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Spectrum of Invasive Yeast Infections in Neonates, Children and Adults in Pakistan Over Five Years: 2015-2019

Salima Rattani*, Saba Memon**, Kauser Jabeen*, Joveria Farooqi*

*Department of Pathology and Laboratory medicine, Aga Khan University Hospital, Karachi, Pakistan
**Department of Microbiology, Karachi University, Karachi, Pakistan

Abstract

Background
There is a widening population of hosts susceptible to invasive yeast infections secondary to prolonged hospitalization, immunocompromised, use of medical devices, broad-spectrum antibiotics, and survival of extremely vulnerable populations. Here we describe the changing spectrum of invasive yeast infections in neonates, children, and adults in the last 5 years in Pakistan.

Methods
Records of archived yeast isolates from Jan 2015-May 2019 kept at the Aga Khan University Clinical Laboratories, section of Microbiology in Karachi, were retrieved for analysis. Frequencies of different *Candida* and non-*Candida* species isolated over the last five years were computed and compared for age groups using chi-square test.

Results
A total of 1119 non-duplicate isolates were identified, of which 992 were invasive specimens. *Candida* species made up 86.7%, 88.2% and 88.1% of all invasive yeasts in neonates, children, and adults, respectively. Rare *Candida* were most common group in neonates, *C. tropicalis* predominated in children and *C. auris* topped the list of invasive yeasts in adults. The distribution of invasive yeasts between neonatal, pediatric and adult age groups was different significantly ($p<0.001$). Change in spectrum of invasive yeast over the years was significant for neonates ($p=0.017$), and adults ($p=0.003$). Fluconazole resistance in yeast isolated from neonates, children and adults was found to be 8%, 23% and 36% respectively.

Conclusion
It is essential to monitor the spectrum and antifungal resistance trends of yeast infections in neonates and children as they are the population where there is greatest diversity of invasive species, not limited to Candida species.

Corresponding Author: Joveria Farooqi,
Department of Pathology and Laboratory Medicine,
Aga Khan University,
Stadium Road, Karachi, Pakistan
Email: joveria.farooqi@aku.edu

Keywords
Invasive yeast infections, spectrum, Candida, antifungal resistance, neonates, children, adults.

Introduction
The incidence of invasive yeast infections is on the rise due to expanding population of susceptible hosts. These infections can occur secondary to prolonged hospitalization, use of medical devices, drugs or diseases affecting immune response, broad-spectrum antibiotics, and survival of extremely vulnerable populations like preterm babies, patients with solid organ or hematological malignancies and geriatrics.[1-3]

The spectrum of invasive fungal infections includes fungemia, intra-abdominal infections, septic arthritis, iatrogenic and neonatal meningitis. *Candida* species are most commonly involved, however *Cryptococcus* species, *Trichosporon* species, *Malassezia* species and others are also implicated.[3]

In a previous study from our center on spectrum of candidiasis (2006-2009), neonatal and pediatric age group differed greatly from the adults.[1] To explore this difference in neonatal and pediatric group further, we conducted a review of our laboratory data from the last 5 years.

Methods
This study reviewed laboratory data of the Aga Khan University Hospital (AKUH) laboratory, Karachi, Pakistan. Records of reported yeast isolates from Jan 2015-May 2019 were retrieved for analysis.

Invasive yeast was defined as isolates yielded from blood, CSF, wounds, intraabdominal collections, central venous lines, and other sterile fluids positive for yeast (*Candida* species, *Ustilago* species, *Trichosporon* species, *Rhodotorula* species and *Cryptococcus* species). Duplicate cases were excluded if more than one culture from a single patient was positive for the same organism. The data was divided into 3 groups based on patient age including neonates (0 to 30 days of life), pediatric patients (>1 month to <18 years) and adults (≥18 years).

Conventional phenotypic methods were used to identify yeasts isolated from different cultures. These included productions of

a germ tube, morphology on BBL BiGGY Agar (BD) and chrome agar, growth with cycloheximide, urease production, and morphology on cornmeal/Tween 80 agar. API 20C AUX® (bioMe´rieux) was used to generate an identification profile for isolates which could not be identified by these methods, that is, species other than *Candida albicans, C. tropicalis, C. parapsilosis* and *C. glabrata*. Those candida species other than *C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, C. krusei* and *C. auris* were termed "rare *Candida* species". For *Ustilago* species, identification was done using phenotypic methods including microscopic morphology (string bean pseudohyphae), growth on different media (initially mucoid, later dry membranous colonies) and susceptibility pattern (resistance to echinocandins and flucytosine).

Antifungal susceptibility testing for fluconazole and voriconazole was performed by disc diffusion for *C. albicans, C. tropicalis, C. parapsilosis* and interpreted according to CLSI M60. For all other non-*C. albicans* candida species antifungal susceptibility testing was performed by broth microdilution with fluconazole, itraconazole, voriconazole, posaconazole, anidulafungin, caspofungin, micafungin and amphotericin B as described by the Clinical and Laboratory Standards Institute (CLSI) using Sensititre™ YeastOne™ YO9 AST Plate. *Candida glabrata, Candida krusei* MIC were reported according to CLSI guidelines.[4] For other candida species, fluconazole was considered resistant at an MIC of ≥4 µg/ml in accordance with clinical breakpoints for fungi by EUCAST.[5] In the absence of clinical breakpoints, epidemiological cut-off values (ECVs) were used to interpret MICs as those conforming to wild-type or non-wild-type for a particular antifungal agent against a specific species based on CLSI guidelines.[5,6]

Data was exported from laboratory information system to Microsoft Excel 2010 from the relevant study period and duplicates were removed as described. Data was then imported into Stata/SE version 12.1 (2012) for analysis. Frequencies of different *Candida* and non-*Candida* species isolated over the last 5 years were computed and compared for age groups using chi-square test. Hypothesis of a change in the spectrum over the years among each age group was also tested.

Results

A total of 1119 non-duplicate isolates were identified, of which 992 were invasive specimens: from blood (n=833), CSF (n=51), wounds (n=47), intra-abdominal collections (n=36), central venous lines (17) and other sterile fluids (8). The patients were divided into neonates (n=259), pediatric age group (n=253) and adults (n=477). *Candida* species made up 86.7%, 88.2% and 88.1% of all invasive yeasts in neonates, children, and adults, respectively. The most common group in neonates was a diverse group of rare *Candida* species (including *C. lusitaniae, C. guilliermondii* and *C. pelliculosa, C. utilis, C. kefyr, C. famata, C. rugosa, Kodamaea ohmeri* and unidentified *Candida* species); *Ustilago* species were the most common non-candida yeast (Table 1). In children, the most common species was *C.*

Table 1: Spectrum of invasive yeasts in neonates 0 to 30 days of life

Organism	2015	2016	2017	2018	2019	2015-May 2019	%
Candida albicans	11	2	8	4	3	28	10.0%
Candida krusei	3	0	2	0	2	7	2.5%
Candida glabrata	0	0	0	1	0	1	0.4%
Candida parapsilosis	9	7	17	4	3	40	14.2%
Candida tropicalis	9	16	29	22	1	77	27.4%
Rare *Candida* species	24	21	37	23	20	125	*44.5%
Candida auris	0	2	1	0	0	3	1.1%
All Candida	56	48	94	54	29	281	86.7%
Ustilago species	2	6	10	10	5	33	±10.2%
Trichosporon species	2	1	1	0	0	4	1.2%
Rhodotorula species	2	0	3	1	0	6	1.9%
Malassezia species	0	0	0	0	0	0	0.0%
Cryptococcus neoformans	0	0	0	0	0	0	0.0%
Cryptococcus non-neoformans	0	0	0	0	0	0	0.0%
Kloeckera species	0	0	0	0	0	0	0.0%
All Non-Candida	6	7	14	11	5	43	13.3%
All Yeasts	62	55	108	65	34	324	

p-value: 0.017

*most common *Candida* species causing invasive yeast infections

±most common non-*Candida* species causing invasive yeast infections

Rare *Candida* species: *Candida* species other than *C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, C. krusei* and *C. auris*

tropicalis; *Ustilago* was again the most common non-candida yeast (Table 2). Most notably in adults, *C. auris* topped the list of invasive yeasts, while *C. neoformans* was the most common non-candida yeast (Table 3). There was a significant difference in the distribution of invasive yeasts between neonatal, pediatric and adult age groups ($p<0.001$). Change in spectrum of invasive yeast over the years was significant for neonates ($p=0.017$), and adults ($p=0.003$); however, we could not demonstrate a significant difference in spectrum within infants and children ($p=0.417$). Fluconazole resistance in yeast isolated from neonates was found to be 7.9% while it was 22.5% in infants and children, and 36.3% in adults.

Discussion

Candida species were the most common of all invasive yeasts in neonates, children, and adults. The spectrum of invasive yeast infections has changed significantly over the years with rare *Candida* species (including *C. lusitaniae, C. guilliermondii* and *C. pelliculosa* to name a few) being the most common cause in neonates and *C. auris* being the most common invasive yeast infection in adults. However, there was no significant change in spectrum of these infections among infants and children.

The spectrum of invasive yeast infections is changing. Studies show candidemia to be the most common cause of invasive yeast infection worldwide[7,8] similar to our study.

Candida albicans had been the predominantly reported *Candida* species associated with neonatal candidemia in Pakistan before 2006[2]. Later studies indicated that *C. tropicalis* became the most common candida species identified in neonates with candidemia[1,9]. In contrast, study from India shows *C. parapsilosis* and *C. glabrata* to be the most common *Candida* species as a cause of neonatal candidemia during recent years.[10] Our study shows rare *Candida* species (including *C. lusitaniae, C. guilliermondii* and *C. pelliculosa* to name a few) to be the most common cause of invasive yeast infections followed by *C. tropicalis* (27.4%) and *C. parapsilosis* (14.2%). *C. glabrata* (0.4%) was rarely seen in our neonatal population.

Studies from developed countries show *C. parapsilosis* followed by *C. tropicalis* to be the most common yeast causing infections in children specifically in patients with hematologic malignancies, *C. albicans* in patients needing intensive care followed by *C. parapsilosis* and *C. albicans* in children with solid organ transplants.[11,12] Older studies from developing countries (before 2015) show *C. albicans* to be most common amongst children[1,13] in contrast to our study where *C. tropicalis* was found to be the most common cause of invasive yeast infection in children in our population.

Some studies show *C. albicans* to be the most common cause of invasive yeast infection in adults.[14,15] Studies from Pakistan report non-*C. albicans candida* species like *Candida tropicalis*

Table 2: Spectrum of invasive yeasts in Children >1 month to <18y

Organism	2015	2016	2017	2018	2019	2015-May 2019	%
Candida albicans	8	2	10	3	6	29	16.9%
Candida krusei	0	0	1	1	1	3	1.7%
Candida glabrata	3	0	1	0	2	6	3.5%
Candida parapsilosis	4	1	13	5	2	25	14.5%
Candida tropicalis	7	10	14	9	9	49	*28.5%
Rare *Candida* species	7	3	6	8	5	29	16.9%
Candida auris	2	5	7	11	6	31	18.0%
All Candida	31	21	52	37	31	172	88.2%
Ustilago species	5	1	3	3	6	18	±9.2%
Trichosporon species	0	0	1	0	1	2	1.0%
Rhodotorula species	0	0	1	0	0	1	0.5%
Malassezia species	0	0	0	0	0	0	0.0%
Cryptococcus neoformans	0	0	0	0	1	1	0.5%
Cryptococcus non-neoformans	0	0	1	0	0	1	0.5%
Kloeckera species	0	0	0	0	0	0	0.0%
All Non-Candida	5	1	6	3	8	23	11.8%
All Yeasts	36	22	58	40	39	195	

p-value: 0.417

*most common *Candida* species causing invasive yeast infections

±most common non-*Candida* species causing invasive yeast infections

Rare *Candida* species: *Candida* species other than *C. albicans, C. tropicalis, C. parapsilosis, C. glabrata, C. krusei* and *C. auris*

Table 3: Spectrum of invasive yeasts in Adults

Organism	2015	2016	2017	2018	2019	2015-May 2019	%
Candida albicans	17	9	28	17	13	84	20.0%
Candida krusei	0	0	3	3	0	6	1.4%
Candida glabrata	7	5	21	15	25	73	17.4%
Candida parapsilosis	16	7	32	11	10	76	18.1%
Candida tropicalis	17	12	15	9	9	62	14.8%
Rare *Candida* species	4	4	8	4	6	26	6.2%
Candida auris	6	7	40	26	14	93	*22.1%
All Candida	67	44	147	85	77	420	88.1%
Ustilago species	0	0	2	1	0	3	0.6%
Trichosporon species	3	0	1	4	1	9	1.9%
Rhodotorula species	3	0	0	0	2	5	1.0%
Malassezia species	0	0	0	0	0	0	0.0%
Cryptococcus neoformans	7	2	12	10	7	38	±8.0%
Cryptococcus non-neoformans	0	0	0	1	0	1	0.2%
Kloeckera species	1	0	0	0	0	1	0.2%
All Non-Candida	14	2	15	16	10	57	11.9%
All Yeasts	81	46	162	101	87	477	

p-value: 0.003
*most common *Candida* species causing invasive yeast infections
±most common non-*Candida* species causing invasive yeast infections
Rare *Candida* species: *Candida* species other than *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. krusei* and *C. auris*

and *C. parapsilosis* were the most common cause of invasive yeast infections in adults.[1,16,17] In contrast our study found an increase in isolation of *Candida auris* in adults as a cause of invasive yeast infections.

Ustilago species were the most common non-candida species causing invasive yeast infections in neonates and children. These are yeast-like fungi which are plant pathogens and have rarely been incriminated in human infections.[19] According to a few case reports, it causes invasive fungal infections globally and is resistant to flucytosine, fluconazole and echinocandins, this pathogen assumes a greater clinical significance.[19] However further patient details are needed before determining its clinical significance.

Cryptococcus neoformans, at 8%, was found to be the most common non-candida species causing invasive yeast infections in adults like other studies.[20-22] Globally, most cases of cryptococcosis are seen in HIV patients. A previous study from our center identified a large group of non-HIV risk factors for cryptococcosis, e.g., transplant, use of immunosuppressive agents, chronic infections like hepatitis B and C, autoimmune diseases, and malignancies.

There was a very high incidence of fluconazole resistance in yeasts isolated in adults followed by children. This could possibly be due to an emergence of *C. auris* infections in adults (22.3%) and children (18%) versus neonates (1.1%).

The limitations of our study include the retrospective nature of study due to which clinical significance of uncommon organisms, and risk factors could not be assessed for acquiring invasive yeast infections. Another limitation of our study was that all the yeasts were identified by phenotypic methods which might have led to improper identification of rare and unknown *Candida* species.

The strength of this study is that it shows the etiologic spectrum and antifungal resistance of invasive yeasts infection amongst neonates, children, and adults. It is necessary to learn a center's local epidemiology due to differences in the distribution of these species among different pediatric intensive care units and hospitals.

It is essential to monitor the spectrum of yeast infections in neonates and children as they are the population where there is greatest diversity of invasive species, not limited to Candida species. Emerging pathogens are most likely to arise from this population, including resistant strains, hence, it is also important to monitor resistance trends.

References

1. Farooqi JQ, Jabeen K, Saeed N, Iqbal N, Malik B, Lockhart SR, *et al*. Invasive candidiasis in Pakistan: clinical characteristics, species distribution and antifungal susceptibility. *J of Med Micro* 2013;62

(Pt 2):259-68.
2. Ariff S, Saleem AF, Soofi SB, Sajjad R. Clinical spectrum and outcomes of neonatal candidiasis in a tertiary care hospital in Karachi, Pakistan. *J Inf in Dev Countries* 2011;5(03):216-23.
3. Bitar D, Lortholary O, Le Strat Y, Nicolau J, Coignard B, Tattevin P, *et al*. Population-based analysis of invasive fungal infections, France, 2001–2010. *Emerg Infec Dis* 2014;20(7):1149.
4. CLSI. Performance Standards for Antifungal Susceptibility Testing of Yeasts. 2nd ed. CLSI supplement M60. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
5. European Committee on Antimicrobial Susceptibility Testing, Data from the EUCAST MIC distribution website, last accessed 02/07/2020". [cited 2020 02/07/2020]. Available from: http://www.eucast.org.
6. CLSI. Epidemiological Cutoff Values for Antifungal Susceptibility Testing. 3rd ed. CLSI supplement M59. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
7. Guo L-N, Xiao M, Cao B, Qu F, Zhan Y-L, Hu Y-J, *et al*. Epidemiology and antifungal susceptibilities of yeast isolates causing invasive infections across urban Beijing, China. *Future Microbiology* 2017;12(12):1075-86.
8. Yapar N. Epidemiology and risk factors for invasive candidiasis. Therapeutics and clinical risk management. 2014;10:95.
9. Farooqi J, Jabeen K, Naqvi F, Malik F, Zafar A, editors. Spectrum of Neonatal Candidemia in Pakistan: review of laboratory data 2014-2016. MYCOSES; 2017: WILEY 111 RIVER ST, HOBOKEN 07030-5774, NJ USA.
10. Sardana V, Pandey A, Madan M, Goel SP, Asthana AK. Neonatal candidemia: a changing trend. *Indian J Path & Microb* 2012;55(1):132-3.
11. Pana ZD, Roilides E, Warris A, Groll AH, Zaoutis T. Epidemiology of Invasive Fungal Disease in Children. *J Ped Inf Dis Society* 2017;6(suppl_1): S3-S11.
12. Benedict K, Roy M, Kabbani S, Anderson EJ, Farley MM, Harb S, *et al*. Neonatal and pediatric candidemia: results from population-based active laboratory surveillance in four US locations, 2009–2015. *Ped Inf Dis Society* 2018;7(3):e78-e85.
13. Hsu J-F, Lai M-Y, Lee C-W, Chu S-M, Wu I-H, Huang H R, *et al*. Comparison of the incidence, clinical features and outcomes of invasive candidiasis in children and neonates. *BMC Infectious Diseases* 2018;18(1):1-11.
14. Lin S, Chen R, Zhu S, Wang H, Wang L, Zou J, *et al*. Candidemia in adults at a tertiary hospital in China: clinical characteristics, species distribution, resistance, and outcomes. *Mycopathologia* 2018;183(4): 679-89.
15. Agnelli C, Valerio M, Bouza E, Vena A, Guinea J, del Carmen Martínez-Jiménez M, *et al*. Persistent Candidemia in adults: underlying causes and clinical significance in the antifungal stewardship era. *Euro J Clinical Micro & Infec Dis* 2019;38(3):607-14.
16. Kumar S, Kalam K, Ali S, Siddiqi S, Baqi S. Frequency, Clinical Presentation and Microbiological Spectrum of Candidemiain a Tertiary Care Center in Karachi, Pakistan. Age. 2014;35:13.27.
17. Sayeed MA, Farooqi J, Jabeen K, Mahmood SF. Comparison of risk factors and outcomes of Candida auris candidemia with non-Candida auris candidemia: A retrospective study from Pakistan. *Medical Mycology* 2020;58(6):721-9.
18. McNeil JC, Palazzi DL. Ustilago as a cause of fungal peritonitis: case report and review of the literature. *J Ped Inf Dis Society* 2012;1(4): 337-9.
19. Prakash A, Wankhede S, Singh PK, Agarwal K, Kathuria S, Sengupta S, *et al*. First neonatal case of fungaemia due to P seudozyma aphidis and a global literature review. *Mycoses* 2014;57(1):64-8.
20. Luxmi S, Salahuddin N, Herekar F, Sultan F, Irshad H, Ansari NB. Clinical and demographic pattern of HIV positive patients in two tertiary care hospitals in Pakistan: 20 years experience. *Inf Dis J Pak* 2012;21:485-91.
21. Jabeen K, Farooqi J, Mirza S, Denning D, Zafar A. Serious fungal infections in Pakistan. *Euro J Clinical Micro & Infec Dis* 2017;36(6):949-56.
22. Pfaller MA, Pappas PG, Wingard JR. Invasive fungal pathogens: current epidemiological trends. *Clinical Infec Dis* 2006;43(Supplement_1): S3-S14.