ORIGINAL ARTICLE

Epidemiology and antifungal susceptibility patterns of invasive candidiasis: A single-center study

Amna Younas, Irim Iftikhar, Karam Rasool

Chughtai Institute of Pathology, Lahore Pakistan

ABSTRACT

Background: Invasive candidiasis remains a leading cause of morbidity and mortality. Despite the availability of various antifungal treatments, global reports continue to highlight resistance, treatment ineffectiveness, and outbreaks. This study aimed to analyze the epidemiology of various *Candida* species isolated from invasive samples and assess their antifungal susceptibility profiles.

Material and Methods: The study identified and evaluated a total of 200 *Candida* isolates, recovered over a 6-month period from various clinical specimens. These were assessed against various antifungals according to recommendations.

Results: Among the samples, blood (79%) and pus (12%) were the most commonly encountered sources of *Candida* isolation. *Candida* isolates comprised 14.5% (30) *C. albicans* and 85.5% (170) non-albicans species. Non-albicans species included 15% (30) *C. guilliermondii,* 14.5% (29) *C. lusitaniae,* 11.5% (22) *C. tropicalis*, 2.3% (4) *C. auris,* and 8.5% (17) *C. parapsilosis*. Overall, the strains showed 74% and 91% sensitivity to Fluconazole and Voriconazole, respectively, and 100% sensitivity to Caspofungin and Amphotericin B, with the exception of *C. lusitaniae.*

Conclusion: The susceptibility profile of antifungals is evolving. This study demonstrated a low rate of resistance to four antifungals in invasive candidiasis in Pakistan. None of the isolates were resistant to more than one drug. Early and prompt treatment through the implementation of an antifungal stewardship program and strict infection control is crucial.

Keywords: Invasive candidiasis (IC), Antifungal susceptibility profile, Fluconazole, Candidemia

BACKGROUND

Candida species account for approximately 20% of all microbiological infections in critical patients.[1] Recent studies have reported higher minimum inhibitory concentrations (MICs) to commonly used antifungals against both *C. albicans* and *non-albicans*.[1] The increase in fungal infections corresponds to factors such as advanced age, prolonged length of ICU stays, use of steroids or immunosuppressive drugs, diabetes mellitus (DM), multiple invasive procedures, renal replacement therapy (RRT), and the wide use of invasive devices such as central venous catheters (CVCs) and biofilm formation. Colonization may also lead to invasive disease in 3-25% of cases. The spectrum of invasive candidiasis is broad, including fungemia, intra-abdominal infections, septic arthritis, iatrogenic and neonatal meningitis.[1] Significant geographic variation has been observed in invasive candidiasis, with a poor mortality rate of 40–55%. In a pan-European ICU cohort study (2015–16), *C. albicans* (57%) was the dominant species, followed by *C. glabrata* and *C. parapsilosis*.[2-6] A descriptive epidemiological data from Pakistan in 2012, for invasive *Candida* isolates among adults and neonates revealed *Candida tropicalis* (38% and 36 %, respectively) as the most common species.[7] Despite the availability of antifungals, therapeutic failure may occur due to inadequate responses to common treatments.[8] The Infectious Diseases Society of America (IDSA) guidelines recommend the empiric use of Echinocandins, as it is associated with better survival. Echinocandins have also been found to have limited adverse effects and minimal drug–drug interactions.[8,9] The objective of this cross-sectional study is to observe the frequency of different *Candida* species and determine the susceptibility to antifungals in clinical isolates during a 6-month period (April - September 2023) in the microbiology department of Chughtai Institute of Pathology, Lahore, Pakistan.

MATERIAL AND METHODS

This study included sterile and invasive samples (such as blood, ascitic fluid, pus aspirates, deep or debrided tissue, CVP tips, and CSF) with yeast growth, encompassing both genders across all age

Correspondence: Dr. Amna Younas, Registrar, Chughtai Institute of Pathology, Lahore Pakistan

Email: amnayounas.9209@cll.edu.pk

This article can be cited as: Younas A, Iftikhar I, Rasool K. Epidemiology and antifungal susceptibility patterns of invasive candidiasis: A single-center study. Infect Dis J Pak. 2024; 33(1): 13-17. DOI: https://doi.org/10.61529/idjp.v33i1.263

Receiving date: **30 Oct 2023** Acceptance Date: **15 Mar 2024**
Revision date: **07 Mar 2024** Publication Date: **30 Mar 2024**

groups. CVP tips with blood culture positive with the same pathogen were included in this study. All duplicate isolates and non-invasive samples were excluded.

A cross-sectional observational study was conducted over a 6-month period at Chughtai Institute of Pathology (CIP) in the Microbiology, BSL-2 laboratory, under the assigned IRB number. The study focused on the analysis of various *Candida* species isolated from clinical invasive samples, alongside their antifungal susceptibility profiles, from April to September 2023.

Two hundred yeast isolates from invasive samples were included, processed for identification using MALDI-TOF, VITEK MS, and their antifungal susceptibility profiles were determined. *Candida* isolates that couldn't be identified to the species level using VITEK MS were labeled as "*Candida* species." Antifungal susceptibilities were determined by the minimum inhibitory concentration (MICs) using E-strip for Fluconazole, Voriconazole, Amphotericin, and Caspofungin, as guided by CLSI M60. However, Amphotericin was not tested for *C. lusitaniae,* and Fluconazole was not tested for *C. krusei.* Quality control strains were also employed to standardize the testing. Antifungal breakpoints were species-specific, and the results were reported as sensitive (S), resistant (R), or susceptible dose-dependent (SDD). Patient privacy was maintained by using case numbers exclusively. Statistical Analysis: Descriptive analysis was performed, calculating the frequencies of *C. albicans* and non-albicans isolation, gender, age groups, and the MIC 50 and 90 percentile of antifungals using SPSS 21.

RESULTS

A total of 200 yeasts from various invasive samples were studied, with the majority originating from Punjab (82.5%). *Candida* isolates were most commonly obtained from blood (79%), followed by pus (12%), ascitic fluid (3%), CSF (2.5%), tissue (2%), and CVP tips (1.5%). The study revealed the isolation of 14.5% *C. albicans* and 85.5% *non-albicans* species. Among the *non-albicans* species, *C. guilliermondii* was the most frequent, accounting for 15% of cases. Additionally, 14.5% of *C. lusitaniae,* 11.5% of *C. tropicalis*, 8.5% of *C. parapsilosis*, and 2.3% of *C. auris* were isolated from clinical samples, with other *Candida* species accounting for 18.5% of cases. Antifungal sensitivities were also assessed. Amphotericin B and Caspofungin were found to be 100% effective in all isolates, both *C. albicans* and *non-albicans*. Of the 193 (96%) isolates tested for fluconazole, 143 (74%) were found to be sensitive, 17 (8.8%) were resistant, and 33 (17%) showed susceptible dose-dependent results. Total of fifteen Fluconazole-resistant isolates of *C. lusitaniae and C. guilliermondii* were observed to have MIC > 64ug/ml. Similarly, isolates were tested for voriconazole, with 182 (91%) found to be sensitive, 16 (8%, *C. lusitaniae, C. auris*) resistant, and 2 (1%) showing susceptible dose-dependent results. Fifteen isolates of *C. guilliermondii* and *C. lusitaniae* exhibited high voriconazole MIC (> 32 ug/ml). Amphotericin B and Caspofungin displayed 100% sensitivity.

Table-I: Demographic data from provinces of pakistan

Province	Frequency	Percent
AJK	2	1.0
Baluchistan	2	1.0
KPK	25	12.5
Punjab	165	82.5
Sindh	6	3.0
Total	**200**	**100.0**

Table-II: Over all percentile of MICs of *Candida* species against different antifungals

Species(n)	Antifungal agents	MIC90	MIC50
Candida albicans (n=29)	Amphotericin B	0.6	0.125
	Caspofungin	0.25	0.25
	Fluconazole	0.925	0.315
	Voriconazole	0.25	0.12
Candida guilliermondii (n=30)	Amphotericin B	0.5	0.1575
	Caspofungin	1.05	0.75
	Fluconazole	32	24
	Voriconazole	0.44	0.19
Candida lusitaniae (n=29)	Amphotericin B	NT	NT
	Caspofungin	1.5	1
	Fluconazole	64	28
	Voriconazole	0.625	0.22
Candida parapsilosis (n=17)	Amphotericin B	0.5	0.1875
	Caspofungin	1	0.75
	Fluconazole	1.3	0.38
	Voriconazole	0.198	0.12
Candida tropicalis (n=22)	Amphotericin B	0.38	0.125
	Caspofungin	0.25	0.25
	Fluconazole	0.788	0.25
	Voriconazole	0.952	0.12
Candida spp. (n=73)	Amphotericin B	0.75	0.25
	Caspofungin	0.75	0.38
	Fluconazole	32	3.5
	Voriconazole	0.75	0.125

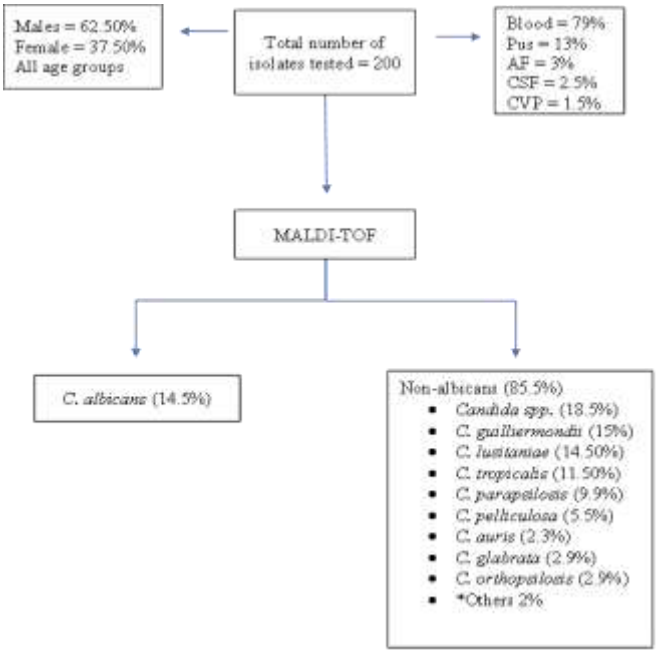

Figure-I: Work flow chart showing the categorization of samples and of *Candida* isolates.
***Others 2% of the total isolates of *C. lipolyica, C. blankii, C. boidinii, C. rugosa* and *C. metapsilosis* were also identified.**

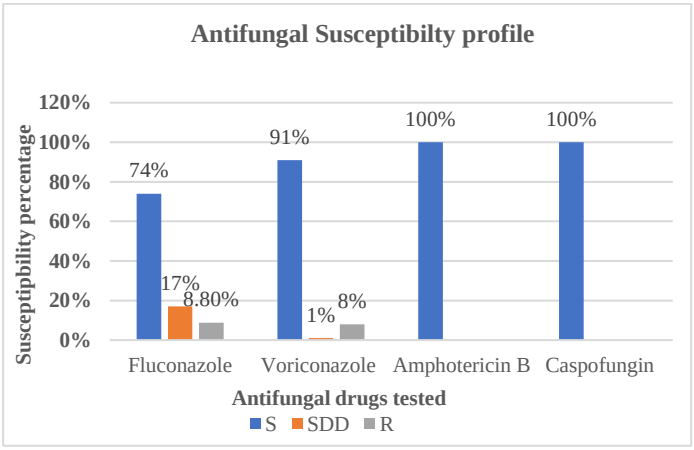

Figure-II: Showing antifungal susceptibilty

DISCUSSION

Candida species, which are the most common cause of invasive candidiasis (IC) across all age groups, present extensive disease manifestations with high mortality rates.[10] This study provides a concise overview of the distribution of *Candida* species and their antifungal susceptibilities in invasive samples from various geographical regions in Pakistan, mainly from Punjab (82.5%), as the laboratory is based in Lahore, the capital city of the province (Table-I).

The distribution of *non-albicans* species, as documented by Papas *et al*., particularly in ICU patients, has been observed globally. It was proposed that *non-albicans* species, including *C. lusitaniae, C. guilliermondii*, and *C. parapsilosis*, were the leading cause of IC, especially in patients with prior exposure to antibiotics, a central venous catheter, recent abdominal surgery or colonization (5-30%).[10] Some studies have suggested that colonization can be used as an indication to initiate prophylaxis and reduce IC.[11,12,13] However, this has not shown any benefits in reducing mortality. Similarly, a study in Switzerland by Egiimann revealed that the shift in ICU fungal distribution resulted from empirical treatment with Caspofungin and Azoles. This led to increased *C. glabrata* without reducing invasive candidiasis-related mortality or prevalence.[14] Our study also demonstrated that *non-albicans* species were the major cause of IC (85.5%). Another study published in 2021 by Ratani *et al*. on the "Spectrum and antifungal resistance in *Candida* species isolated from blood culture of neonates" similarly indicated non-albicans species (86.7%) as the major cause of IC.[15] In contrast to our study, they noted *C. tropicalis* as the most frequently isolated pathogen, while *C. guilliermondii* (15%) was the dominant isolate in our study. This finding might be attributed to the different sample types and age groups included. Our results also differ from the study in Iran on blood cultures from the Pediatric Intensive Care Unit (PICU), which reported 53.6% *C. albicans*, 24.7% *C. parapsilosis*, and 8.5% *C. tropicalis*.[16]

The gold standard for diagnosing IC is culture, yet the sensitivity of blood culture and ascitic fluid is reported to be insignificant. This could be due to the common practice of empiric antifungal therapy. Literature review has shown that the sensitivities of blood and ascitic fluid are only 75% and 5-20%, respectively.[17,18] Similarly, the isolation of *Candida* from sterile sites, such as pus from the abdomen, poses challenges and takes 2–3 days.[17] Our study also indicated low frequency in terms of the isolation of *Candida* from blood (79%) and pus (3%).

A worldwide transformation in the antifungal susceptibility profile, especially in Fluconazole, has been observed. This might be attributed to the common practice of frequently using Fluconazole as an empiric treatment. This study shows reduced sensitivity against Fluconazole (74%) compared to Amphotericin B (100%) and Voriconazole (91%) (Figure-1). These calculations did not include Fluconazole susceptibility profiles for *C. krusei* and *C. auris* as they are intrinsically resistant and have high MICs, respectively.

Approximately 8% of susceptible dose-dependent (SDD) isolates were observed for both Fluconazole and Voriconazole. We detected a >64 ug/ml MIC for Fluconazole in total of 12 isolates of *C. lusitaniae*, 2 isolates of *C. guilliermondii*, and 1 *Candida species*. High MICs of Fluconazole against *C. lusitaniae* may be due to mutations, as discussed by Shawn *et al*. Additionally, prior use and persistent candidemia may contribute to high MICs. Similarly, in a "20 years of antifungal surveillance program from 135 medical centers in the USA, Canada, and Europe," an increase in the isolation of *Candida glabrata* and *Candida parapsilosis* and a decrease in *C. albicans* was observed, along with a gradual emergence of resistance to azoles (Fluconazole and Voriconazole).[19] Unlike our findings, Merhendi *et al*. reported 100% sensitivity to Fluconazole and Voriconazole.[20,21]

An observational study in the USA, Texas, and Columbia on the susceptibility profile of *C. auris* noted 33% and 1% resistance to Amphotericin B and Echinocandins, respectively. [22,23] We reported 4 pan-sensitive *C. auris* isolates from blood and one from a tissue sample. The difference in susceptibility profiles might be due to the different clades, the selective pressure of antifungals, and the methodology of antifungal testing. To date, little information is available for treating infections due to pan-resistant isolates. More research, knowledge, and data are required for the accurate diagnosis and treatment of resistant isolates.[24]

The emergence of *non-albicans* species as predominant causes of invasive candidiasis (IC), particularly in intensive care units (ICUs), underscores the urgent need for targeted infection prevention and control measures. Evidence from this study underscores the need for rigorous surveillance mechanisms and guidelines to ensure judicious use of antifungal agents, thereby minimizing the risk of antimicrobial resistance and improving patient safety. Moreover, addressing diagnostic limitations and enhancing laboratory capacities for *Candida* detection are essential components of comprehensive public health strategies against IC. Policymakers should prioritize investments in diagnostic infrastructure and workforce training to facilitate early detection, prompt treatment initiation and effective management. This study underscores the importance of closing knowledge gaps surrounding *Candida* epidemiology and antifungal resistance mechanisms through targeted research and innovation. Policymakers should prioritize research funding and interdisciplinary collaborations to advance our understanding of IC dynamics and inform evidence-based policy responses.[25]

Several limitations exist in this study. We were unable to identify all inpatient units and the sources for pus samples due to a lack of clinical history. Furthermore, the mechanism of antifungal resistance in our community remains unknown. Discrepancies in the frequencies of *C. albicans* and *non-albicans* might be because of the short duration of the study. Due to small sample size, *Candida* species vise, antifungal susceptibility pattern could not be calculated. Differences in antifungal susceptibilities can also be attributed to the variation in testing methodologies, as broth microdilution is the recommended methodology.

CONCLUSION

This study reveals that there is a rise in *non-albicans candida* species. It is also concluded that there were no pan-resistant *Candida* isolates. However molecular-based assays should be employed to detect the mechanisms of antifungal resistance in our community. DNA sequence analysis of FKS genes should be performed to identify hotspot mutations. The use of antifungals should be restricted and improved through antifungal stewardship programs. A multicenter study should be conducted to observe the trends of antifungals and prevalence over a period of 5-10 years in our community.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Amna Younas: Study design. Study performance. Manuscript drafting, revision and final review of manuscript.

Irim Iftikhar: Conception of work. Study design. Critical review for important intellectual content. Final approval of version.

Karam Rasool: Acquisition, analysis and interpretation of data. Final review.

REFERENCES

1. Bilgin MK, Talan L, Evren E, Altıntas ND. Retrospective evaluation of risk factors for invasive candida infections in

a medical Intensive Care Unit. Infect Dis Clin Microbiol. 2022; 4(1): 62-71.
DOI: https://doi.org/10.36519/idcm.2022.56

2. Paiva J-A, Pereira JM, Tabah A, Mikstacki A, de Carvalho FB, Koulenti D, *et al*. Characteristics and risk factors for 28-day mortality of hospital acquired fungemias in ICUS: Data from the EUROBACT study. Crit Care. 2016; 20(1): 53. DOI: https://doi.org/10.1186/s13054-016-1229-1
3. Bassetti M, Giacobbe DR, Vena A, Trucchi C, Ansaldi F, Antonelli M, *et al*. Incidence and outcome of invasive candidiasis in Intensive Care Units (ICUS) in Europe: Results of the EUCANDICU project. Crit Care. 2019;23(1): 219.
DOI: https://doi.org/10.1186/s13054-019-2497-3
4. Koehler P, Stecher M, Cornely OA, Koehler D, Vehreschild MJGT, Bohlius J, *et al*. Morbidity and mortality of candidemia in Europe: An epidemiologic meta-analysis. Clinical Microbiology and Infection. 2019;25(10):1200–12.
DOI: https://doi.org/10.1016/j.cmi.2019.04.024
5. Flevari A, Theodorakopoulou M, Velegraki A, Armaganidis A, Dimopolus G. Treatment of invasive candidiasis in the elderly: A review. Clin Interv Aging. 2013; 1199-208. DOI: https://doi.org/10.2147/cia.s39120
6. Kett DH, Azoulay E, Echeverria PM, Vincent JL. Candida bloodstream infections in intensive care units: Analysis of the extended prevalence of infection in Intensive Care Unit Study. Crit Care Med. 2011; 39(4): 665–70.
DOI: https://doi.org/10.1097/ccm.0b013e318206c1ca
7. Farooqi JQ, Jabeen K, Saeed N, Iqbal N, Malik B, Lockhart SR, Zafar A, Brandt ME, Hasan R. Invasive candidiasis in Pakistan: clinical characteristics, species distribution and antifungal susceptibility. J Med Microbiol. 2013; 62(Pt 2): 259-68.
DOI: https://doi.org/10.1099/jmm.0.048785-0
8. Bels DD, Maillart E, Van Bambeke F, Redant S, Honoré PM. Existing and emerging therapies for the treatment of invasive candidiasis and Candidemia. Expert Opin Emerg Drugs. 2022; 27(4): 405–16.
DOI: https://doi.org/10.1080/14728214.2022.2142207
9. Koehler P, Stecher M, Cornely OA, Koehler D, Vehreschild MJGT, Bohlius J, *et al*. Morbidity and mortality of candidaemia in Europe: An epidemiologic meta-analysis. Clin Microbiol Infect. 2019; 25(10): 1200-12. DOI: https://doi.org/10.1016/j.cmi.2019.04.024
10. Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. Invasive candidiasis. Nat Rev Dis Primers. 2018; 4(1): 18026.
DOI: https://doi.org/10.1038/nrdp.2018.26
11. Garbino J, Lew DP, Romand JA, Hugonnet S, Auckenthaler R, Pittet D. Prevention of severe candida infections in nonneutropenic, high-risk, critically ill patients: A randomized, double-blind, placebo-controlled trial in patients treated by selective digestive decontamination. Intensive Care Med. 2002; 28(12): 1708–17.
DOI: https://doi.org/10.1007/s00134-002-1540-y
12. Logan C, Martin-Loeches I, Bicanic T. Invasive candidiasis in critical care: Challenges and future directions. Intensive Care Med. 2020; 46(11): 2001–14.
DOI: https://doi.org/10.1007/s00134-020-06240-x
13. Senn L, Eggimann P, Ksontini R, Pascual A, Demartines N, Bille J, *et al*. Caspofungin for prevention of intra-abdominal candidiasis in high-risk surgical patients. Intensive Care Med. 2009; 35(5): 903–8.
DOI: https://doi.org/10.1007/s00134-009-1405-8
14. Ferreira D, Grenouillet F, Blasco G, Samain E, Hénon T, Dussaucy A, *et al*. Outcomes associated with routine systemic antifungal therapy in critically ill patients with candida colonization. Intensive Care Med. 2015; 41(6): 1077–88.
DOI: https://doi.org/10.1097/ccm.0b013e318236f297
15. Rattani S, Farooqi J, Hussain AS, Jabeen K. Spectrum and antifungal resistance of Candidemia in neonates with early- and late-onset sepsis in Pakistan. Pediatric Infect Dis J. 2021; 40(9): 814–20.
DOI: https://doi.org/10.1097/INF.0000000000003161
16. Bhat J, Charoo B, Ashraf Y, Qazi I. Systemic candida infection in preterm babies: Experience from a tertiary care hospital of North India. J Clin Neonatol. 2019; 8(3): 151. DOI: https://doi.org/10.4103/jcn.JCN_9_19
17. Clancy CJ, Nguyen MH. Finding the "missing 50%" of invasive candidiasis: How nonculture diagnostics will improve understanding of disease spectrum and transform patient care. Clin Infect Dis. 2013; 56(9): 1284–92.
DOI: https://doi.org/10.1093/cid/cit006
18. Clancy C, Nguyen MH. Non-culture diagnostics for invasive candidiasis: Promise and unintended consequences. J Fungi. 2018; 4(1): 27.
DOI: https://doi.org/doi10.3390/jof4010027
19. Pfaller MA, Diekema DJ, Turnidge JD, Castanheira M, Jones RN. Twenty years of the Sentry Antifungal Surveillance Program: Results for Candida species from 1997–2016. Open Forum Infect Dis. 2019; 6(Supplement_1): S79-S94.
DOI: https://doi.org/10.1093/ofid/ofy358
20. Mirhendi H, Charsizadeh A, Eshaghi H, Nikmanesh B, Arendrup MC. Species distribution and antifungal susceptibility profile of candida isolates from blood and other normally sterile foci from pediatric ICU patients in Tehran, Iran. Medl Mycol. 2020; 58(2): 201-6.
DOI: https://doi.org/10.1093/mmy/myz047
21. Lockhart SR, Pham CD, Kuykendall RJ, Bolden CB, Cleveland AA. Candida lusitaniae MICs to the echinocandins are elevated but FKS-mediated resistance is rare. Diagn Microbiol Infect Dis. 2016; 84(1): 52-4.
DOI: https://doi.org/10.1016/j.diagmicrobio.2015.08.012
22. Lyman M, Forsberg K, Reuben J, Dang T, Free R, Seagle EE, *et al*. Notes from the field: Transmission of Pan-resistant and echinocandin-resistant candida auris in health care facilities—Texas and the District of Columbia, January–April 2021. MMWR Morb Mortal Wkly Rep. 2021; 70(29): 1022-3.
DOI: https://doi.org/10.15585/mmwr.mm7029a2
23. Forsberg K, Lyman M, Chaturvedi S, Schneider EC, Fischer J, Baynham DF, *et al*. 155. public health action-based system for tracking and responding to U.S. candida drug resistance: AR Lab Network, 2016–2019. Open Forum Infect Dis. 2020; 7(Supplement_1): S206-7.
DOI: https://doi.org/10.1093/ofid/ofaa439.465
24. Baalaaji AR M. Invasive candidiasis in children: Challenges remain. Indian J Crit Care Med. 2022; 26(6): 667–8.
DOI: https://doi.org/10.5005/jp-journals-10071-24250
25. Riera FO, Caeiro JP, Angiolini SC, Vigezzi C, Rodriguez E, Icely PA, *et al*. Invasive candidiasis: Update and current challenges in the management of this mycosis in South America. Antibiotics (Basel). 2022; 11(7): 877.
DOI: https://doi.org/10.3390/antibiotics11070877