Content available at: https://www.ipinnovative.com/open-access-journals

IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Original Research Article

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

Umamaheswari K[1,*], Gnanasekar Ranganathan[1], Arun Kumar Chellappa[2]

[1]*Dept. of Biotechnology, University of Madras, Guindy Campus, Chennai, Tamil Nadu, India*
[2]*Dept. of Dental Surgery, Arignar Anna Memorial Cancer Hospital & Research Institute, Kanchipuram, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 05-11-2022
Accepted 01-12-2022
Available online 28-12-2022

Keywords:
Candida metapsilosis
Wickerhamiella pararugosa
Yarrowia lipolytica. Susceptibility testing
Anidulafungin

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.

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.[1,2] High prevalence of yeast carriage (>90%) has been reported in oral squamous cell carcinoma patients when compared to healthy and oncotherapy groups.[3] 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.[4] In a retrospective investigation, high mortality rate up to 43% was allied with *C. rugosa, C. hemulonii, C. guilliermondii, C. famata, C. lusitaniae* outbreaks.[5] 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.[6,7]

* *Corresponding author*.
E-mail address: rk_uma@yahoo.com (Umamaheswari K).

https://doi.org/10.18231/j.ijmmtd.2022.064

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.[8,9] Exclusively, the ITS1-4 segment is also an easy target for amplification with multiple copies, thus an authentic barcode.[10]

High prevalence of intrinsic resistance among the rare yeast is frequently reported and the limited data of species-specific 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[4,6,7,11] 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.[12] 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°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.[13] 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)[6]. A total reaction volume of 50μl containing ~100ng/ml of genomic DNA, 10pmol of each primer, and 10x PCR assay mix with 25mM MgCl2, 0.3μl of Taq 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 first-line 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.[4] *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 species-specific CBPs, ECVs outlined in CLSI document[14–16]. 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.[12,17,18]

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}>32μg/ml, >8μg/ml, >8 μg/ml, >1μg/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 >1μg/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 0.03-0.125μg/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.[4,11,19] 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.[20] *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.[19] 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 non-pathogenic 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.[11,21]

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.[4] In the present study CBPs for the unusual clinical isolates of Y. *lipolytica, W. pararugosa, C.lusitaniae* and *C. metapsilosis* ranged from 0.125μg/ml to >16μg/ml for the azoles tested.

C. albicans and *M. guilliermondii* both showed high MIC values to FLC which was in concordance with earlier reports.[12,18] *Y. lipolytica* isolates were found to be less susceptible towards FLC in our study and the MIC_{90} 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 isolates tested).		Antifungals Susceptibility Profile (MIC in μg/ml)			
		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} μg/ml	>32	>8	>8	>1
Y. lipolytica (17)	MIC range	0.5-32	0.06-16	0.06-16	0.06-4
	MIC_{90} μg/ml	>8	1	>1	>1
M. guilliermondii (9)	MIC range	0.25-16	0.06-1	0.03-1	0.06-8
	MIC_{90} μg/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_{90} μg/ml	16	<4	<4	<1
C. tropicalis (4)	MIC range	2-64	0.125-16	0.03-16	0.03-1
	MIC_{90} μg/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} μg/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} μg/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} μg/ml	3	0.125	0.06	0.09
C. lusitaniae (1)	MIC range	-	-	-	-
	MIC_{90} μg/ml	2	0.125	0.5	1
W. pararugosa (1)	MIC range	-	-	-	-
	MIC_{90} μg/ml	0.5	0.125	0.5	1

Species-wise antifungal susceptibility profile of the candida isolates

echinocandins and higher MIC cutoff towards FLC.[19] 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.[12,17] 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.[22] 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.[23] 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 regional-wise 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.

6. Source of Funding

DBT, Govt of India for funding this research work.

7. Conflict of Interest

None.

Acknowledgment

The authors acknowledges DBT, Govt of India for funding this research work, Pfizer, NY, USA for the provision of antifungal drugs and the Director, Arignar Anna Memorial Cancer Hospital, Kancheepuram, Tamil Nadu, India for clinical specimens.

References

1. Li D, Xia R, Zhang Q, Bai C, Li Z, Zhang P. Evaluation of candidemia in epidemiology and risk factors among cancer patients in a cancer center of China: an 8-year case-control study. *BMC Infect Dis*. 2017;17(1):536. doi:10.1186/s12879-017-2636-x.
2. Soni P, Soni LK, Verma SK. Non Candida albicans Candida (NCAC) of oral cavity in head and neck cancer patients under cancer therapy: Prevalance, species identification and antimycotic sensitivity pattern. *Int J Med Microbiol Trop Dis*. 2018;4(2):88–93.
3. Berkovits C, Tóth A, Szenzenstein J, Deák T, Urbán E, Gácser A. Analysis of oral yeast microflora in patients with oral squamous cell carcinoma. *Springer plus*. 2016;5(1):1257. doi:10.1186/s40064-016-2926-6.
4. Falagas ME, Roussos N, Vardakas KZ. Relative frequency of albicans and the various non-albicans Candida spp among candidemia isolates from in patients in various parts of the world: a systematic review. *Int J Infect Dis*. 2010;14(11):954–66.

5. Tak V, Mathur P, Varghese P, Gunjiyal J, Xess I, Misra MC. The Epidemiological Profile of Candidemia at an Indian Trauma Care Center. *J Lab Physicians*. 2014;6(2):96–106.
6. Fujita S, Senda Y, Nakaguchi S, Hashimoto T. Multiplex PCR Using Internal Transcribed Spacer 1 and 2 Regions for Rapid Detection and Identification of Yeast Strains. *J Clin Microbiol*. 2001;39(10):3617–39.
7. Sanguinetti M, Posteraro B. Susceptibility Testing of Fungi to Antifungal Drugs. *J Fungi*. 2018;4(3):110. doi:10.3390/jof4030110.
8. Ciardo DE, Schar G, Böttger EC, Altwegg M, Bosshard PP. Internal Transcribed Spacer Sequencing versus Biochemical profiling for Identification of medically important yeasts. *J Clin Microbiol*. 2006;44(1):77–84.
9. Taverna CG, Bosco-Borgeat ME, Murisengo OA, Davel G, Boité MC, Cupolillo E. Comparative analyses of classical phenotypic method and ribosomal RNA gene sequencing for identification of medically relevant Candida species. *Mem Inst Oswaldo Cruz*. 2013;108(2):178–85.
10. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *PNAS*. 2012;109(16):6241–7.
11. Merseguel KB, Nishikaku AS, Rodrigues AM, Padovan AC, Ferreira RC, Melo AS. Genetic diversity of medically important and emerging Candida species causing invasive infection. *BMC Infect Dis*. 2015;15:57. doi:10.1186/s12879-015-0793-3.
12. Stavrou A, Hansen AP, Lackner M, Flörl CL, Boekhout T. Elevated minimum inhibitory concentrations to antifungal drugs prevail in 14 rare species of candidemia-causing Saccharomycotina yeasts. *Med Mycol*. 2020;58(7):987–95.
13. Lõoke M, Kristjuhan K, Kristjuhan A. Extraction of genomic DNA from yeasts for PCR-based applications. *Biotechniques*. 2011;50(5):325–33.
14. Borman AM, Muller J, Szekely A. MIC distributions for amphotericin B, fluconazole, itraconazole, voriconazole, flucytosine and anidulafungin and 35 uncommon pathogenic yeast species from the UK determined using the CLSI broth microdilution method. . *J Antimicrob Chemother*. 2020;75(5):1194–1205.
15. Clinical and Laboratory Standards Institute document M60-Performance standard for antifungal susceptibility testing of yeast; 2020. p. 27–31.
16. Clinical and Laboratory Standards Institute- Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; 2017. p. M2–S4.
17. Ingroff AE, Turnidge J. The role of epidemiological cutoff values (ECVs/ECOFFs) in antifungal susceptibility testing and interpretation for uncommon yeasts and moulds. *Rev Iberoam Micol*. 2016;33(2):63–70.
18. Tseng TY, Chen TC, Ho CM, Lin PC, Chou CH, Tsai CT. Clinical features, antifungal susceptibility, and outcome of Candida guilliermondii fungemia: An experience in a tertiary hospital in mid-Taiwan. *J Microbiol Immunol Infec*. 2018;51(4):552–60.
19. Zhao Y, Chan JF, Tsang CC, Wang H, Guo D, Pan Y. Clinical characteristics, laboratory identification, and in vitro antifungal susceptibility of Yarrowia (Candida) lipolytica isolates causing Fungemia: a multicenter, prospective surveillance study. *J Clin Microbiol*. 2015;53(11):3639–84.
20. Khan Z, Ahmad S, Sweih NA, Khan S, Joseph L. Candida lusitaniae in Kuwait: Prevalence, antifungal susceptibility and role in neonatal fungemia. *PLoS ONE*. 2019;14(3):213532. doi:10.1371/journal.pone.0213532.
21. Tavanti A, Davidson AD, Gow N, Maiden M. Odds FC. Candida orthopsilosis and Candida metapsilosis spp. nov. To replace Candida parapsilosis Groups II and III. *J Clin Microbiol*. 2005;43(1):284–92.
22. Arastehfar A, Daneshnia F, Zomorodian K, Najafzadeh MJ, Khodavaisy S, Zarrinfar H. Low Level of Antifungal Resistance in Iranian Isolates of Candida glabrata Recovered from Blood Samples in a Multicenter Study from 2015 to 2018 and Potential Prognostic Values of Genotyping and Sequencing of PDR1. *Antimicrob Agents Chemo*. 2019;63(7):2503–21.
23. Leaw SN, Chang CH, Sun HF, Barton R, Bouchara JP, Chang TC. Identification of Medically Important Yeast Species by Sequence Analysis of the Internal Transcribed Spacer Regions. *J Clin Microbiol*. 2006;44(3):693–9.
24. Borman AM, Muller J, Quantick JW, Szekely A, Patterson Z, Palmer MD. MIC distributions for amphotericin B, fluconazole, itraconazole, voriconazole, flucytosine and anidulafungin and 35 uncommon pathogenic yeast species from the UK determined using the CLSI broth microdilution method. *J Antimicrob Chemother*. 2020;75(5):1194–205.

Author biography

Umamaheswari K, Associate Professor https://orcid.org/0000-0003-1528-4002

Gnanasekar Ranganathan, Research Student

Arun Kumar Chellappa, Dental Surgeon

Cite this article: Umamaheswari K, Ranganathan G, Chellappa AK. Unusual non-albicans candida yeast in South India: Incidence in cancer patients and antifungal susceptibility towards first-line drugs. *IP Int J Med Microbiol Trop Dis* 2022;8(4):331-335.