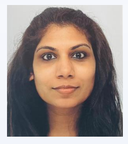
Uncovering *Candidozyma auris* Epidemiology in London Hospitals With Nanopore Sequencing



Darshana Patel[1], Mark Tan[2], Jonathan Edgeworth[2], Paul Jones[1]

Advanced Biomedical Scientist

[1]HCA Laboratories, Microbiology

[2]Guy's & St Thomas' Hospital and Centre for Clinical Infection & Diagnostics Research (CIDR)

Key Words: *C. auris,* multidrug resistance, Nanopore sequencing, Whole Genome Sequencing (WGS), nosocomial outbreaks

Introduction

Candidozyma auris is an emerging multidrug-resistant yeast posing a high risk to hospitalised patients.

- Causes severe invasive infections in immunocompromised patients (bloodstream, wound, and intra-abdominal).
- Commonly resistant to fluconazole; emerging resistance to amphotericin B & echinocandins, a WHO fungal priority pathogen.
- First identified in 2009 from a Japanese patient's ear discharge; now reported in >40 countries.
- Highly transmissible in hospitals via surfaces and direct contact; outbreaks reported in (USA & UK).

Six geographical clades (1-6):

- Clade 1 (South Asia), Clade 3 (South Africa), & Clade 4 (South America) → invasive outbreaks
- Clade 2 (East Asia) → ear infections
- Clade 5 (Iran) and Clade 6 (Bangladesh) -> emerging clades; limited data available.

The *C. auris* Genome:

- 7 chromosomes (12.1–12.5 Mb).
- Large inter-clade genomic diversity -> Several thousand SNPs between clades.
- Small intra-clade genomic diversity -> <100 SNPs differences between two isolates within the same clade.

Drug resistance genes:

- *ERG11* (azole resistance), *TAC1b* (efflux pump upregulation, multidrug resistance), FKS1 (echinocandin resistance), *CDR1*/*CDR2*, *MDR1* (azole efflux pumps, HSP90 & biofilm-associated genes (stress response, drug tolerance)

Current benefits and targets for nanopore whole genome sequencing (WGS):

- Trace nosocomial outbreaks
- Track clade-specific evolution
- Correlate genotypic to phenotypic resistance

Objectives

- Conduct nanopore WGS and genome assembly on *C. auris* isolates
- Identify single nucleotide polymorphisms and other genomic variations
- Understand the phylogenetics of the isolates and infer outbreak patterns

Methods

Isolate Collection & Identification

- A total of 71 *C. auris* isolates were collected from multiple clinical sites (July 2019 - October 2023)
- Identification was performed on CHROMagar™ Candida Plus and confirmed by MALDI-ToF MS (Bruker).

DNA Extraction & Sequencing

- Mechanical cell lysis through bead beating was performed to extract genomic DNA from sub-cultured colonies.
- Performed multiplexed nanopore WGS using the rapid barcoding kit (RBK114.96) and the MinION flow cell (FLO-MIN114) on the MinION MK1B platform.

Data Analysis

- Demultiplexed with MinKNOW v23.07.12 and basecalled Dorado (v0.7.3).
- Reads were mapped to the *C. auris* Clade 1 (South Asia) reference genome with minimap2 (v2.1.1-r341).
- Variants were detected with BCFtools (v1.17).
- Phylogenetic analysis was performed with Parsnp (v1.2).
- Maximum-likelihood phylogenetic tree was generated to compare isolates with all global Clades (1-6).

Downstream Analysis

- Genome annotation by SnpEff (v. 5.2f) was carried out as part of the downstream analysis.

Results

Isolate Distribution Across Hospital Settings

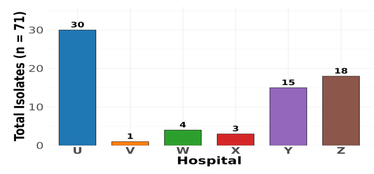


Figure 1: The bar chart illustrates the distribution of 71 total *C. auris* isolates across six hospitals (U-Z) between July 2019 and October 2023. Isolates were from various body sites, including the nose, groin, axilla, throat, catheter sites, urine, tissue biopsy, and blood culture during screening.

In Vitro Antifungal Susceptibility Profile

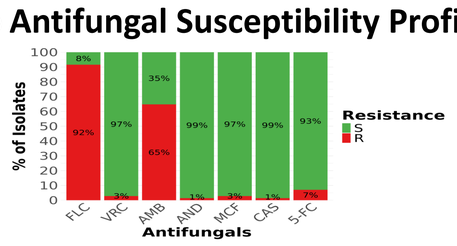


Figure 2: The stacked bar chart shows antifungal susceptibility profile (%)

- High resistance was observed to fluconazole (92%) and amphotericin B (65%), while echinocandins remained effective with only two resistant isolates (ys016, ys068).
- Resistance to 5-flucytosine was low (7%), and voriconazole showed minimal resistance 3%.

Genome Coverage Across Thresholds

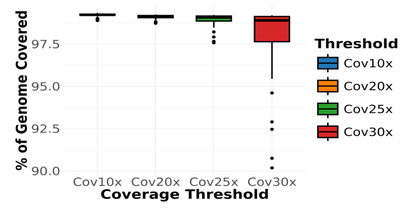


Figure 3: The box plot displays genome coverage across different sequencing depths (10x, 20x, 25x, and 30x).

- At 10x, 20x, and 25x thresholds (dark blue, purple, and red), most isolates achieved high genome coverage (97.5 –100%), with few outliers. However, at 30x, coverage dropped in some isolates, indicating uneven sequencing.
- QC-passed isolates (52/71) maintained excellent coverage across 20x, 25x, and 30x depths and taken forward for downstream analyses.

Mutation Frequency in *ERG11, CDR1*, and *FKS1*

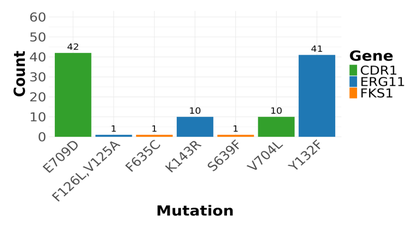


Figure 4: Resistance-Associated Mutations Identified in *C. auris* isolates by Whole-Genome Analysis

Sequenced reads were mapped to antifungal resistance genes (*ERG11, CDR1, CDR2, MDR1, TAC1b,* and *FKS1*). Most of these genes exhibited one or more non-synonymous amino acid changes.

- ***ERG11***: Mutations Y132F (found in 41 isolates) and K143R (in 10 isolates) were common. One isolate (ys029) carried dual mutations (V125A, F126L) in this gene. These mutations are known to be associated with azole resistance.
- ***CDR1*:** E709D (found in 42 isolates) and V704L (in 10 isolates), are known to be associated with efflux-mediated resistance.
- ***FKS1*:** S639F (found in ys068) is a known hotspot for echinocandin resistance. F635C mutation (ys016), less common, was linked to high echinocandin resistance and has been reported in pan-resistant isolates in other studies.

Phylogenetic Analysis Reveals Clustering with South Asian Clade 1

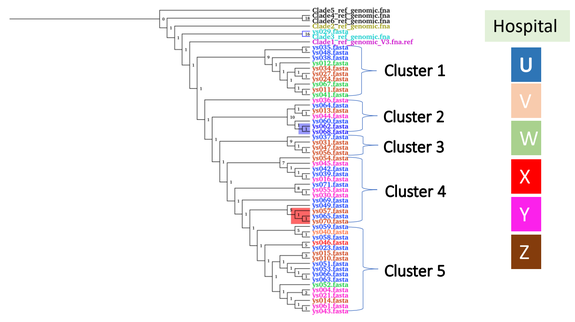


Figure 5: Phylogenetic Analysis of *C. auris* isolates. A maximum-likelihood tree was generated and colour- coded by hospital site, revealing five distinct clusters. This analysis compared 52 QC-passed *C. auris* genomes to six global reference clades.

- **Clade Distribution:** Most isolates (51/52) clustered with the South Asian Clade 1 (Indian/Pakistan origin), with SNP distances of 1 to 16.
- **Clade 3:** One isolate (ys029) aligned with South African Clade 3, showing 16 SNPs from its reference.
- **SNP Diversity:** All isolates exhibited low SNP diversity (≤29), regardless of specimen type, hospital site, or hospital stay, which hindered the ability to accurately assess patient-to-patient transmission.

Discussion/Conclusion

Phylogenetic and antifungal susceptibility analysis of isolates from six hospitals provided insights into the pathogen's epidemiology, genetic diversity, and resistance mechanisms. High resistance to fluconazole and amphotericin B was observed, while echinocandins remained effective, with only two resistant isolates. Genomic analysis identified key mutations in ERG11 and CDR1 genes linked to fluconazole resistance, and FKS1 associated with echinocandin resistance, consistent with phenotypic data, except for a few isolates that were fluconazole sensitive despite resistance-associated mutations. Most isolates clustered with South Asian Clade 1, with one isolate aligned with South African Clade 3, suggesting limited geographical spread or a possible transmission link. SNP typing for contact tracing remains challenging due to low SNP diversity among isolates. Further work should focus on analysing repeat regions to better understand transmission dynamics.

References

- Parums, Editorial: The WHO Fungal Priority Pathogens List in Response to Emerging Fungal Pathogens During the COVID-19, Medical Science Monitor (2022).
- Sharma and Kadosh, Perspective on the Origin, Resistance, and Spread of the Emerging Human Fungal Pathogen *Candida auris*, PLOS Pathogens (2023).
- Du et al., *Candida auris*: Epidemiology, Biology, Antifungal Resistance, and Virulence, PLOS Pathogens (2020).
- Lockhart et al., Simultaneous Emergence of Multidrug-Resistant *Candida auris* on 3 Continents, Clinical Infectious Diseases (2017).
- Jacobs et al., *Candida auris* Pan-Drug-Resistant to Four Classes of Antifungal Agents, Antimicrobial Agents and Chemotherapy (2022).

Acknowledgement

This project was funded by the CIDR research laboratory.