

Susceptibility pattern of anti-candida drugs in the pediatric patients with acute leukemia

Soheila Zareifar MD^{1,*}, Parisa Badiie MD², Pedram Haddadi MD³, Babak Abdolkarimi MD¹

1. Hematology Research Center, Pediatric Hematology/Oncology Department, Shiraz, Iran

2. Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

3. Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran

*Corresponding author: Dr. Soheila Zareifar, Hematology Research Center, Namazi Hospital, Shiraz, Iran. E mail: zareifars@sums.ac.ir

Received: 14 July 2015

Accepted: 27 January 2016

Abstract

Background: Pediatric patients on chemotherapy are vulnerable to invasive fungal infection especially *Candida* species. Resistance to antifungal agents has increased in *Candida* spp., especially in non-*albicans* species. This study aims to assess the susceptibility of *Candida* spp. strains isolated from children with acute leukemia less than 18 years.

Materials and Methods: This prospective cross-sectional study was conducted during March 2011 to March 2012. Participants were 188 children aging from 1 month to 18 years, who had acute leukemia, were admitted in Amir Oncology Hospital affiliated to Shiraz University of Medical Science, Shiraz, Iran.

Identification of *Candida* strains was performed using germ tube and chlamyospore production tests on an Application Programming Interface (API) 20 C AUX yeast identification system. Susceptibility testing for 7 antifungal agents was performed by the agar-based E-test method. Fungal cultures were carried out from nose, oropharynx, stool, and urine specimens.

Results: A total of 229 yeasts were isolated. *C. albicans* was the most common species found, followed by *C. krusei*, *C. parapsilosis*, *C. glabrata*, and other *Candida* species. *Candida glabrata* was the most highly resistant of the yeasts isolated, being 100% resistant to fluconazole and itraconazole, 88% to posaconazole, and 75% to amphotericin B and ketoconazole.

Conclusion: In this study, caspofungin was the most effective antifungal agent against the colonized *Candida* spp. found, followed by conventional amphotericin B. Knowledge about susceptibility patterns of colonized *Candida* spp. can be of help to clinicians managing pediatric patients on chemotherapy.

Key Words: Acute Leukemia, Antifungal Drugs, Cancer, Neutropenia, Pediatric

Introduction

Despite progress in supportive care and treatment strategy of children with malignant disorders, invasive fungal infections associated with significant morbidity and mortality in these patients, especially those with hematological malignancies, increases in related healthcare costs. Choosing the appropriate treatment is not always simple because of the possibility of drug interactions and side effects (1). These infections are influenced by various factors including, the use of chemoprophylaxis, central venous catheters and local epidemiology (2). *Candida* has been observed as a primary

fungal pathogen, particularly in immunosuppressed patients (3). *Candida albicans* has been the most common causal agent in these infections, affecting 48% of patients (4). Recent studies have showed a reduction in the rates of *C. albicans* infection and a relative shift toward non-*albicans Candida* spp (5-8). Early recognition, prompt diagnosis and treatment are also serving as a diagnostic tool to manage patients and avert *Candida* transmission to others (9, 10). Nonetheless, several studies revealed increasing resistance of *Candida* spp. to common used antifungal agents (6, 11,

12). Distribution and susceptibility patterns of predominant *Candida* spp. information could be of help to clinicians involved in the management of these patients. The aim of this study was to determine the antifungal susceptibility pattern of the isolated fungi in children with acute leukemia on chemotherapy.

Materials and Methods

A prospective study was conducted between March 2011 and March 2012 to investigate drug susceptibility patterns in isolated fungi from patients treated in a large tertiary-care referral pediatric hematology/oncology center in Southern Iran. The study included 188 pediatric patients (101 boys and 87 girls). All of patients had acute leukemia including acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL), and had undergone courses of chemotherapy and/or hospitalizations. All the eligible participants were included after taking consents from their parents or their legal guardians. The study was reviewed and approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran. Neutropenia was considered as less than 1500 cells/mL and severe Neutropenia as absolute neutrophil count (ANC) less than 500 cells/mL or an ANC that is expected to reduce to <500 cells/mL during the next 48 hours. About 90% of patients had a prior history of antifungal prophylaxis consumption. On admission, specimens were obtained from nose, oropharynx, stool, and urine for fungal surveillance cultures. Samples were placed on Sabouraud dextrose agar (Merck, Germany) and incubated at 24°C for 10 days. The purity of the isolate was evaluated by culturing the isolate on potato dextrose agar (OXOID LTD, Basingstoke, Hampshire, England) twice for 48h at 35°C. Identification of *Candida* strains was performed using germ tube, chlamydospore production tests, and carbohydrate assimilation reactions on the

API 20 C AUX system (bioMérieux, France) according to the manufacturer's instructions. Two *Candida* spp., including *Candida parapsilosis* (ATCC 22019) and *Candida krusei* (ATCC 6258) were used as quality controls.

Susceptibility testing for ketoconazole, fluconazole, itraconazole, voriconazole, amphotericin B, caspofungin, and posaconazole was performed using an agar-based E-test method (bioMérieux, Sweden). RPMI 1640, supplemented with 1.5% agars and 2% glucose and buffered to pH 7.0 with 0.165 M morpholine propane sulfonic acid (MOPS), was used to prepare the plates. These were inoculated by dipping a sterile swab into an inoculum suspension adjusted to the turbidity of a 0.5 McFarland standard (106 cells/ mL) and streaking the agar in three directions. The minimum inhibitory concentration (MIC) endpoints were determined after 24h and 48h of incubation at 35°C. Concentrations of each drug ranged from 0.002-32 µg/mL, with the exception of fluconazole, with 0.016-256 µg/mL concentrations.

The MIC for ketoconazole, itraconazole, fluconazole, voriconazole, posaconazole, and caspofungin, was based on significant inhibition of 80% of growth. For amphotericin B, the MIC was determined as the point of complete inhibition (100%). The resistance breakpoints for the antifungal were as follows: ketoconazole ≥ 4.0 , fluconazole ≥ 64 , itraconazole ≥ 1.0 , voriconazole ≥ 8.0 , amphotericin B > 1.0 and caspofungin > 2.0 micrograms per milliliter (13-18). The resistant breakpoint for posaconazole has not been established as per Clinical & Laboratory Standards Institute (CLSI) document M27-A3 (12). The MIC50 and MIC90 (i.e., the MIC at which 50% and 90% of isolates are inhibited) were also calculated. Statistical analysis was performed using SPSS software (version 17, Chicago, Illinois, USA) and were analyzed using descriptive statistics and cross tabulation.

Results

During the study period, overall, 229 yeasts were isolated and 88 of 188 children were found to be colonized with *Candida* spp., yielding an overall colonization rate of 46.8%. Colonization of the oral cavity was significantly more frequent than in the other body sites investigated. The highest percentage of *Candida* colonization was found in those with acute lymphoblastic leukemia.

Candida spp. were isolated from more than one body site in 26 (29.5%) colonized patients, and in some cases, more than one species was isolated from each site. *C. albicans* was the most common species found, being detected in 117 patients (51.2%), followed by *C. krusei* in 18 patients (7.9%), *C. glabrata* in 14 (6.3%), *C. tropicalis* in 11 (4.7%), *C. famata* in 11 (4.7%), *C. parapsilosis* in 8 (3.5%), *C. dubliniensis* in 6 (2.4%), *C. kefyr* in 4 (1.8%), *Cryptococcus* (Cr) in 16 (7%) including *C. terreus* and *C. laurentii* and

other *Candida* species, including *C. guilliermondii*, *C. rugosa*, *C. lusitanae*, *C. lambica*, as well as *Rhodotorula* spp. in 24 patients (10.5%).

Candida albicans, the species most frequently isolated, was sensitive to amphotericin B, fluconazole, voriconazole, itraconazole, ketoconazole, posaconazole, and caspofungin at rates of 97%, 58.5%, 57%, 72%, 31%, 48%, and 97%, respectively. *Candida glabrata* was the most resistant species among the isolated yeasts, with resistance rates of 100% to fluconazole and itraconazole, 88% to posaconazole, and 75% to amphotericin B and ketoconazole. All the fungal species exhibited highest sensitivity to caspofungin. The detected 16 *Cryptococcus* isolates were divided into two subspecies; *Cr. terreus* and *Cr. laurentii*. The results of antifungal susceptibility testing by E-test in *Candida* isolates are shown in Table I. The lowest MIC₉₀ was observed for caspofungin.

Table I: Antifungal Susceptibility of *Candida* spp isolated from pediatric patients with acute leukemia

Antifungal agent	Species (no. isolates)	No isolates	MIC ₅₀ (µg/mL)	MIC ₉₀ (µg/mL)	Range	Resistance No. (%)
Amphotericin	<i>C. albicans</i>	117	0.5	0.75	0.047-1.5	4 (3%)
	<i>C. krusei</i>	18	4	6	0.38-6	16 (80%)
	<i>C. glabrata</i>	14	3	6	0.25-6	12 (75%)
	<i>Cryptococcus spp</i>	16	0.38	1	0.016-2	2 (11%)
Fluconazole	<i>C. albicans</i>	117	1.5	256	0.094-256	54 (41.5%)
	<i>C. krusei</i>	18	96	256	24-256	16 (80%)
	<i>C. glabrata</i>	14	96	256	32-256	16 (100%)
	<i>Cryptococcus spp</i>	16	2	16	2-256	6 (33%)
Voriconazole	<i>C. albicans</i>	117	0.125	32	0.003-32	56 (43%)
	<i>C. krusei</i>	18	0.5	2	0.125-32	2 (10%)
	<i>C. glabrata</i>	14	1.5	2	0.75-32	4 (25%)
	<i>Cryptococcus spp</i>	16	0.125	3	0.016-3	0
Itraconazole	<i>C. albicans</i>	117	0.032	32	0.016-32	36 (28%)
	<i>C. krusei</i>	18	0.75	4	0.19-32	6 (30%)
	<i>C. glabrata</i>	14	32	32	4-32	16 (100%)
	<i>Cryptococcus spp</i>	16	0.75	32	0.023-32	8 (44%)
Ketoconazole	<i>C. albicans</i>	117	0.125	32	0.016-32	90 (69%)
	<i>C. krusei</i>	18	6	8	1-32	14 (70%)
	<i>C. glabrata</i>	14	6	32	2-32	12 (75%)
	<i>Cryptococcus spp</i>	16	1.5	12	0.032-32	6 (33%)
Posaconazole	<i>C. albicans</i>	117	0.047	32	0.008-32	*
	<i>C. krusei</i>	18	0.75	8	0.008-32	
	<i>C. glabrata</i>	14	32	32	1-32	
	<i>Cryptococcus spp</i>	16	0.032	4	0.032-32	
Caspofungin	<i>C. albicans</i>	117	0.094	0.125	0.032-8	4 (3%)
	<i>C. krusei</i>	18	0.125	0.094	0.032-4	4 (20%)
	<i>C. glabrata</i>	14	0.094	0.19	0.064-0.19	0
	<i>Cryptococcus spp</i>	16	0.094	1.5	0.032-4	1 (5%)

Species present in small numbers are not mentioned in the table.

Discussion

In the present study, 46.8% of pediatric patients were colonized with *Candida* spp. Patients on chemotherapy and documented prior *Candida* colonization are at considerable risk of developing systemic candidiasis (19). There is evidence that *Candida* colonization is a risk factor for developing invasive *Candida* infection (20) and candidemia (5) in hospitalized patients. In one study, the reported rate of nosocomial candidemia increased more than 2-fold over the 9-year study period (3), while in another research, nosocomial candidemia was diagnosed in 6.9% of colonized neonates, compared with 0.76% of non-colonized neonates (21). It is Reported colonization rates are 12.1% in neonates (21), 12.4% in infants (22), and 55.2% in adults with hematological malignancies (8). In current study, the most commonly colonized sites belonged to the oral cavity and rectum, in keeping with the findings of other studies (8, 22).

Among the *Candida* species isolated in the present report, 51.2% were identified as *C. albicans*. In other studies, *C. albicans* detection rates have been reported at 50% (23), 64.2% (22), 48.6% (24), 42% (21), and 55% (12). In blood cultures, *C. albicans* isolates accounted for 37.2% 2 and 39.2% (25) of *Candida* spp. found. The isolation rate of non-*albicans* *Candida* in this study was 48.8%, whereas rates of 35.8% (21) and 78.2% (26) have been reported in the literature. In our study, *Cryptococcus* spp. accounted for 7% of the isolates. Colonization with this yeast has been less extensively described. The increased use of antifungal in patients on chemotherapy, mainly for prophylaxis, is considered the strongest contributory factor to the changes in species distribution, which have subsequently affected mortality and the choice of empirical treatment (27).

Resistance to antifungal agents is associated with high mortality rate in at-risk hospitalized patients especially on chemotherapy. It can be categorized into

primary, acquired, and clinical resistance. Primary resistance to antifungal agents is known as intrinsic and occurs when the organism is naturally resistant to the antifungal agent without exposure to anti-fungal, such as *C. krusei*, which is known to be universally resistant to fluconazole (28). Acquired resistance develops during treatment, and often occurs as a result of genetic mutations (29). Clinical resistance, i.e., failure of anti-fungal therapy, depends on a variety of factors, like the host immune system condition, pharmacokinetics of the antifungal agent, and the species involving in fungal infection. Intrinsic resistance to amphotericin B is rare and acquired resistance during therapy is even less common (30, 31). *C. glabrata* and *C. krusei* tend to have higher MICs than *C. albicans*, and a small proportion of them have been found to be resistant to amphotericin B with MIC ≥ 2 $\mu\text{g}/\text{mL}$ (32). *Candida glabrata* with amphotericin B MIC ≥ 2 $\mu\text{g}/\text{mL}$ was reported in less than 1% of USA and in 4.4% of European isolates 33. Furthermore, difficulty in the treatment of infection with *C. glabrata*, that is often resistant to many azole antifungal agents, especially fluconazole, is also reported 34. Recent studies have revealed that the MICs of triazoles, voriconazole, itraconazole and fluconazole, for *C. glabrata* were higher than those seen for most other *Candida* species (12, 23, 24).

Amphotericin B is recommended as first-line therapy for invasive mycoses, and is most commonly used in pediatrics (26). The use of this drug is limited by the toxicity of the conventional formulation and the high cost of the lipid emulsions. In the present study, susceptibility testing revealed that all isolates of *C. albicans* were more sensitive to amphotericin B and caspofungin than to the other antifungal studied. However, resistance to amphotericin B was seen in 80% of *C. krusei* and 75% of *C. glabrata* isolates. In one study, amphotericin B resistance was

found in nearly 20% of *C. parapsilosis* isolates (35).

Triazole antifungal drugs alter the fungal cell membrane by inhibiting ergosterol synthesis through an interaction with 14-demethylase, which leads to alterations in cellular permeability and a loss of membrane fluidity and integrity (36). The currently available triazole antifungals include fluconazole, voriconazole, itraconazole, ketoconazole, and posaconazole. Resistance to azoles was found in all *Candida* spp., with species-specific trends. Gene mutations related to ATP dependent pumps CDR genes in *Candida* spp. appears to confer resistance to multiple azoles and have been associated with fluconazole treatment, and cross-resistance with other azoles may also be possible (36,37) which was well documented by molecular methods (38). According to previous studies, resistance to older azoles is most commonly demonstrated in *C. krusei* and *C. glabrata* (23, 24). In the present study, fluconazole resistance was observed in 41.5%, 80%, and 100% of *C. albicans*, *C. krusei*, and *C. glabrata* isolates and resistance to itraconazole was found in nearly 28%, 30% and 100% of *C. albicans*, *C. krusei*, and *C. glabrata* strains, respectively percentages that are consistent with the findings in previous reports (4,23). Cross-resistance between the newer and older azoles has been a concern, particularly for pairs of congeners, such as fluconazole - voriconazole and itraconazole- posaconazole (39, 40). In the present study, voriconazole and fluconazole resistance was documented in 10% of *C. krusei* and 25% of *C. glabrata* isolates. Posaconazole is the newest orally administered triazole antifungal with an extended spectrum of activity. Due to cross activity between this antifungal agent and other azoles, in this study, the MICs for many *Candida* spp. were more than 2 µg/mL.

Our susceptibility data showed that the newer antifungal agents are effective for

the treatment of yeast infections. Echinocandins such as caspofungin are active against many species of fungi and have been approved by the U.S.A Food and Drug Administration (FDA) for the treatment of candidemia and invasive candidiasis. In our study, caspofungin was the most effective agent, with lower MIC50 and MIC90 values against all the *Candida* spp. studied.

Conclusion

Knowledge about the susceptibility patterns of colonized *Candida* spp. can be of help to clinicians who must decide which is the best option for the management of their high-risk patients. In pediatric patients with hematologic malignancies and especially neutropenia, we found that caspofungin was the best antifungal agent against *Candida* colonization, followed by conventional amphotericin B.

Acknowledgments

Our sincere thanks go to Dr. Hassan Khajehei for copyediting the manuscript and to K. Shashok (Author AID in the Eastern Mediterranean) for improving the use of English in the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding/Support

The present article was extracted from the thesis written by Pedram Haddadi and was financially supported by Shiraz University of Medical Sciences grant no. 89-01-0102328.

References

1. Akan H, Antia VP, Kouba M, Sinkó J, Tanase AD, Vrhovac R, et al. Preventing invasive fungal disease in patients with hematological malignancies and the recipients of hematopoietic stem cell transplantation: practical aspects. *J*

- Antimicrob Chemother 2013; 68(3): iii5-16.
2. Celebi S, Hacimustafaoglu M, Ozdemir O, Ozkaya G. Nosocomial candidaemia in children: results of a 9-year study. *Mycoses* 2008; 51: 248-57.
 3. Badiie P, Kordbacheh P, Alborzi A, Zakernia M, Haddadi P. Early detection of systemic candidiasis in the whole blood of patients with hematologic malignancies. *Jpn J Infect Dis* 2009; 62: 1-5.
 4. San Miguel LG, Cobo J, Otheo E, Sánchez-Sousa A, Abaira V, Moreno S. Secular trends of candidemia in a large tertiary-care hospital from 1988 to 2000: emergence of *Candida parapsilosis*. *Infect Control Hosp Epidemiol* 2005; 26: 548-52.
 5. Maródi L, Johnston RB Jr. Invasive *Candida* species disease in infant and children: occurrence, risk factor, management, and innate host defense mechanism. *Curr Opin Pediatr* 2007; 19: 693-7.
 6. Saiman L, Ludington E, Dawson JD, Patterson JE, Rangel-Frausto S, Wiblin RT, et al. Risk factors for *Candida* species colonization of neonatal intensive care unit patients. *Pediatr Infect Dis J* 2001; 20: 1119-24.
 7. Kaufman D, Boyle R, Hazen KC, Patrie JT, Robinson M, Donowitz LG. Fluconazole prophylaxis against fungal colonization and infection in preterm infants. *N Engl J Med* 2001; 345: 1660-6.
 8. Pammi M, Eddama O, Weisman LE. Patient isolation measures for infants with candida colonization or infection for preventing or reducing transmission of candida in neonatal units. *Cochrane Database Syst Rev*. 2011 Nov 9; (11):CD006068. doi: 10.1002/14651858.CD006068.pub3.
 9. Zomorodian K, Rahimi MJ, Pakshir K, Motamedi M, Ghiasi MR, Rezashah H. Determination of antifungal susceptibility patterns among the clinical isolates of *Candida* species. *J Glob Infect Dis* 2011; 3: 357-60.
 10. Shokohi T, Bandalizadeh Z, Hedayati MT, Mayahi S. In vitro antifungal susceptibility of *Candida* species isolated from oropharyngeal lesions of patients with cancer to some antifungal agents. *JJM* 2011; 4: 19-26.
 11. Badiie P, Alborzi A. Susceptibility of clinical *Candida* species isolates to antifungal agents by E-test, Southern Iran: A five-year study. *Iran J Microbiol*. 2011; 3: 183-8.
 12. Pfaller MA, Boyken L, Hollis RJ, Messer SA, Tendolkar S, Diekema DJ. In vitro susceptibility of *Candida* spp. to caspofungin: four years of global surveillance. *J Clin Microbiol* 2006; 44: 760-3.
 13. Rex JH, Alexander B D, Andes D, Arthington-Skaggs B, Brown S D, Chaturvedi V, Ghannoum M A, Espinel-Ingroff A, Knapp CC, Ostrosky-Zeichner L, Pfaller M.A, Sheehan D J, Walsh T J. Clinical and Laboratory Standards Institute. M27-A3. Reference method for broth dilution antifungal susceptibility testing of yeast, 3rd ed. Clinical 2008; 28(14). Replaces M27-A2. Vol. 22 No. 15. ISBN 1-56238-666. www.clsi.org
 14. Blignaut. E, Messer S, Hollis RJ, Pfaller MA. Antifungal susceptibility of South African oral yeast isolates from HIV/AIDS patients and healthy individuals. *Diagn Microbiol Infect Dis* 2002; 44: 169-74.
 15. Davey. KG, Holmes AD, Johnson EM, Szekeley A, Warnock DW. Comparative evaluation of fungitest and broth microdilution methods for antifungal drug susceptibility testing of *Candida* species and *Cryptococcus neoformans*. *J Clin Microbiol* 1998; 36: 926-30.
 16. Swinne D, Wattle M, Van der Flaes M, Nolard N. In vitro activities of voriconazole (UK-109, 496), fluconazole, itraconazole and amphotericin B against 132 non-albicans blood stream yeast isolates. *Mycoses* 2004; 47: 177-83.
 17. Sabatelli F, Patel R, Mann PA, Mendrick CA, Norris CC, Hare R, et al. In vitro activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large

collection of clinically important molds and yeasts. *Antimicrob Agents Chemother* 2006; 50: 2009-15.

18. Maa SH, Lee HL, Huang YC, Wu JH, Tsou TS, MacDonald K, et al. Incidence density and relative risk of nosocomial infection in Taiwan's Only Children's Hospital, 1999-2003. *Infect Control Hosp Epidemiol* 2008; 29: 767-70.

19. Badiie P, Alborzi A, Joukar M. Molecular assay to detect nosocomial fungal infections in intensive care units. *Eur J Intern Med* 2011; 22: 611-5.

20. Farmaki E, Evdoridou J, Pouliou T, Bibashi E, Panagopoulou P, Filioti J, et al. Fungal colonization in the neonatal intensive care unit: risk factors, drug susceptibility, and association with invasive fungal infections. *Am J Perinatol* 2007; 24: 127-35

21. Lopes MM, Barros R, Peres I, Serelha M, Neto MT, Cabrita J, et al. Surveillance of nosocomial fungal infections in a portuguese paediatric hospital incidence and risk factors. *J Mycol Méd* 2006; 16: 212-9.

22. Badiie P, Alborzi A, Davarpanah MA, Shakiba E. Distributions and antifungal susceptibility of *Candida* species from mucosal sites in HIV positive patients. *Arch Iran Med* 2010; 13: 282-7.

23. Badiie P, Alborzi A, Shakiba E, Farshad S, Japoni A. Susceptibility of *Candida* species isolated from immunocompromised patients to antifungal agents. *EMHJ* 2011; 17: 425-30.

24. Bakir M, Cerikcioglu N, Barton R, Yagci A. Epidemiology of candidemia in Turkish tertiary care hospital. *APMIS* 2006; 114: 601-10.

25. Pasqualotto AC, Nedel WL, Machado TS, Severo LC. A 9-year study comparing risk factors and the outcome of paediatrics and adults with nosocomial candidaemia. *Mycopathologia* 2005; 160: 111-6.

26. Filioti J, Spiroglou K, Panteliadis CP, Roilides E. Invasive candidiasis in pediatric intensive care patients:

epidemiology, risk factors, management, and outcome. *Intensive Care Med* 2007; 33: 1272-83.

27. Wingard JR, Merz WG, Rinaldi MG, Johnson TR, Karp JE, Saral R. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. *N Engl J Med* 1991; 325(18):1274-77.

28. MasiáCanuto M, Gutiérrez Rodero F. Antifungal drug resistance to azoles and polyenes. *Lancet Infect Dis* 2002; 2(9): 550-63.

29. Sanglard D, Odds FC. Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. *Lancet Infect Dis* 2002; 2(2): 73-85.

30. Dannaoui E, Lortholary O, Dromer F. In vitro evaluation of double and triple combinations of antifungal drugs against *Aspergillus fumigatus* and *Aspergillus terreus*. *Antimicrob Agents Chemother* 2004; 48(3): 970-8.

31. Rex JH, Walsh TJ, Sobel JD, Filler SG, Pappas PG, Dismukes WE, et al. Practice guidelines for the treatment of candidiasis. *Infectious Diseases Society of America. Clin Infect Dis* 2000; 30(4): 662-78.

32. Pfaller MA, Messer SA, Boyken L, Tendolkar S, Hollis RJ, Diekema DJ. Geographic variation in the susceptibilities of invasive isolates of *Candida glabrata* to seven systemically active antifungal agents: a global assessment from the ARTEMIS Antifungal Surveillance Program conducted in 2001 and 2002. *J Clin Microbiol* 2004; 42(7): 3142-6.

33. Fidel PL Jr, Vazquez JA, Sobel JD. *Candida glabrata*: review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*. *Clin Microbiol Rev* 1999; 12(1): 80-96.

34. Zaoutis TE, Foraker E, McGowan KL, Mortensen J, Campos J, Walsh TJ, et al. Antifungal susceptibility of *Candida* spp. isolated from pediatric patients: A

survey of 4 children's hospitals. *Diagn Microbiol Infect Dis.* 2005; 52: 295-8.

35. Carrillo-Muñoz AJ, Giusiano G, Ezkurra PA, Quindós G. Antifungal agents: Mode of action in yeast cells. *Rev Esp Quimioter* 2006; 19: 130–9.

36. Perea S, López-Ribot JL, Kirkpatrick WR, McAtee RK, Santillán RA, Martínez M, et al. Prevalence of molecular mechanisms of resistance to azole antifungal agents in *Candida albicans* strains displaying high-level fluconazole resistance isolated from human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother* 2001; 45(10): 2676-84.

37. Posteraro B, Sanguinetti M, Fiori B, La Sorda M, Spanu T, Sanglard D, et al.

Caspofungin activity against clinical isolates of azole cross-resistant *Candida glabrata* overexpressing efflux pump genes. *J Antimicrob Chemother* 2006; 58(2); 458-61.

38. White TC, Holleman S, Dy F, Mirels LF, Stevens DA. Resistance mechanisms in clinical isolates of *Candida albicans*. *Antimicrob Agents Chemother.* 2002; 46:1704–13.

39. Pfaller MA, Messer SA, Hollis RJ, Jones RN, Diekema DJ. In vitro activities of ravuconazole and voriconazole compared with those of four approved systemic antifungal agents against 6,970 clinical isolates of *Candida* spp. *Antimicrob Agents Chemother* 2002; 46:1723-7.