitis in South Africa, 2014-19: a cross-sectional study. Lancet Glob

Health. 2022;10:e1170-8. https://doi.org/10.1016/S2214-109X(22)00246-7

- Shuping L, Mpembe R, Mhlanga M, Naicker SD, Maphanga TG, Tsotetsi E, et al.; for GERMS-SA. Epidemiology of culture-confirmed candidemia among hospitalized children in South Africa, 2012-2017. Pediatr Infect Dis J. 2021; 40:730–7. https://doi.org/10.1097/INF.000000000003151
- Welsh RM, Bentz ML, Shams A, Houston H, Lyons A, Rose LJ, et al. Survival, persistence, and isolation of the emerging multidrug-resistant pathogenic yeast *Candida auris* on a plastic health care surface. J Clin Microbiol. 2017;55:2996–3005. https://doi.org/10.1128/JCM.00921-17
- Muñoz JF, Gade L, Chow NA, Loparev VN, Juieng P, Berkow EL, et al. Genomic insights into multidrug resistance, mating and virulence in *Candida auris* and related emerging species. Nat Commun. 2018;9:5346. https://doi.org/10.1038/ s41467-018-07779-6
- Escandón P, Chow NA, Caceres DH, Gade L, Berkow EL, Armstrong P, et al. Molecular epidemiology of *Candida auris* in Colombia reveals a highly related, countrywide colonization with regional patterns in amphotericin B resistance. Clin Infect Dis. 2019;68:15–21.
- Maphanga TG, Naicker SD, Kwenda S, Muñoz JF, van Schalkwyk E, Wadula J, et al.; for GERMS-SA. *In vitro* antifungal resistance of *Candida auris* isolates from bloodstream infections, South Africa. Antimicrob Agents Chemother. 2021;65:e0051721. https://doi.org/10.1128/ AAC.00517-21
- 16. Chris Hani Baragwanath Academic Hospital [cited 2022 Sep 10]. https://www.chrishanibaragwanathhospital.co.za
- Berkow EL, Lockhart SR, Ostrosky-Zeichner L. Antifungal susceptibility testing: current approaches. Clin Microbiol Rev. 2020;33:e00069-19. https://doi.org/10.1128/ CMR.00069-19
- Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts, 4th edition (M27). Wayne (PA): The Institute; 2017.
- Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antifungal Susceptibility Testing of Yeasts, 2nd edition (M60). Wayne (PA): The Institute; 2020.
- 20. Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets. Bioinformatics. 2011;27:863–4. https://doi.org/10.1093/bioinformatics/btr026
- Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25:1754– 60. https://doi.org/10.1093/bioinformatics/btp324
- Sahl JW, Lemmer D, Travis J, Schupp JM, Gillece JD, Aziz M, et al. NASP: an accurate, rapid method for the identification of SNPs in WGS datasets that supports flexible input and output formats. Microb Genomics. 2016;2:e000074.
- 22. Kumar S, Nei M, Dudley J, Tamura K. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief Bioinform. 2008;9:299–306. https://doi.org/10.1093/bib/bbn017
- 23. Rambaut A, Lam TT, Max Carvalho L, Pybus OG. Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). Virus Evol. 2016;2:vew007. https://doi.org/10.1093/ve/vew007
- 24. Drummond AJ, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol. 2007;7:214. https://doi.org/10.1186/1471-2148-7-214
- Choudhuri S. Phylogenetic analysis. In: Bioinformatics for beginners. San Diego: Academic Press; 2014. p. 209–18.
- 26. Frias-De-Leon MG, Hernandez-Castro R, Vite-Garin T, Arenas R, Bonifaz A, Castanomn-Olivares L, et al.

Antifungal resistance in *Candida*: molecular determinants. Antibiotics. 2020;9:1–16.

- Healey KR, Kordalewska M, Jiménez Ortigosa C, Singh A, Berrío I, Chowdhary A, et al. Limited ERG11 mutations identified in isolates of *Candida auris* directly contribute to reduced azole susceptibility. Antimicrob Agents Chemother. 2018;62:e01427-18. https://doi.org/10.1128/ AAC.01427-18
- Adam RD, Revathi G, Okinda N, Fontaine M, Shah J, Kagotho E, et al. Analysis of *Candida auris* fungemia at a single facility in Kenya. Int J Infect Dis. 2019;85:182–7. https://doi.org/10.1016/j.ijid.2019.06.001
- Moema I, Ismail H, Van Schalkwyk E, Shuping L, Govender NP. Outbreak of culture-confirmed *Candida auris* bloodstream infection in the neonatal unit of a public-sector hospital, South Africa, July through September 2017. 2017 [cited 2023 Aug 21]. https://www. tephinet.org/learning/fead/outbreak-culture-confirmedcandida-auris-bloodstream-infection-neonatal-unit-public
- van Schalkwyk E, Iyaloo S, Naicker SD, Maphanga TG, Mpembe RS, Zulu TG, et al. Large outbreaks of fungal and bacterial bloodstream infections in a neonatal unit, South Africa, 2012–2016. Emerg Infect Dis. 2018;24:1204–12. https://doi.org/10.3201/eid2407.171087
- Michalski C, Kan B, Lavoie PM. Antifungal immunological defenses in newborns. Front Immunol. 2017;8:281. https://doi.org/10.3389/fimmu.2017.00281
- Chow NA, Muñoz JF, Gade L, Berkow EL, Li X, Welsh RM, et al. Tracing the evolutionary history and global expansion of *Candida auris* using population genomic analyses. mBio. 2020;11:e03364–19. https://doi.org/10.1128/mBio.03364-19

**S DISEASES** 

CDC

- Turbett ISE, Becker DSM, Belford MTB, Kelly RTM, Desrosiers MTL, Oliver RNE, et al. Evaluation of *Candida auris* acquisition in US international travellers using a culture-based screening protocol1. J Travel Med. 2022;29:taab186. https://doi.org/10.1093/ jtm/taab186
- 34. Chow NA, Gade L, Tsay SV, Forsberg K, Greenko JA, Southwick KL, et al.; US Candida auris Investigation Team. Multiple introductions and subsequent transmission of multidrug-resistant *Candida auris* in the USA: a molecular epidemiological survey. Lancet Infect Dis. 2018;18:1377–84. https://doi.org/10.1016/S1473-3099(18)30597-8
- 35. Yadav V, Heitman J. On fruits and fungi: a risk of antifungal usage in food storage and distribution in driving drug resistance in *Candida auris*. MBio. 2022;13:e0073922. https://doi.org/10.1128/mbio.00739-22
- Miot J, Leong T, Takuva S, Parrish A, Dawood H. Cost-effectiveness analysis of flucytosine as induction therapy in the treatment of cryptococcal meningitis in HIV-infected adults in South Africa. BMC Health Serv Res. 2021;21:305. https://doi.org/10.1186/s12913-021-06268-9
- Bravo Ruiz G, Lorenz A. What do we know about the biology of the emerging fungal pathogen of humans *Candida auris*? Microbiol Res. 2021;242:126621.

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strep'to-mi'sin

In the late 1930s, Selman Waksman, a soil microbiologist working at the New Jersey Agricultural Station of Rutgers University, began a large-scale program to screen soil bacteria for antimicrobial activity. By 1943, Albert Schatz, a PhD student working in Waksman's laboratory, had isolated streptomycin from *Streptomyces griseus* (from the Greek *strepto*-["twisted"] + *mykēs* ["fungus"] and the Latin *griseus*, "gray").

In 1944, Willam H. Feldman and H. Corwin Hinshaw at the Mayo Clinic showed its efficacy against *Mycobacterium tuberculosis*. Waksman was awardeda Nobel Prize in 1952 for his discovery of streptomycin, al-though much of the credit for the discovery has since been ascribed to Schatz. Schatz later successfully sued to be legally recognized as a co-discoverer of streptomycin.

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#### **References**:

- 1. Comroe JH Jr. Pay dirt: the story of streptomycin. Part I. From Waksman to Waksman. Am Rev Respir Dis. 1978;117:773-81.
- 2. Wainwright M. Streptomycin: discovery and resultant controversy. Hist Philos Life Sci. 1991;13:97–124.

https://wwwnc.cdc.gov/eid/article/25/3/et-2503\_article

Article DOI: http://doi.org/10.3201/eid2910.230181

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# Candida auris Clinical Isolates Associated with Outbreak in Neonatal Unit of Tertiary Academic Hospital, South Africa

Appendix

## **Supplementary Methods**

## **Transmission Route Reconstruction**

To reconstruct potential transmission routes in the hospital, an algorithm called SeqTrack was used (1). SeqTrack is within the adegenet package in R, it uses a parsimony phylogeny approach to determine ancestries based on an alignment of SNPs and sampling dates of the isolates to create a schematic diagram of proposed transmission routes between the patients. The mutational rate (mu) obtained from the root-to-tip regression analysis, the length (haplo.length) of analyzed sequences in number of nucleotides and a matrix of pairwise distances and collections was used to plot a series of trees and the tree with the maximum parsimony was chosen. Only one sample per person (whether colonization or disease) was used. The data from the isolates was assessed to investigate whether the isolates formed distinct clusters due to epidemiologic variables such as year of isolation or ward location with regard to their genetic relationships.

## **Supplementary Results**

## **Outbreak Reconstruction for Clade III**

The potential infection chains or transmission route of *C. auris* clade in the hospital were determined using SeqTrack. The transmission network shows the most likely source of infection/colonization for each case. Only isolates from clade III were included in this analysis, which was the clade responsible for the outbreak in the neonatal ward. This analysis allowed us

to track clade III strains across the hospital wards and introduction into the neonatal ward (Appendix Figure).

Case 1 (Isolate 4867 on the phylogenetic trees) was the index case of this tree and was isolated from a patient in an adult medical ward. Two transmission events occurred from Case 1 that gave rise to two clusters. The first cluster begins with Case 37 (1172) from an unknown ward. This cluster contained 18 cases with an average of 12.2 pairwise differences and therefore most (n = 6) had <12 SNPs in this cluster and likely had a very recent common ancestor and transmission. The second cluster from Case 1 begins with Case 6 (227) from the adult burn unit. This cluster contained infections from the neonatal ward outbreak with all (n = 83) cases having <12 SNPs and an average of 1.3 pairwise differences. Case 18 (594) seems to be the index case/ancestral case for most infections involved in this outbreak. From this ancestor, the weight is as low as zero in most branches, which indicates rapid transmission of the pathogen between the cases acharacteristic of an outbreak. This is contrary to the high number of mutations between the cases earlier in the transmission tree; these cases were sporadic and are further apart in their sampling dates. Overall, infections appear to have been introduced into the neonatal unit from the adult burn unit, adult medical department and an unknown ward.

The Bayesian analysis using BEAST estimated that the TMRCA of the outbreak strain occurred in 2018. This estimation was supported by the date of isolation for Case 6 (227) and Case 10 (292), ancestors of the neonatal ward outbreak, which were both isolated in 2018.

This exploratory analysis is limited by the inclusion of only one sample per patient and that only a small subset of isolates from the hospital were collected which does not capture the full scope of transmission in the hospital. Furthermore, our study does not include environmental isolates which form a crucial part in the transmission of the pathogen and therefore introducing missing links in the network and a looser interpretation.

### References

 Jombart T, Eggo RM, Dodd PJ, Balloux F. Reconstructing disease outbreaks from genetic data: a graph approach. Heredity. 2011;106:383–90. <u>PubMed https://doi.org/10.1038/hdy.2010.78</u>



**Appendix Figure.** Reconstructed transmission tree of 118 South African Clade III Candida auris isolates from this study. Each case is represented by a node on the tree and arrows indicate plausible ancestries/transmissions. Mutations between the nodes are indicated by the color arrows (red = no/few mutations; light gray = many mutations) and the numbers in blue represent the weight of mutations when compared to the ancestries. Time is represented by y-axis (up: older; down: younger).