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Antifungal resistance profile and phenotypic virulence characters of Candida auris isolates: A study from South India.

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ABSTRACT
Background: *Candida auris* is an emerging multidrug-resistant yeast of global concern, linked to nosocomial outbreaks worldwide within a decade of its discovery. Aim: This study aimed to evaluate the virulence traits and antifungal resistance profile of *C. auris* isolates from a South Indian hospital. **Methods:** 111 *C. auris* isolates (70 from blood, 41 from urine) collected between 2018 and 2023 were analyzed. Antifungal susceptibility testing, thermotolerance (42°C and 45°C), enzymatic activity, biofilm production, and aggregation assays were performed. **Results:** High resistance rates were observed, with 84% of isolates resistant to fluconazole (Minimum Inhibitory Concentration (MIC) ≥32 mg/L) and 82% to amphotericin B. Most isolates (96%) exhibited thermotolerance at 42°C, but none grew at 45°C. Enzymatic activity varied among strains. All isolates formed biofilms, with blood isolates demonstrating significantly higher biofilm biomass ($p < 0.05$). Biofilm production did not correlate with antifungal resistance. **Conclusion:** The study highlights the predominance of multidrug-resistant *C. auris* strains in South India, with notable virulence traits that may contribute to nosocomial spread.

INTRODUCTION:

Candida auris has rapidly emerged as a global health threat, with outbreaks in over 40 countries since its first detection in Japan in 2009[1]. Whole-genome sequencing shows at least five phylogenetically distinct clades with geographic associations: South Asia (Clade I), East Asia (Clade II), South Africa (Clade III), South America (Clade IV), and Iran (Clade V)[2]. A recent study from Singapore reported a sixth clade, highlighting ongoing evolution[3]. Recognition of clades and studying virulence factor and resistance profiles of different clades is important for understanding transmission, resistance trends, and outbreak potential.

Clade I strains dominate in South Asia, including India, and are now a major cause of ICU candidemia[4]. Contributing factors include prolonged hospitalisation, broad-spectrum antifungal use, and the COVID-19 pandemic[5]. Indian isolates show high resistance, mortality, and nosocomial clustering[1,4,5]. Given India's diverse conditions[6], regional studies are essential. Ample research exists, comparing the virulence of *Candida auris* with other *Candida* species[7,8,9,10]. In this work, we examined antifungal resistance and virulence traits of South Indian *C. auris* isolates, focusing on site of origin (blood vs urine) rather than interspecies comparison.

MATERIALS AND METHODS:

Isolate Collection and Ethical Approval:
We studied 111 *C. auris* clinical isolates (70 blood, 41 urine) collected at a South Indian tertiary hospital (2019–2023). The study was IRB-approved (IEC-NI/19/DEC/72/116); informed consent was waived. Identification was by MALDI-TOF MS (scores ≥2.0), with confirmation by species-specific PCR targeting the ITS region. Primers used were from an institutional project under preparation. *C. albicans* ATCC 90028 and other clinical *Candida* strains served as quality controls.

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1108-1116
DoI-10.004687/1000-9035.2025.148

Antifungal Susceptibility Testing:
Antifungal susceptibility was determined by broth microdilution (CLSI M27-A3)[11]. Inocula (0.5–2.5 × 10^3 cells/ml) were prepared in RPMI 1640 with MOPS buffer, and 100 µl was added to 96-well plates. The eight antifungals tested were amphotericin B, anidulafungin, caspofungin, micafungin, fluconazole, itraconazole, posaconazole, and voriconazole (MIC ranges as per CLSI). [Drug-free wells served as growth controls and uninoculated medium as sterility controls.] MICs were read at 24 h and interpreted using CDC tentative breakpoints (Table 1)[12]

Thermotolerance Assay:
For assessing thermotolerance, a standard inoculum (1–10 × 10^5 colony-forming units [CFU]) of yeast cells was plated onto Sabouraud dextrose agar (SDA) using a modified quadrant-streaking method adapted from Ben-Ami et al., 2017[13]. Plates were incubated for three days at 37 °C, 42 °C, and 45 °C. Growth was assessed by noting the extent of colony development across the quadrants.

Biofilm Formation:
Biofilm formation was measured using the XTT reduction assay. Strains grown on SDA (24 h, 37 °C) were suspended to 1 × 10^7 cells/mL (0.5 McFarland), diluted 1:20, and 200 µL inoculated into 96-well plates. After attachment (overnight, 37 °C) and PBS washing, biofilms were induced with Yeast Nitrogen Broth for 24 h. XTT-menadione solution was added, incubated (3 h, 37 °C, dark), and absorbance recorded. Biofilm strength was categorized as weak, moderate, or strong. Only metabolic activity was assessed; no biofilm antifungal testing was done.

Aggregate formation:
Fresh cultures were suspended in sterile water, mounted on slides, and examined microscopically (40×). Isolates were classified as weak (single cells predominate) or strong (≥50% cells in clusters) aggregators, following published methods[7,15]. Classification was by two independent observers; no quantitative imaging was done.

Enzymatic activity:
Enzymatic activity was assessed using standard plate assays. [16,17,18,19,20]

- Phospholipase: 10 µL inocula (1 × 10^7 cells/mL) were plated on SDA with NaCl, $CaCl_2$, and egg yolk emulsion; opacity zones were measured after 7 days at 37 °C.
- Proteinase: isolates were plated on Staib's medium containing bovine serum albumin and incubated 10 days at 37 °C; proteolysis zones were visualized with amido black.
- Hemolysin: SDA with 7% sheep blood + 3% glucose; clearance zones measured.
- Esterase: medium with peptone, NaCl, $CaCl_2$, agar, and 0.5% Tween 80; incubated 10 days at 37 °C.

Statistical analysis:
Data normality was assessed using the Kolmogorov–Smirnov test. Since the data were not normally distributed, non-parametric Mann–Whitney U tests were used to compare continuous variables between blood and urine isolates. Chi-square tests were performed to examine associations between the source of isolation (blood vs. urine) and categorical outcomes, such as antifungal resistance profiles. A p-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS software.

RESULTS:

Antifungal Susceptibility Testing: Table 2 shows the results of antifungal susceptibility tests for the 111 *C. auris* isolates tested against Amphotericin B, Fluconazole, Itraconazole, Voriconazole, Posaconazole, Caspofungin, Micafungin and Anidulafungin. According to CDC breakpoints for *C. auris*, the resistance rates were 84% for Fluconazole, 82% for Amphotericin B, 7.5 % for Caspofungin, 6.5% for Anidulafungin and 4.5% for Micafungin. *Candida albicans* ATCC 90028 exhibited expected susceptibility profiles for all antifungal agents tested, confirming the validity of the MIC results.

Despite numerical differences in resistance rates between blood and urine isolates, chi-square tests revealed no statistically significant associations between anatomical source and resistance status for any antifungal agent (all $p > 0.05$) (Table 3). This suggests that resistance mechanisms in our cohort are conserved across infection sites, possibly reflecting clade-wide resistance traits or regional antifungal pressure patterns. Furthermore, >70% of the isolates exhibited multidrug resistance spanning two or more antifungal classes which are worrying trends. While MIC_{50} values were identical between blood and urine isolates for most antifungals, bloodstream infections demonstrated higher MIC_{90} extremes (Table 4) (Figure 1).

Minimum inhibitory concentrations (MIC_{50} and MIC_{90}) for *Candida auris* isolates against fluconazole, amphotericin B, caspofungin, anidulafungin, and micafungin are shown. The shaded bars represent the MIC range. The blue lines indicate MIC_{50} values, and black dashed lines indicate MIC_{90} values. The red vertical lines represent tentative CDC breakpoints for C. auris (Centers for Disease Control and Prevention, 2024). Note the high MICs for fluconazole and amphotericin B exceeding CDC breakpoints, while

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1108-1116
DoI-10.004687/1000-9035.2025.148

echinocandin MICs remain mostly below the resistance thresholds.

Thermotolerance: Table 5 presents the thermotolerance assay results for 111 isolates of *C. auris*. All 111 isolates of C. auris demonstrated robust growth at 37 °C on SDA plates, with majority of them (97%) maintaining their proliferative capacity at 42 °C. None of the isolates survived at 45 °C on SDA plates suggesting an upper thermal limit for this pathogen under standard conditions. ATCC *C. albicans* demonstrated robust growth at 30 °C and 37 °C, with no growth at 42 °C, confirming typical thermotolerance and validating assay conditions. Despite minor numerical differences, no statistically significant association was observed between anatomical source (blood vs urine) and thermotolerance patterns (Chi square test, $p > 0.223$).

Biofilm formation: Biofilm formation ability of the 111 *C. auris* isolates was determined using the XTT reduction assay which is used for assessing metabolic activity of biofilm. All strains were able to produce biofilm but with considerable inter-isolate variation in both the colonizing and invasive group (Figure 1). A statistically significant difference in biofilm absorbance values was observed between blood and urine isolates ($p < 0.05$), indicating variation in biofilm-forming capacity based on the source of isolation. Antifungal resistance in the biofilms was not checked using the XTT assay. But when comparing the antifungal resistance profiles of the isolates with their biofilm production levels, no significant correlation was observed ($p = 0.621$). While moderate biofilm strength was the most common in both MDR (58.2%) and non-MDR (62.5%) groups, the distribution differences were not statistically significant. Blood isolates showed significantly higher biofilm metabolic activity than urine isolates (Mann–Whitney U test, $p < 0.001$).

Aggregate formation: Some clinical isolates of *Candida auris* can form multicellular morphology due to aggregation caused by defects in cell division. Aggregation scoring revealed that 27% of blood isolates and 39% of urine isolates exhibited strong aggregation, while the rest were weakly aggregating. The ATCC *Candida* strain did not form strong aggregates under microscopy. The association between strength of aggregation and source of isolation was not significant ($p = 0.673$) when tested using chi-square test.

Enzymatic activity: The tested isolates of *Candida auris* produced phospholipase, proteinase, esterase and hemolysin in a strain dependent manner (Table 7 and Figure 3). 41% of the tested *C. auris* strains possessed phospholipase activity and 55% tested positive for proteinase activity. Hemolysis was observed in 88% of isolates while esterase production was low (1%). The control strains yielded satisfactory results. Hemolysin and Proteinase production were significantly higher in blood isolates.

Table 7 summarizes the relationship between site of origin and virulence traits of the 111 Candida auris isolates analyzed in this study.

DISCUSSION:

C. auris has emerged as a major nosocomial threat due to its multidrug resistance, persistence in the hospital environment, and ability to cause invasive infections. In the COVID-19 pandemic era, increased ICU admissions, prolonged ventilation, and widespread steroid use have contributed to new clusters of multidrug-resistant *C. auris* outbreaks [5]. Accurate and timely detection of *Candida auris* remains a diagnostic challenge due to its frequent misidentification by conventional biochemical methods[21]. Improved laboratory workflows now include MALDI-TOF MS and species-specific PCR assays targeting the ITS or D1/D2 rDNA regions to achieve reliable species-level identification [2,5]. In addition, differential chromogenic media, such as CHROMagar Candida Plus have been developed and validated to facilitate early detection and presumptive differentiation of *C. auris* from other Candida species directly from clinical or surveillance samples[22]. Novel technologies like Raman spectroscopy and surface-enhanced Raman scattering (SERS) have shown promise for rapid and label-free identification of *C. auris* with high sensitivity, although these methods remain mainly in the research phase[23].

Globally, *Candida auris* has now been reported in more than 50 countries across six continents, establishing itself as a significant nosocomial pathogen[2,23]. In South Asia, India and Pakistan remain hotspots for Clade I strains with high resistance and outbreak potential[4,5,25]; East Asia is represented by Clade II, with early cases and genotyping reports from Japan and South Korea[1,26]; South Africa harbors Clade III, which has been extensively characterized during regional outbreaks in public hospitals[27]. South America, especially Venezuela and Colombia, reports Clade IV strains with regional amphotericin B resistance trends[28]. Iran is the origin of Clade V[29]. Recently, genomic and phenotypic data from Singapore have confirmed a distinct sixth clade with unique local characteristics[3]. In the United States, imported cases and local transmission have led to repeated clusters, emphasizing cross-border spread[21,24].

The emergence of multidrug-resistant *C. auris* highlights the urgent need for novel antifungal agents and innovative treatment approaches. Echinocandins remain the first-line therapy for most *C. auris* infections; however, emerging resistance underscores the importance of alternative strategies[30]. Combination regimens, such as echinocandins with amphotericin B or azoles, have shown synergistic effects in vitro, offering potential avenues for overcoming resistance[10,27]. Additionally, new antifungal classes, such as fosmanogepix (APX001) and ibrexafungerp (SCY-078), have demonstrated promising activity against *C. auris* biofilms and resistant isolates in preclinical studies[31].

The virulence traits of *Candida auris* present unique challenges for clinical management and infection control. *C. albicans* is widely considered the model species for studying Candida virulence factors. Recent genomic evidence shows that nearly 29% of the *C. auris* genome is similar to *C. albicans*[19], potentially explaining many shared traits. However, important differences exist: *C. auris* does not form chlamydospores[1], produces weaker pseudohyphae and hydrolytic enzymes overall[17], yet exhibits unique mechanisms for immune evasion and stress tolerance, such as neutrophil inhibition[32]. A comparative overview of these virulence characteristics relative to *C. albicans* is detailed in Table 8.

In this study, instead of comparing with other species of Candida, we compared the antifungal resistance profiles and phenotypic virulence factors of blood and urine isolates of *C. auris* from South India. Although we did not have detailed travel or inter-hospital transfer data, regional patient referrals and the absence of robust screening protocols suggest that unrecognized inter-facility spread may occur.

Our finding of high fluconazole (84%) and amphotericin B (82%) resistance aligns closely with previous ICU-based surveillance from India and South Asia[4,5,15]. This trend reflects the dominance of Clade I isolates, which frequently harbor ERG11 and TAC1B mutations conferring high-level azole resistance[27]. Although echinocandins remain the most active class against *C. auris*, the emergence of echinocandin resistance through FKS1 hotspot mutations has been documented globally, including in Indian isolates[5,9]. While our cohort's echinocandin resistance remains low (6.5–7.5%), these sporadic cases highlight the risk of future therapeutic failure if resistance expands further[9]. Importantly, the comparable resistance rates and MIC_{50} values observed between bloodstream and urinary isolates suggest that colonizing strains may serve as hidden reservoirs for MDR clones within healthcare facilities[13,28]. This is consistent with reports that environmental persistence and asymptomatic colonization enable silent transmission and outbreaks[13,34]. Therefore, our results underscore the need for strengthened infection prevention and control (IPC) measures, robust antifungal stewardship, and active screening to limit the spread of *C. auris* in critical care settings[21,24].

Thermotolerance testing confirmed the ability of *C. auris* to grow at elevated temperatures up to 42 °C, as originally described by Satoh et al. and later confirmed in diverse settings[1,13]. This trait likely facilitates environmental persistence and nosocomial transmission by supporting survival on hospital surfaces[34]. Moreover, such thermotolerance, combined with high saline tolerance, has been leveraged to develop selective culture and enrichment methods to improve surveillance and outbreak control[27].

Regarding virulence traits, biofilm formation showed significant variation by source, with bloodstream isolates demonstrating significantly higher biofilm metabolic activity than urinary isolates. Strong biofilm-forming ability may contribute to the persistence of *C. auris* in catheter-associated infections and hospital surfaces, complicating eradication and enhancing antifungal tolerance[7,10,15]. Previous studies have shown that *C. auris* can form dense biofilms with extracellular matrix, conferring protection against commonly used antifungals and disinfectants[15].

Transcriptomic analyses have revealed that biofilm-associated cells upregulate genes linked to efflux pumps and stress responses, which further contribute to antifungal resistance[10]. A recent meta-analysis highlights biofilm formation as one of the key mechanisms that differentiates *C. auris* from other non-albicans *Candida* species in terms of treatment challenges and hospital persistence[19].
Although antifungal susceptibility was not assessed within biofilms in this study, the lack of correlation between planktonic resistance and biofilm biomass underscores the importance of dedicated biofilm resistance testing to guide clinical management and infection control.

Certain isolates of *Candida auris* do not release their daughter cells after budding, forming aggregate strains that cannot be easily disrupted[25]. Aggregation is a complex phenomenon that can be altered by several external factors like salt content, antifungal, temperature and chemicals and could be passed in vivo in animal studies[15]. Aggregation —

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1108-1116
DoI-10.004687/1000-9035.2025.148

linked to cell division defects[7] — was more common among urinary isolates in this study, but did not correlate significantly with biofilm production or antifungal resistance. This suggests that aggregate formation may be strain-specific or influenced by factors other than the anatomical site of infection like genomic traits[37]. While aggregation may reduce pathogenicity in vivo[7], it can facilitate environmental survival and spread[38]. Future work using quantitative imaging and animal models is needed to clarify its role in transmission and virulence.

Among the several enzymes that help with its invasiveness, hydrolases are the largest group of enzymes found in the *C. auris* genome, followed by transferases and oxidoreductases[25,37]. Hydrolytic enzyme production (proteinase, phospholipase, hemolysin) was strain-dependent comparable to prior reports[17]. Phospholipase and hemolysin activities were slightly more prevalent among bloodstream isolates, suggesting their possible role in tissue invasion and bloodstream dissemination.

The findings of this study have direct relevance for clinical practice, particularly in high-risk hospital settings. The demonstration of widespread multidrug resistance, high biofilm-forming capacity, and robust thermotolerance among *C. auris* isolates in the South Indian region underscores the need for enhanced infection prevention measures, targeted screening, and strict environmental decontamination protocols. Since urinary isolates can act as reservoirs for MDR strains, even colonizing isolates should be monitored and managed carefully to limit transmission. Furthermore, knowledge of local resistance patterns and virulence traits can guide empirical antifungal choices, promote appropriate antifungal stewardship, and inform hospital preparedness strategies to contain potential outbreaks.

Our findings also reinforce that future work should include high-resolution genotyping (e.g., microsatellite typing) to understand local transmission dynamics more precisely. Combining phenotypic resistance data with genotypic markers such as ERG11 mutations could guide clinicians in selecting effective antifungal combinations and preventive strategies. Recent evidence also highlights the need for innovative combinational therapies and new antifungal agents to tackle pan-resistant strains.

This study provides one of the few comprehensive regional datasets on *Candida auris* from South India, combining antifungal susceptibility testing with detailed phenotypic characterization of virulence traits across invasive and colonizing isolates. The use of standardized identification methods, quality controls, and phenotypic assays strengthens the reliability of the results. However, there are important limitations. The study did not include higher-resolution genotyping methods such as microsatellite or whole-genome sequencing beyond ITS/D1/D2 regions, which would help clarify intra-clade diversity and transmission dynamics. The classification of aggregate formation was qualitative and not supported by digital imaging or cell counting, which may introduce observer bias. Antifungal susceptibility within biofilms was not tested, although this is a clinically relevant aspect given the role of biofilms in antifungal tolerance. Finally, correlations with detailed patient clinical outcomes or treatment response could not be explored, which limits conclusions about the clinical impact of these phenotypic traits. Future studies addressing these gaps are needed to strengthen preparedness for *C. auris* surveillance and outbreak response in India.

CONCLUSION:

Candida auris is a pathogen that is here to stay and its detailed virulence mechanisms have played a major role in its rise to prominence. This study highlights the importance of analyzing its virulence factors from the perspective of site of origin of the isolates as well. While urinary isolates are generally considered as colonizers rather than invaders, they might play a bigger role in maintenance of spread within hospitals by persisting on medical devices and on biotic surfaces. The problem of antifungal resistance in *Candida auris* was serious since the beginning, and is only getting worse with the emergence of pan drug resistant isolates. To our knowledge, this study is the first to examine the virulence traits and resistance profile of *C. auris* isolates using a comprehensive collection of clinical *C. auris* samples from the geographical region of South India. Based on our findings, we recommend routine screening and species-level identification of all *Candida* isolates in high-risk units, especially ICUs, to detect *C. auris* early. In addition to continued prospective surveillance, we recommend that retrospective analysis of previously stored *Candida* isolates be undertaken wherever feasible to detect potential misidentification, strengthen local epidemiological understanding, and guide targeted infection control measures for *C. auris*. In India, where awareness of emerging fungal pathogens remains limited and laboratory capacity is often constrained, there is a pressing need for more dedicated funding and coordinated national surveillance to detect, report, and contain possible ongoing C. auris transmissions and outbreaks. Improved molecular diagnostics, routine screening of high-risk patients, and investment in mycology research infrastructure are vital steps to strengthen

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1108-1116
DoI-10.004687/1000-9035.2025.148

preparedness against this globally emerging threat. Hospitals should also maintain preparedness plans for potential outbreaks, including robust contact tracing, environmental cleaning, and staff education. Future studies should combine phenotypic data with high-resolution genotyping to track transmission routes more precisely and inform public health response.

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CONFLICT OF INTEREST:
The authors declare that they have no conflicts of interest relevant to this study.

TABLES:
Table 1: Current tentative CDC breakpoints for *Candida auris*

Antifungal agent	Tentative CDC MIC breakpoint for resistance (µg/mL)
Amphotericin B	≥ 2
Fluconazole	≥ 32
Anidulafungin	≥ 4
Caspofungin	≥ 2
Micafungin	≥ 4

Source: CDC *Candida auris* antifungal recommendations.

Table 2: In vitro antifungal susceptibility pattern of Candida auris isolates (The numbers in bold are considered as resistant isolates according to CDC breakpoints)

Agent	Number isolates with the following MIC (mg/L)							
	0.25	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**
Fluconazole			1	1	2	7	7	**93**
Amphotericin B			20	**4**	**5**	**30**	**34**	**18**
	0.062	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
Posaconazole	36	23	14	15	8	10	0	5
Voriconazole	3	3	5	23	57	12	0	8
Caspofungin	13	10	20	27	33	**8**		
Micafungin			6	2	5	1	**5**	
n				9	2	9		
Anidulafungin		3	13	20	37	32	**6**	

Table 3: Comparison of Antifungal Resistance between Blood and Urine *Candida auris* isolates

	Blood isolates (n=70) resistance %	Urine isolates (n=41) resistance %	P value (chi-square test)
Fluconazole	82.8	85.36	0.449
Amphotericin B	75.7	92.7	0.25
Caspofungin	12.8	4.9	0.183
Anidulafungin	5.7	4.9	0.987
Micafungin	8.6	2.4	0.237

Table 4: Minimum Inhibitory Concentrations (MIC_{50} and MIC_{90} in µg/mL) of *Candida auris* isolates from blood and urine, stratified by antifungal agent.

	Blood isolates		Urine isolates		Total isolates	
	MIC 50	MIC 90	MIC 50	MIC 90	MIC 50	MIC 90
Fluconazole	64	256	64	128	64	256
Amphotericin B	16	32	8	32	8	32
Caspofungin	0.5	4	0.5	1	0.5	1
Anidulafungin	1	4	1	2	1	2
Micafungin	1	4	1	1	1	2

Table 5: Thermotolerance assay results of *Candida auris* isolates at different temperatures using the quadrant growth method.

Temperature of incubation	% of isolates grown in each quadrant			
	+	++	+++	++++
37°C	100	100	100	100
42°C	96.5	96.5	93	92
45°C	0	0	0	0

'+' to '++++' indicate the extent of colony growth based on the quadrant streak method: + = growth in first quadrant only; ++ = growth in first and second quadrants; +++ = growth in three quadrants; ++++ = growth in all four quadrants. Values represent the percentage of isolates demonstrating growth up to the indicated quadrant at each incubation temperature. No growth was observed at 45 °C for any isolate.

Table 6: shows the number of strains and the resulting enzymatic activity among blood and urine derived isolates of *Candida auris*. (S- strongly positive, W- weakly positive, N-negative.)

	Phospholipase			Proteinase			Hemolysin			Esterase		
	S	W	N	S	W	N	S	W	N	S	W	N
Blood	**14**	**32**	**24**	**13**	**26**	**31**	**21**	**39**	**10**	**0**	**2**	**68**
Urine	**6**	**11**	**24**	**10**	**12**	**19**	**15**	**13**	**13**	**0**	**0**	**41**

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1108-1116
DoI-10.004687/1000-9035.2025.148

Table 7: Association of Phenotypic Virulence Traits and Antifungal Resistance with Site of Isolation

Parameter	Test Used	p-value	Significant Association	Notes
Antifungal Resistance	Mann–Whitney U / Chi-square	> 0.05	No	Fluconazole, Amphotericin B, Caspofungin, Anidulafungin, Micafungin all non-significant
Biofilm Absorbance	Mann–Whitney U	< 0.001	Yes	Higher in blood isolates
Biofilm Strength	Chi-square	< 0.001	Yes	Stronger biofilm associated with site
Aggregation Strength	Chi-square	0.673	No	Not associated
Phospholipase	Chi-square	0.245	No	Not associated
Proteinase	Chi-square	< 0.001	Yes	Stronger activity in blood isolates
Hemolysin	Chi-square	< 0.001	Yes	Stronger activity in blood isolates
Esterase	Chi-square	0.416	No	Not associated
Thermotolerance	Chi-square	0.542	No	Not associated
MDR Status	Chi-square	0.769	No	Not associated
MDR vs Biofilm Strength	Chi-square	0.621	No	Not associated

Table 8: Studies investigating the virulence characteristics of *Candida auris* in comparison with *Candida albicans.*

Virulence trait	Function	*C. albicans* comparison
Thermotolerance	Survival in physiological temperatures	More robust (42 °C) than C. albicans (40 °C) [33]
Osmotolerance	Persistence on dry surfaces	Higher than *C. albicans* [34]
Morphological plasticity	Adaptation under stress	Filamentation rare; pseudohyphae under salt stress; C. albicans shows robust hyphae [35]
Phenotypic switch	Unknown function	Switching observed; more similar to *C. glabrata* than *C. albicans* [36]
Aggregate formation	Tolerance to antifungals; biofilm seeding	*C. auris* forms aggregates due to incomplete budding; *C. albicans* does not aggregate like this [7]
Biofilm formation	Escape from antifungals; surface persistence	Weaker biofilms than *C. albicans* but more resistant due to matrix composition [15,3]
Antifungal resistance	Drug resistance	First MDR *Candida* species; higher resistance than *C. albicans* [27]
Serine aspartyl proteases	Host tissue invasion, adhesion	Lower expression and activity than *C. albicans* [33]
Lipases	Tissue invasion, immune evasion	Weaker than *C. albicans*; strain-dependent [31]
Cell wall stress response	Survival under antifungal pressure	Strong stress response; upregulated chitin synthesis; overlaps with *C. albicans* pathways [37]
Drug efflux pumps	Resistance mechanism	Multiple ABC transporters; similar strategy to *C. albicans* but broader substrate range [25]
Iron acquisition	Essential nutrient scavenging	Uses siderophore uptake pathways like *C. albicans* but fewer heme receptors [37]
Immune evasion	Escaping neutrophil attack, NETs	Better evasion of neutrophil extracellular traps than *C. albicans* [32,38]
Chlamydospore formation	Stress survival	Absent in *C. auris*; present in *C. albicans* [1]
Matrix composition	Protection in biofilms	Different matrix composition; less β-glucan than *C. albicans* [10]

ILLUSTRATIONS (Figures):

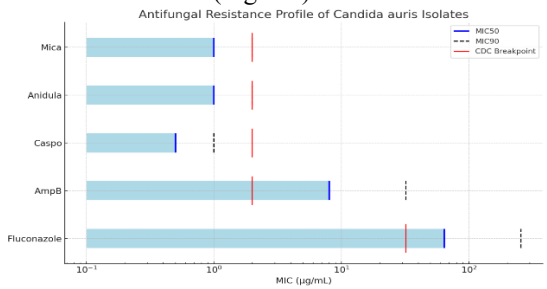

Fig 1: Antifungal resistance profile of *Candida auris* isolates from South India.

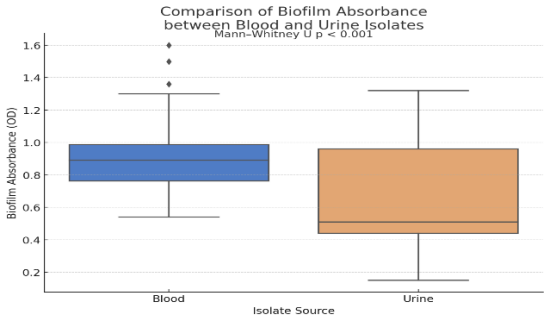

Fig 2: Box plot comparing biofilm absorbance (OD) between bloodstream and urinary isolates of *Candida auris*.

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1108-1116
DoI-10.004687/1000-9035.2025.148

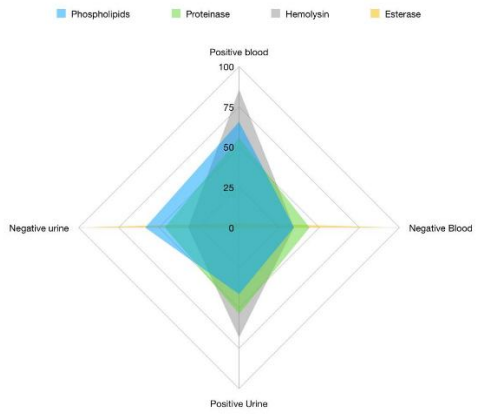

Fig 3: Percentage of isolates among the blood and urine group that were positive and negative for the various enzyme production tests.

REFERENCES:

1. Satoh K, Nishikawa Y, Arakawa Y, Tsuyama M, Yamamoto M, Okano A, et al. Global emergence of Clade V *Candida auris*. Emerg Infect Dis. 2023;29(2):263–271.
2. 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 Apr 28;11(2):10-128.
3. Suphavilai C, Ko KK, Lim KM, Tan MG, Boonsimma P, Chu JJ, Goh SS, Rajandran P, Lee LC, Tan KY, Ismail BB. Detection and characterisation of a sixth Candida auris clade in Singapore: a genomic and phenotypic study. The Lancet Microbe. 2024 Sep 1;5(9).
4. Rudramurthy SM, Chakrabarti A, Paul RA, Soman R, Rajeev N, Kaur H, et al. *Candida auris* candidaemia in Indian ICUs: analysis of risk factors. Mycoses. 2021;64(9):1042–1047.
5. Chowdhary A, Tarai B, Singh A, Sharma A. Multidrug-resistant Candida auris infections in critically ill coronavirus disease patients, India, April–July 2020. Emerging infectious diseases. 2020 Nov;26(11):2694.
6. Chakrabarti A, Chatterjee SS, Shivaprakash MR. Epidemiology and pathogenesis of *Candida* infections in India: challenges and perspectives. J Fungi. 2014;1(1):60–9.
7. Borman AM, Szekely A, Johnson EM. Comparative pathogenicity of United Kingdom isolates of the emerging pathogen Candida auris and other key pathogenic Candida species. MSphere. 2016 Aug 31;1(4):10-128.
8. Wurster S, Bandi A, Beyda ND, Albert ND, Raman NM, Raad II, Kontoyiannis DP. Drosophila melanogaster as a model to study virulence and azole treatment of the emerging pathogen Candida auris. Journal of Antimicrobial Chemotherapy. 2019 Jul 1;74(7):1904-10.
9. Singh R, Kaur M, Chakrabarti A, Shankarnarayan SA, Rudramurthy SM. Biofilm formation by *Candida auris* isolated from colonising sites and candidemia cases. *Mycoses*. 2019;62(8):706–709.
10. Kean R, Delaney C, Sherry L, Borman A, Johnson EM, Richardson MD, et al. Transcriptome assembly and profiling of *Candida auris* reveals novel insights into biofilm-mediated resistance. *mSphere*. 2018;3(4):e00334-18.
11. Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard. 3rd ed. CLSI document M27-A3. Wayne (PA): CLSI; 2008.
12. Centers for Disease Control and Prevention (CDC). Antifungal susceptibility testing and interpretation for *Candida auris*. Available from: https://www.cdc.gov/fungal/candida-auris/c-auris-antifungal.html. Accessed 30 September 2024.
13. Ben-Ami R, Berman J, Novikov A, Bash E, Shachor-Meyouhas Y, Zakin S, et al. Multidrug-resistant *Candida haemulonii* and *C. auris*, Tel Aviv, Israel. *Emerg Infect Dis*. 2017;23(2):195–203.
14. Silva-Dias A, Miranda IM, Branco J, Monteiro-Soares M, Pina-Vaz C, Rodrigues AG. Adhesion, biofilm formation, cell surface hydrophobicity, and antifungal planktonic susceptibility: relationship among *Candida* spp. *Front Microbiol*. 2015;6:205.
15. Sherry L, Ramage G, Kean R, Borman A, Johnson EM, Richardson MD. Biofilm-forming capability of highly resistant *Candida auris*. *Emerg Infect Dis*. 2017;23(2):328–331.
16. Price MF, Wilkinson ID, Gentry LO. Plate method for detection of phospholipase activity in *Candida albicans*. *Sabouraudia*. 1982;20(1):7–14.
17. Kumar D, Banerjee T, Pratap CB, Tilak R. Itraconazole-resistant *Candida auris* with phospholipase, proteinase and hemolysin activity from a case of vulvovaginitis. *J Infect Dev Ctries*. 2015;9(4):435–437.
18. Staib F. Serum proteinase of *Candida albicans*. Its role in pathogenicity. *Sabouraudia*. 1965;4(3):187–193.
19. Osei Sekyere J. *Candida auris*: A systematic review and meta-analysis of current updates on an emerging multidrug-resistant pathogen. *MicrobiologyOpen*. 2018;7(4):e00578.
20. Samaranayake LP, Raeside JM, MacFarlane TW. Factors affecting the phospholipase activity of *Candida* species in vitro. *J Med Microbiol*. 1984;17(1):13–18.
21. Jeffery-Smith A, Taori SK, Schelenz S, Jeffery K, Johnson EM, Borman A, et al. *Candida auris*: a review of the literature. *Clin Microbiol Rev*. 2018;31(1):e00029-17.
22. Bentz ML, Sexton DJ, Welsh RM, Litvintseva AP. Evaluation of CHROMagar Candida Plus for the detection of *Candida auris* with a panel of 206 fungal isolates and 83 colonization screening skin-swabs. *Microbiol Spectr*. 2024;12(2):e03564-23.
23. Radhakrishnan VS, Pathak A, Rego S, Sharma R, Capoor MR. Rapid identification of *Candida auris* by Raman spectroscopy. *Mycoses*. 2019;62(8):708–712.
24. Centers for Disease Control and Prevention. *Candida auris* antifungal susceptibility and surveillance. Available from: https://www.cdc.gov/fungal/candida-auris/c-auris-antifungal.html.
25. Sharma C, Chowdhary A. The changing face of candidemia: emergence of multidrug-resistant *Candida auris*. *Curr Opin Infect Dis*. 2017;30(6):528–535.
26. Kwon YJ, Shin JH, Byun SA, Choi MJ, Won EJ, Lee D, et al. *Candida auris* clinical isolates from South Korea: identification, antifungal susceptibility, and genotyping. *J Clin Microbiol*. 2019;57(4):e01624-18.
27. Lockhart SR, Et al. Simultaneous emergence of multidrug-resistant *Candida auris* on three continents confirmed by whole-genome sequencing and epidemiological analyses. *Clin Infect Dis*. 2017;64(2):134–140.
28. 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(1):15–21.
29. Badali H, Chiller T, Meis JF, Chow NA, de Groot T. Potential fifth clade of *Candida auris*, Iran, 2018. *Emerg Infect Dis*. 2019;25(9):1780–1781.
30. Jacobs SE, J. J. *Candida auris* pan-drug-resistant to four classes of antifungal agents. *Antimicrob Agents Chemother*. 2022;66(7):e00053-22.
31. Larkin E, Hager C, Chandra J, Mukherjee P, Retuerto M, Salem I, et al. The emerging pathogen *Candida auris*: growth phenotype, virulence factors, activity of antifungals, and effect of SCY-078, a novel glucan synthesis inhibitor, on growth morphology and biofilm formation. *Antimicrob Agents Chemother*. 2017;61(5):e02396-16.
32. Johnson CJ, Davis JM, Huttenlocher A, Kernien JF, Nett JE. Emerging fungal pathogen *Candida auris* evades neutrophil attack. *mBio*. 2018;9(4):e01403-18.
33. Wang X, Bing J, Zheng Q, Zhang F, Liu J, Yue H, et al. The first isolate of *Candida auris* in China: clinical and biological

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Volume 35 Issue 3, Year of Publication 2025, Page 1108-1116
DoI-10.004687/1000-9035.2025.148

aspects. *Emerg Microbes Infect*. 2018;7(1):93.
34. Welsh RM, Bentz ML, Shams A, Houston H, Lyons A, Rose LJ, Litvintseva AP. Survival, persistence, and isolation of the emerging multidrug-resistant pathogenic yeast *Candida auris* on a plastic health care surface. *J Clin Microbiol*. 2017;55(10):2996–3005.
35. Kim SH, Iyer KR, Pardeshi L, Muñoz JF, Robbins N, Cuomo CA, et al. Genetic analysis of *Candida auris* implicates Hsp90 in morphogenesis and azole tolerance and Cdr1 in azole resistance. *mBio*. 2019;10(6):e00346-19.
36. Bentz ML, Shams A, Smith RM, Sims EH, Fredricks DN, Harrington SM, et al. Phenotypic switching and stress adaptation of *Candida auris* in a murine model of disseminated infection. *mBio*. 2018;9(5):e01464-18.
37. Muñoz JF, Gade L, Chow NA, Loparev VN, Juieng P, Farrer RA, et al. Genomic insights into multidrug-resistance, mating and virulence in *Candida auris* and related emerging species. *Nat Commun*. 2018;9(1):5346.
38. Horton MV, Nett JE. *Candida auris* aggregation, biofilm formation, and environmental persistence. *J Fungi*. 2020;6(3):138.