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# **Original Research Article**

# Unusual non-albicans candida yeast in South India: Incidence in cancer patients and antifungal susceptibility towards first-line drugs

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# ABSTRACT

**Background:** Unusual Ascomycetes yeasts are frequently reported in obstinate infections among the cancer population. Emergence of resistance among the non-albicans yeasts towards promising first-line azoles queries over treatment options. The present study probes on the recent trends in incidence of *C. albicans* and emerging non- albicans yeasts in cancer patients and to determine the species-wise antifungal susceptibility towards first-line Triazoles and Echinocandin.

**Materials and Methods:** A total of 53 isolates recovered from the clinical specimens collected from 87 cancer patients were presumptively identified on CHROMagar and speciated by sequencing internal transcribed spacer (ITS) target. The species-wise antifungal susceptibility were determined for the first-line triazoles (Fluconazole, Voriconazole, Posaconazole) and Echinocandin (Anidulafungin) following CLSI guidelines.

**Results:** The proportion of Candida isolates were predominantly non-albicans Candida (NAC) species (85%) and *C. albicans* (15%). All isolates were speciated using ITS sequencing and the spectrum of NAC species isolated were dominated with *Yarrowia lipolytica* (17) followed by *Meyerozyma guilliermondii* (9), *C. metapsilosis* (5), C. tropicalis (4), *C. parapsilosis* (3), *Pichia kudriavzevii* (3), *C. glabrata* (2) and one isolate of each *Clavispora lusitaniae* and *Wickerhamiella pararugosa*. The unusual NAC species showed varied resistance profile towards first-line azoles and were susceptible to Anidulafungin.

**Conclusion:** The changing spectrum, high prevalence and pattern of low level azoles susceptibility among unusual NAC species recovered from the cancer population are alarming.

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# 1. Introduction

The hyperendemicity of the unusual non-albicans ascomycetes yeast infections among the cancer population is less determined of its potential threat. The risk for candidiasis among the cancer patients is increased as a consequential to immunosuppression caused due to radiation and/or chemotherapy which further exacerbates the criticality.<sup>1,2</sup> High prevalence of yeast carriage (>90%) has been reported in oral squamous cell carcinoma patients

when compared to healthy and oncotherapy groups.<sup>3</sup> C. albicans was the most predominant pathogenic yeast in the clinical domain and in the recent past the shift in global emergence and incidence rate of non-albicans Candida (NAC) species infections are alarming.<sup>4</sup> In a retrospective investigation, high mortality rate up to 43% was allied with *C. rugosa, C. hemulonii, C. guilliermondii, C. famata, C. lusitaniae* outbreaks.<sup>5</sup> This species drift in epidemics relates with the adapting pathogenicity and aggregating resistance against the first-line azoles and echinocandins amongst the emerging unusual NAC species reported.<sup>6,7</sup>

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Misinterpretation of the NAC species by conventional methods is quiet common and the line of species demarcation has always been misleading diagnosis. This lacuna over reporting of conventional methods is alleviated by nucleic acid sequencing based molecular approaches facilitating higher resolution among closed species complexes. Currently the ITS region of the ribosomal nuclear DNA is an authentic universal barcode signature demarking genetic diversity amongst medically important yeast. The non-coding ITS1-4 region is the most favored phylogenetic loci with vastly conserved domain showing interspecies variations enabling reliable speciation.<sup>8,9</sup> Exclusively, the ITS1-4 segment is also an easy target for amplification with multiple copies, thus an authentic barcode.<sup>10</sup>

High prevalence of intrinsic resistance among the rare yeast is frequently reported and the limited data of speciesspecific epidemiological cutoff values (ECV), clinical break points (CBP) hinders broad spectrum antifungal regimen. Assigning species-specific ECVs for first-line antifungals against rare isolates would help in monitoring the emergence of decreased susceptibility, chiefly among the more susceptible C. albicans, C. tropicalis, and C. parapsilosis. Exemplified recent records shows elevated minimum inhibitory concentration (MIC) for first line antifungals against a significant proportion of unusual NAC isolates included C. lusitaniae, C. intermedia, C. rugosa, W. pararugosa and Y. lipolytica which interrogate the spontaneous development of cross resistance<sup>4,6,7,11</sup>These factors emphasize the need for large-scale epidemiological surveillances to monitor species-specific resistant patterns in order to correlate between in vitro susceptibility and positive clinical outcome.<sup>12</sup> In the present study we investigated the spectrum of unusual non-albicans Ascomycetes prevalent among the cancer population and the species-specific MIC for the first-line antifungals.

# 2. Materials and Methods

# 2.1. Clinical isolates and mycological investigations

All the clinical specimens were collected from oral and cervical cancer patients registered in the Department of Surgical Oncology, Arignar Anna Memorial Cancer Hospital, Kancheepuram, Tamil Nadu, India and the study was approved by Institutional Human Ethical Committee, University of Madras. In total 87 non-repeat cancer patients (male-45, female-42) suggestive of candidiasis were recruited in the study. The swabs from the oral (63) and vaginal (24) infected site were collected aseptically, transported in cold chain at 4°C. The swab was inoculated onto Sabouraud's Dextrose agar (SDA) followed by incubation at 37°C for 24-48hrs. A total of 53 specimens were positive for direct microscopy, culture and all the isolates were phenotyped presumptively using CHROMagar

medium (CHROMagar, Paris, France). The pure culture isolates were preserved in glycerol stock maintained at  $-20^{\circ}$ C until further processing.

# 2.2. Molecular characterization by ITS sequencing

Total genomic DNA was extracted from the Candida isolates using lithium acetate (LioA3) extraction protocol.<sup>13</sup> PCR for the amplification of the ITS region was performed using forward primer ITS1 (5'-TCCGTAGGTGAACCTGCG-3') and reverse ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Sigma-Aldrich, USA)<sup>6</sup>. A total reaction volume of  $50\mu$ l containing ~100ng/ml of genomic DNA, 10pmol of each primer, and 10x PCR assay mix with 25mM MgCl2, 0.3µl of Tag polymerase and sterile PCR grade water were used for PCR reactions performed in a thermocycler (T100, Bio Rad, USA). Thermocyclic PCR amplification was optimized under the following conditions: an initial denaturation at 95°C for 10 min; cycling denaturation at 94°C for 1min, annealing at 56°C for 30 sec, and extension at 72°C for 1min for 35 cycles; and a final extension step at 72°C for 7 min. The PCR was performed with the inclusion of positive and negative controls. The amplicon of 250-880bp size generated were electrophoresed and subjected to purification using Microcon Centrifugal Filter Units (Millipore, Billerica, MA), according to the manufacturer's instructions. Cycle sequencing reactions were performed on both strands per isolate with ITS 1 and ITS 4 as sequencing primers by using Big Dye Terminator version 3.1 kit (Applied Biosystems, USA). The ITS domain consensus sequences were then subjected to Basic Local Alignment Search Tool (BLAST) deposited at Genbank database and the referred homology matches with >99% was regarded confirmatory at both genus and species level, identities between 93%-98% were considered for genus identification.

# 2.3. In vitro antifungal susceptibility testing

The in vitro antifungal susceptibility towards the firstline triazoles included Posaconazole (POS) (Sigma-Aldrich, USA), Fluconazole (FLC), Voriconazole (VOR), and Echinocandin- Anidulafungin (ANF) (Pfizer, NY, USA) were determined by using broth microdilution assay as per CLSI M27-S4 guidelines.<sup>4</sup>*C. albicans* IFM 40009 was used as quality control strain. The MICs were interpreted as sensitive (S), susceptible- dose dependent (SDD), intermediate resistant (I) and resistant (R) as per the speciesspecific CBPs, ECVs outlined in CLSI document<sup>14–16</sup>. Due to lack of species-specific interpretive CBPs/ECVs for the isolates such as *Y. lipolytica, M. guilliermondii, C. lusitaniae* and *W. pararugosa* the corresponding MIC values were evaluated according to earlier reports.<sup>12,17,18</sup> Umamaheswari K, Ranganathan and Chellappa / IP International Journal of Medical Microbiology and Tropical Diseases 2022;8(4):331–335

# 3. Results

#### 3.1. Prevalence and species distribution of NAC isolates

A total of 53/87 specimens included oral swab (38) and vaginal swab (15) were culture positive for Candida. The overall incidence of NAC species (85%) was proportionately higher compared to C. albicans (15%) in our study. The spectrum of NAC species were dominated with Y. lipolytica (38%) followed by M. guilliermondii (20%), C. metapsilosis (11%), C. tropicalis (9%), C. parapsilosis (7%), P. kudriavzevii (7%), C. glabrata (4%) and one isolate of each C. lusitaniae (2%), W. pararugosa (2%). Reason for high incidence of Y. lipolytica recovery from vaginal site (71%) is unclear whilst all other unusual isolates of C. metapsilosis, W. pararugosa and C. lusitaniae were isolated from oral cavity. The dominance of diverse unusual yeasts was recorded in the present study is indicative of the gradual shift of NAC endemicity in cancer patients.

#### 3.2. Distribution for the first line antifungals

The overall species-wise elevated MICs and reduced pattern of susceptibility towards first-line antifungals was observed for the C. albicans and NAC isolates tested in the present study. Antifungal susceptibility data for all isolates tested are presented in Table 1. The CBPs pattern for all the azoles, ANF varied species-wise and was unique to each individual isolate tested. Seven of the eight isolates of C. albicans showed higher mean  $MIC_{90}>32\mu g/ml$ ,  $>8\mu g/ml$ ,  $>8\mu g/ml$ ,  $>1\mu g/ml$  against FLC, VOR, POS, ANF respectively. Only two strains of M. guilliermondii were FLC, VOR resistant and other isolates were marginally susceptible towards azoles and ANF. Three of the C. tropicalis isolates tested were FLC, VOR, POS resistant and ANF susceptible. Contrarily most of C. parapsilosis, C. krusei, C. glabrata isolates known of prevalent intrinsic resistance were tested susceptible towards all the azoles and ANF. We observed the decreased susceptibility among the more susceptible species of C. abicans, M. guillermondii, C. tropicalis, C. parapsilosis and C. glabrata respective of its species-specific ECVs and CBPs. The hierarchy of first-line antifungals effective against the test isolates was observed as follows: ANF>FLC>VOR>POS.

High CBP for ANF >1 $\mu$ g/ml was recorded against the rare isolates of *C. lusitaniae, C. pararugosa, Y. lipolytica and C. metapsilosis.* Eight *Y. lipolytica* isolates were ANF resistant (47%) and azole susceptible (88%). Varied resistant profile was prominent among the *C. metapsilosis* isolates towards POS, only one isolate was found to be resistant to all the three azoles and all were ANF susceptible. *W. pararugosa* and *C. lusitaniae* isolates were found to be ANF resistant and both were azoles susceptible at lower CBPs. In our study reduced susceptibility towards ANF were detected among the four unusual NAC isolates despite ANF was effective against most of the azoles resistant isolates; MIC range  $0.03-0.125 \mu g/ml$ . In the present study, the susceptibility pattern observed for first-line drugs against unusual NAC species were chiefly variant species-wise thus enforcing the need for right choice of therapeutic regimen.

#### 4. Discussion

The present study reports high incidence of the oral, vaginal Candida infection among the cancer population recruited. We observed a high proportion of Y. lipolytica, M. guilliermondii isolate were predominating over the C. albicans incidence rate which were in concordance with earlier findings.<sup>4,11,19</sup> The other common NAC species frequently isolated were C. glabrata, C. tropicalis, C. parapsilosis and C. krusei. In the recent past the gradual rise in C. lusitaniae cases among cancer patients receiving chemotherapy is prominent.<sup>20</sup> Y. lipolytica are frequently employed in food and pharmaceutical industry citing the probable ecological resource of such unique pathogenic community. The incidence of Y. lipolytica are reviewed in very few case series and epidemiological data on prevalence and antifungal resistance profile is poorly studied elsewhere.<sup>19</sup> The unusual isolates of C. metapsilosis closely related to the C. parapsilosis are poorly distinguished making speciation cumbersome within most of the resource limited laboratories. Genomic explorations have led to the discovery that C. orthopsilosis and C. metapsilosis were derived from the hybridization of species with nonpathogenic lineages. The heterogeneity existing in the ITS region favored discrimination of C. orthopsilosis from other members of the C. parapsilosis complex, which are predominantly clonal with limited genotypic variations. Contrarily, W. pararugosa taxon though not a part of C. rugosa complex frequently misidentified when only phenotypic characteristics are analyzed.<sup>11,21</sup>

Our study revealed an overall reduced susceptibility of the more susceptible isolates of Candida species towards the first-line azoles. Currently, therefore are no CBP or interpretive criteria for rare species and antifungals. The role of ECVs is significant in the potential discrimination of the resistant isolates into NWT wherein the species- specific CBPs are absent.<sup>4</sup> In the present study CBPs for the unusual clinical isolates of Y. *lipolytica*, W. pararugosa, C.lusitaniae and C. metapsilosis ranged from  $0.125\mu$ g/ml to  $>16\mu$ g/ml for the azoles tested.

*C. albicans* and *M. guilliermondii* both showed high MIC values to FLC which was in concordance with earlier reports. <sup>12,18</sup> *Y. lipolytica* isolates were found to be less susceptible towards FLC in our study and the MIC<sub>90</sub> cutoff for VOR and POS were at the highest dilution. Reduced ANF susceptibility was prominent among the isolates of *Y. lipolytica*. Similar susceptibility profile of *Y. lipolytica* isolates is reported earlier showing low MIC cutoff to

Table 1: Species-wise antifungal susceptibility profile of the candida isolates

Species (No of		Antifungals Susceptibility Profile (MIC in $\mu$ g/ml)			
isolates tested ).		Fluconazole (FLC)	Voriconazole (VOR)	Posaconazole (POS)	Anidulafungin (ANF)
C. albicans (8)	MIC range	0.125-64	0.03-16	0.03-16	0.015-8
	$MIC_{90} \mu g/ml$	>32	>8	>8	>1
Y. lipolytica (17)	MIC range	0.5-32	0.06-16	0.06-16	0.06-4
	$MIC_{90} \mu g/ml$	>8	1	>1	>1
M. guilliermondii (9)	MIC range	0.25-16	0.06-1	0.03-1	0.06-8
	$MIC_{90} \ \mu g/ml$	8	0.25	0.25	>2
C. metapsilosis (5)	MIC range	0.25-64	0.03-16	0.06-16	0.06-2
	MIC <sub>90</sub> $\mu$ g/ml	16	<4	<4	<1
C. tropicalis (4)	MIC range	2-64	0.125-16	0.03-16	0.03-1
	$MIC_{90} \mu g/ml$	>32	>8	>8	0.25
C. parapsilosis (3)	MIC range	0.5-1	0.015-1	0.015-1	0.5-2
	$MIC_{90} \ \mu g/ml$	>1	< 0.5	< 0.5	>1
P. kudriavzevii (3)	MIC range	8-16	0.03-0.25	0.125-0.25	0.03-0.125
	$MIC_{90} \mu g/ml$	>8	0.25	< 0.25	0.06
C. glabrata (2)	MIC range	2-4	0.125	0.06	0.06-0.125
	$MIC_{90} \mu g/ml$	3	0.125	0.06	0.09
C. lusitaniae (1)	MIC range	-	-	-	-
	$MIC_{90} \mu g/ml$	2	0.125	0.5	1
W. pararugosa (1)	MIC range	-	-	-	-
	$MIC_{90} \mu g/ml$	0.5	0.125	0.5	1

Species-wise antifungal susceptibility profile of the candida isolates

echinocandins and higher MIC cutoff towards FLC.<sup>19</sup> In our study decreased in vitro susceptibility towards azoles among the isolates of C. albicans, M. guilliermondii, C.tropicalis, Y. lipolytica. C.metapsilosis and C. parapsilosis were in agreement with earlier reports.<sup>12,17</sup> The isolates of P. kudriavzevii, C. glabrata are known of intrinsic resistance to FLC and indigenous development of cross resistance to other azoles thus makes its corresponding CBP's not to be considered irrespective of its susceptibility.<sup>22</sup> Overall the antifungal resistance was low among the common NAC species of C. tropicalis and C. parapsilosis tested in the present study except for the reduced susceptibility towards FLC. However, the susceptibility profile of unusual yeast is poorly investigated globally and recent interpretive MIC CBPs may not be suited for rare isolates.<sup>23</sup> Moreover, the absence of therapeutic clinical data on the individual rare isolate in question would misguide physician in appropriate choice of drug.24

# 5. Conclusion

High incidence rate of unusual yeast isolation in this study clearly shows the species drift towards emerging pathogenic community and insists on the need for regionalwise epidemiological investigation among the high risk cancerous population. The changing antifungal resistance patterns are dependent on individual species and therefore the choice over antifungals needs to be challenged *in vitro* ahead of clinical regimen.

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#### 7. Conflict of Interest

None.

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