Prevalence and antifungal drug resistance of nosocomial Candida species isolated from two university hospitals in Egypt

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Background and Purpose: There is a significant rise in morbidity and mortality of infections caused by Candida. Candida spp. infections are currently ranked fourth among nosocomial infections which are difficult to diagnose and refractory to therapy. Given the differences in susceptibility among various spp., identification of Candida spp. is an important step that leads to the selection of a suitable antifungal.

Materials and Methods: A prevalence study was conducted on 122 Candida isolates. The Candida spp. were identified using Chromogenic agar and polymerase chain reaction (PCR). The antifungal susceptibility (AFS) of Candida spp. to amphotericin B, fluconazole, voriconazole, and caspofungin was determined by the disc diffusion method.

Results: In total, 122 Candida clinical isolates were investigated in this study. Candida albicans with 57.4% (70 isolates) had the highest prevalence rate, while 52 isolates (42.6%) were non-albicans Candida species (NAC). The NAC include Candida krusei (20.4%), Candida tropicalis (6.5%), Candida parapsilosis (5.7%), Candida dublieniensis (4.9%), and Candida glabrata (4.9%). The AFS showed that the resistance rates of Candida spp. to fluconazole and voriconazole were 13.1% (16 isolates) and 9.8% (12 isolates), respectively. Moreover, only five isolates (4.1%) were resistant to caspofungin.

Furthermore, there was no resistance against amphotericin B. The spp. that showed the highest resistance were C. glabrata and C. tropicalis, while the lowest resistance was observed in C. albicans and C. dublieniensis.

Conclusion: In conclusion, rapid identification of clinical Candida isolates and standard AFS are essential procedures for controlling the rise of resistant NAC spp. in clinical settings. Usage of fluconazole should be restricted, especially in patients with recurrent Candida infections.

Keywords: Antifungal drug resistance, Azoles and caspofungin, Candida albicans, Non-albicans, Nosocomial infections

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Introduction

There has been a significant rise in morbidity and mortality of infections caused by Candida, especially in recent years, due to the continuous rise in the number of infections, particularly in hospitalized patients [1]. Hospital-acquired (nosocomial) infection is more prevalent in certain patient groups, including transplant recipients, cancer patients, and other patients who receive immunosuppressive therapy [2]. Nosocomial infection is defined as an infection that is not apparent when the patient is admitted to the hospital but appears at least 48 h after the admission [3].

Candida spp. cause infections that range from non-life-threatening mucosal illnesses to invasive fatal infections, such as bloodstream infections [4]. Nosocomial infection by Candida is a problematic issue worldwide. Candida spp. are currently ranked fourth and sixth among the causative agents of nosocomial bloodstream infections (BSI) in the USA and Europe, respectively [2, 5]. Egypt showed the highest Candida BSI burden, compared to the other Middle East neighboring countries [6].

Candida identification procedures usually start with the germ tube test that can differentiate C. albicans and C. dublieniensis from other Candida spp. [7]. Further tests, such as culturing on cornmeal agar, carbohydrate fermentation, and carbohydrate assimilation tests, are performed for the detection of other spp. [8]. Moreover, several chromogenic culture media have been developed to allow rapid identification of mixed Candida spp. [9]. The molecular approaches have the potential to detect Candida spp. with increased sensitivity and specificity [10]. The sequences of internal transcribed spacer (ITS) regions 1 and ITS2 have been used in various Polymerase chain reaction (PCR)-based methods for identification of medically
important Candida spp. [11].

Identification of Candida to the spp. level is an important step that leads to the selection of suitable antifungals. Changes in Candida spp. distribution may impact treatment recommendations due to differences in susceptibility to antifungal agents among different spp. [12]. Antifungal agents available for the treatment of invasive candidiasis are restricted to polyenes, azoles, and the most recent echinocandin class [13]. Emergence of multidrug-resistant strains that are insensitive to several classes of antifungals is a major concern worldwide [14]. Studies on the prevalence rate of infections and antifungal susceptibility testing can help the selection of a proper treatment strategy that limits the emergence of resistance [15].

In the current study, different Candida spp. were identified in clinical specimens using Chromogenic agar and PCR-based methods. Furthermore, the antifungal drug resistance of the identified spp. was determined by the disc diffusion method.

Materials and Methods

Isolation and phenotypic identification of Candida species

All the clinical specimens were collected from different sources (blood, urine, and sputum) in clinical laboratories at Mansoura and Zagazig University Hospitals, Egypt. Candida identification started with a microscopical examination followed by growth on chromogenic agar and PCR [7]. The clinical specimens were collected under ethical standards and due to retrospective nature of the study, consent form were not applicable to the study. First, the specimens were inoculated on Sabouraud dextrose agar (SDA) plates and incubated at 37 °C for 24-72 h. The colonies were examined microscopically after Gram staining. Colonies that were proved microscopically to be Candida, were sub-cultured on HiChrome agar (HiMedia Laboratories, Mumbai, India) and incubated aerobically at 30 °C. They were inspected after 24-72 h, and the colony color was recorded and interpreted according to the manufacturer instructions. Briefly, C. albicans and C. dubliniensis gave pale green colonies, C. krusei gave purple fuzzy colony, C. tropicalis gave blue to purple colony, while other Candida spp. gave creamy white colonies.

Confirmation of Candida species identification by Polymerase chain reaction

The DNA of Candida spp. was extracted by colony PCR method [16]. A pure colony of each isolate was picked up from SDA and inoculated into 30 μl of Tris-EDTA (TE) buffer. The mixture was heated at 100 °C in a water bath for 10 min and subsequently centrifuged at 10000 rpm for 2 min. Finally, the supernatants were transferred to a fresh Eppendorf tube.

The primers used for the identification of Candida spp. were purchased from Operon Biotechnologies (GmbH Biocompus Cologne, Germany). Table 1 summarizes the sequences of these primers. During the PCR, the species-specific primers formed a pair with the universal primer UNI2 for all tested Candida spp., with the exception of C. lusitaniae, in which species-specific primer (Clus) paired with UNI1 [11].

The target DNA was amplified in a 20 μL reaction mixture containing 1 μL DNA samples, 10 μL of my Taq red mix (Bioline Co., UK), 1 μL of each forward and reverse primers, and up to 20 μL of nuclease-free water. The cycling conditions included heating at 95 °C for 3 min, followed by 30 cycles at 94 °C for 60 sec, 52 °C for 30 sec, 65 °C for 45 sec, and finally heating at 65 °C for 7 min [7]. The PCR products, as well as 100 bp molecular DNA ladder (Bioline Co., UK), were separated on 1% agarose gel, stained with ethidium bromide (Merck, Hohenburnn, Germany) and visualized by a UV transilluminator.

Determination of antifungal susceptibility by disk diffusion method

Candida isolates were tested for their susceptibility to different antifungal agents by disk diffusion method according to Clinical and Laboratory Standard Institute guidelines [17]. Briefly, Three to five well-isolated colonies were inoculated into 4-5 mL Sabouraud dextrose broth (Oxoid, Hampshire, England) and the broth was incubated for 24 h at 37°C. The turbidity of the suspension was adjusted to the turbidity of 0.5 McFarland turbidity standards (10^7 cells/mL).

A sterile cotton swab was dipped into the prepared suspension (within 15 min of adjusting the turbidity) and rotated firmly against the inside of the tube to remove excess fluid. Afterward, it was used to streak over Muller Hinton agar (MHA) (Oxoid, Hampshire, England) plate (containing 2% glucose and 0.5 μg/mL methylene blue). The antifungal disks were placed on the MHA plates using sterile forceps. Disks were pressed firmly against the agar surface to ensure contact and antifungal diffusion. The plates were

| Candida spp. | Primer | Sequence (5’ to 3’) | Size (bp) |
|-------------|--------|---------------------|----------|
| Universal primers | UNI1 | GTCAAACTGGTCAATTAA | 507 |
| | UNI2 | TTCTTTTCCTCCGCTTTAAT | 446 |
| C. albicans | Calb | AGCCGCCAGCAACTGACT | 169 |
| | Ckru | CTGCCGAGCGAAGCTAGACT |  |
inverted and incubated at 37 °C for 24-48 h. The diameter of inhibition zones around each antifungal disk was measured in millimeters and interpreted as susceptible, intermediate, or resistant according to interpretative criteria of CLSI [17].

The tested disks included amphotericin B (AMB, 10μg) as polyene drug, fluconazole (FLU, 25μg) as representative of the first generation azoles, voriconazole (VOR, 1μg) as representative of the second generation azoles, and caspofungin (CASP, 5μg) as representative of the echinocandin drug class. The antifungal disks were obtained from Bioanalyse, (Ankara, Turkey), and the standard strain, *C. albicans* ATCC 10231, was used as the reference strain.

**Results**

**Identification of Candida species isolates**

In total, 122 non-duplicate *Candida* clinical isolates were identified in the present study. These isolates were from different clinical sources; 21 (17.2 %), 45 (36.9%), 56 (45.9 %) isolates were from blood, urinary tract infection, and respiratory tract infection, respectively. In HiChrome agar, 72 isolates (59%) produced light green colonies (*C. albicans* or *C. dubliniensis*), 22 isolates (18%) were identified as *C. krusei* (gave purple colonies), eight (6.5%) were identified as *C. tropicalis* (gave blue colonies), and 20 (16.3%) produced white colonies and were identified as other *Candida* spp. (Figure 1)

![Figure 1](image1.png)

According to the PCR results that are presented in Figure 2, 70 isolates (57.4%) gave a single band of 446 bp and were identified as *C. albicans*, while 52 isolates (42.6%) were non-*albicans*. The non-*albicans* *Candida* spp. included 20.4% *C. krusei* (25 strains gave a single band of 169 bp), 6.5% *C. tropicalis* (eight isolates gave a single band of 507 bp), 5.7% *C. parapsilosis* (seven isolates gave a single band of 370 bp), 4.9% *C. dubliniensis* (six isolates gave a single band of 217 bp), and 4.9% *C. glabrata* (six isolates gave a single band of 839 bp). In the present study, none of the isolates were *C. guilliermondii* or *C. lusitaniae*.

![Figure 2](image2.png)

**Susceptibility to antifungals**

The antifungal susceptibility testing of the 122 *Candida* strains revealed resistance to three antifungal drugs (FLU, VOR, and caspofungin). The data presented in Figure 3 showed that the percentages of resistance to FLU and VOR were 13.1% (16 isolates) and 9.8% (12 isolates), respectively. For caspofungin, five isolates (4.1%) were resistant, and only one isolate (0.8 %) showed intermediate resistance. It should be mentioned that there was no resistance to amphotericin B and *C. albicans* ATCC 10231 was sensitive to all tested antifungals.

![Figure 3](image3.png)
In total, 22 isolates were resistant to antifungals, and each strain showed resistance to only one drug class (Table 2). Based on the findings, seven isolates of C. albicans showed resistance to azoles with no resistance to caspofungin, five C. krusei isolates were resistant (four were resistant to azoles and one to caspofungin), four C. tropicalis were resistant (one was resistant to azole and three to caspofungin), four C. glabrata were resistant to azoles only. Furthermore, one C. parapsilosis strain was resistant to caspofungin and one C. dubliniensis strain was intermediately resistant to caspofungin.

**Discussion**

The incidence of fungal infections with high morbidity and mortality has increased globally due to the limited antifungal arsenal and the high toxicity of some drugs. Only five antifungal drug classes are available, including polyenes, azoles, and allylamines that target ergosterol in the cell membrane, pyrimidine analogs that target DNA synthesis, and the new echinocandin class that targets β-glucan in the fungal cell wall [18]. Candida spp. are currently ranked fourth among nosocomial BSIs in the USA, accounting for 8-10% of all BSIs acquired in hospitals [19]. Candida infections are ranked as the sixth most common cause of nosocomial infection in Europe [2, 5]. Furthermore, Candida BSIs are more prevalent in Egypt than in other Middle East countries [6].

Given the drastic increase in non-albicans Candida species (NAC) infections and the distinct antifungal susceptibility pattern of these spp., accurate identification becomes essential for proper clinical management [19]. The current study aimed to identify and determine the antifungal susceptibility of different Candida spp. from clinical infections.

Both Chromogenic agar and PCR were used for the identification of Candida to the spp. level in this study. Chromogenic agar is an economic and simple method, and in this study, the sensitivity of chromogenic media in the identification of Candida spp. was about 95% which was consistent with the previously reported 96.3% and 97.5% in a study conducted in Egypt [7, 20]. However, Chromogenic agar was unable to differentiate C. albicans from C. dubliniensis and some spp. have no distinct color on it.

Obviously, the PCR method showed better sensitivity in the detection of Candida spp., which was in agreement with the previously reported results [7, 11]. In the present study, the use of species-specific primers allowed the differentiation of several Candida spp. This method makes it possible to identify the spp. that have non-morphologic, cultural, and biochemical characteristics.

According to the results of the current study, C. albicans was the most prevalent spp. (57.4%) which was comparable to the 62.9 % reported in vaginal infections [21]. Kadry et al. [7] reported a prevalence rate of 70% which is slightly higher than the results of the present study. Moreover, according to a study performed in Egypt, the prevalence rate of C. albicans in BSIs was 40%, which is lower than the findings of the present study [22]. These differences in prevalence could be attributed to the variety of sources from which the clinical samples were isolated.

The NAC levels in this study (42.6%) are in line with the values in the literature that denote the epidemiological shift of Candida pathogens in the last
few decades [2]. Nevertheless, this shift appears to be rising with time as the recently reported prevalence of NAC is around or even exceeds 50% in some cases [6,22,23]. The extensive use of antifungals for prophylaxis has become the leading cause of colonization of NAC and increase of resistance to antifungal drugs [24].

It was reported previously that C. tropicalis and C. parapsilosis were the most prevalent Candida spp. after C. albicans [24]. However, in this study, C. krusei ranked second after C. albicans with a prevalence rate of about 20%, followed by C. tropicalis (6.5%) and C. parapsilosis (5.7%). Moreover, a similar prevalence rate for C. krusei was recently reported in Egypt [6,22]. A lower prevalence of C. krusei was reported (10.7%) in vaginal infections [21], while it had a prevalence rate of 46% in leprosy patients [25]. Besides, it was reported that C. krusei was highly associated with FLU exposure [26]. Several studies have also reported variable prevalence rates for C. tropicalis (8.40%) and C. parapsilosis (3.14%) in different clinical sources [27].

In the present study, both C. dubliniensis and C. glabrata had a prevalence rate of 4.9%. Sharma et al. reported a prevalence rate of 0.5-6.3% for C. glabrata [28], while Yang et al. detected a rate of 13-20% in different clinical samples [29]. Besides, various prevalence rates of C. dubliniensis (0-5.5%) were observed in different clinical sources [29] and different countries around the world. Accordingly, 1.4% was reported in Egypt [30], 9% in Germany [31], and 11% in Sweden [32]. Overall, changes in spp. distribution are attributed to several factors, including different geographical regions, hospital-related factors, sources of the specimen, and type of antifungal therapy.

Regarding antifungal susceptibility, in this study, the highest level of resistance was observed against FLU (13.1%) and VOR (9.8%). The spp. that showed FLU resistance were C. glabrata (66.6%), C. krusei (16%), C. tropicalis (12.5%), and C. albicans (10%). Furthermore, Khairat et al. [22] reported thatazole resistance is higher in NAC spp., compared to C. albicans (44% versus 38.9%). The C. glabrata has intrinsically low susceptibility to azoles, and acquired azole resistance has been documented during treatment [33]. Moreover, C. krusei exhibits intrinsic resistance to FLU [34]. Reported FLU resistance in C. tropicalis is within the range of 0-83% and 4-9% in South Korea [35] and the USA [36], respectively. The FLU is known to be the most commonly prescribed antifungal [34], and its prolonged use in treating Candida infections has led to the emergence of resistance in all Candida spp. [12].

Caspofungin is the first echinocandin drug, and 60% of patients with candidemia are reported to have received an echinocandin drug. Resistance to echinocandins has evolved since 2005 but remains relatively low [37]. In this study, three spp. exhibited resistance to caspofungin, C. tropicalis (37.5%), C. parapsilosis (14.3%), and C. krusei (4%). Only one C. dubliniensis strain was intermediate resistant to caspofungin, while C. albicans and C. glabrata did not show any resistance to caspofungin. Similarly, the results of previous studies have shown that C. albicans had no resistance against caspofungin [38, 39]. The C. dubliniensis normally does not show elevated resistance to echinocandins [40], while C. tropicalis acquires resistance after short-term treatment with caspofungin [41]. Moreover, C. parapsilosis tends to be more tolerant to echinocandins [2].

The polyene drug AmB has been the most potent fungicidal drug for decades; however, its renal toxicity has limited its use, hence liposomal formulations of AmB are used to reduce its toxicity [42]. None of our isolates showed resistance to AmB, which is in line with the results of previous studies performed in Egypt which reported either no resistance [39] or very low (3%) resistance to AmB [22]. It is well known that Candida rapidly develops resistance to azoles and echinocandins. Nevertheless, resistance to AmB remains extremely rare despite decades of use [43].

**Conclusion**

Candida spp. are responsible for many fungal infections in humans and a noticeable increase of NAC infections. Changes in Candida spp. distribution may impact treatment recommendations due to differences in susceptibility to antifungals among the spp. Regarding azoles and echinocandin, intrinsic resistance in some spp. and acquired resistance in other spp. were observed. The AmB remains the gold standard drug for the treatment of Candida infections as resistance to it is very rare. Finally, accurate identification of spp. and standard antifungal susceptibility testing are essential procedures for controlling the rise of resistant Candida strains.

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**Authors’ contribution**

A.M.E. conceived the idea of this research, participated in the data analysis and reviewed the manuscript. H.A.K. performed the experiments, participated in data analysis and wrote the initial manuscript draft. N.Y. participated in the initial idea of this research, provided intellectual input and edited the manuscript. All authors read and approved the final manuscript.

**Conflicts of interest**

The authors declare that they have no conflicts of interest.
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References
1. Xiao Z, Wang Q, Zhu F, An Y. Epidemiology, species distribution, antifungal susceptibility and mortality risk factors of Candidaemia among critically ill patients: a retrospective study from 2011 to 2017 in a teaching hospital in China. Antimicrob Resist Infect Control. 2019; 8:89.
2. Sanguinetti M, Posterao B, Lass-Flörl C. Antifungal drug resistance among Candida species: mechanisms and clinical impact. Mycoses. 2015; 58(Suppl 2):2-13.
3. Aboualigaledhari E, Ghafooruin S, Sadeghifard N, Sekawi Z. Is Candida albicans a cause of nosocomial infection in Iran? Rev Med Microbiol. 2013; 24(4):85-90.
4. Seyoum E, Bitew A, Mihret A. Distribution of Candida albicans and non-albicans Candida species isolated in different clinical samples and their in vitro antifungal susceptibility profile in Ethiopia. BMC Infect Dis. 2020; 20(1):231.
5. Hestsveldt L, Gaustad P, Andersen CT, Haarr E, Hannula R, Haakland HH, et al. Twenty-two years of Candidaemia surveillance: results from a Norwegian national study. Clin Microbiol Infect. 2015; 21(10):938-45.
6. Abass E, Mohamed S, El-Kholy I, Zaki S. Incidence of ICU-acquired candidemia in a tertiary care hospital in Cairo, Egypt. Egypt J Microbiol. 2019; 54(1):55-61.
7. Kadry AA, El-Ganny AM, El-Baz AM. Comparison of methods used in identification of Candida albicans. Res J Pharm Technol. 2018; 11(3):1164-8.
8. Baradkar VP, Mathur M, Kumar S. Chromome candida agar for identification of Candida species. Indian J Pathol Microbiol. 2010; 53(1):93-5.
9. Murray CK, Beckius ML, Green JA, Hospenthal DR. Use of chromogenic medium for the isolation of yeasts from clinical specimens. J Med Microbiol. 2005; 54(Pt 10):981-5.
10. Chang HC, Leaw SN, Huang AH, Wu TL, Chang TC. Rapid identification of yeasts in positive blood cultures by a multiplex PCR method. J Clin Microbiol. 2001; 39(10):3466-71.
11. Carvalho A, Costa-De-Oliveira S, Martins ML, Pina-Vaz C, Rodrigues AG, Ludovico P, et al. Multiplex PCR identification of eight clinically relevant Candida species. Med Mycol. 2007; 45(7):619-27.
12. Bassetti M, Peghin M, Timsf JT. The current treatment landscape: candidiasis. J Antimicrob Chemother. 2016; 71(Suppl 2):i13-22.
13. Cannon RD, Lamping E, Holmes AR, Niimi K, Barett PV, Kenyua MV, et al. Efflux-mediated antifungal drug resistance. Clin Microbiol Rev. 2009; 22(2):291-321.
14. Perlin DS, Shor E, Zhao Y. Update on antifungal drug resistance. Curr Clin Microbiol Rep. 2015; 2(2):84-95.
15. Bhatthacharjee P. Epidemiology and antifungal susceptibility of Candida species in a tertiary care hospital, Kolkata, India. Curr Microbiol. 2016; 2(2):20-7.
16. Cornet M, Sendil B, Fradin C, Gaillardin C, Pouilain D, Nguyen HV. Molecular identification of closely related Candida species using two ribosomal intergenic spacer fingerprinting methods. J Mol Diagn. 2011; 13(1):12-22.
17. Clinical and Laboratory Standards Institute (CLSI). Method for anti-fungal disk diffusion susceptibility testing of yeasts: approved guideline. 2nd ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
18. Scorzon L, de Paula E Silva AC, Marcos CM, Assato PA, de Melo WC, de Oliveira HC, et al. Antifungal therapy: new advances in the understanding and treatment of mycosis. Front Microbiol. 2017; 8:36.
19. Pfaffer MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007; 20(1):133-63.
20. Daef E, Moharram A, Eldin SS, Elsherbiny N, Mohammed M. Evaluation of chromogenic media and semi-nested PCR in the identification of Candida species. Braz J Microbiol. 2014; 45(1):255-62.
21. Hassan MH, Ismail MA, Moharram AM, Shoreit M. Prevalence of vaginal infection by multidrug resistant Candida species among different ages in Egypt. Am J Microbiol Res. 2017; 5(4):78-85.
22. Khairat SM, Sayed AM, Nabihi M, Soliman NS, Hassan YM. Prevalence of Candida blood stream infections among children in tertiary care hospital: detection of species and antifungal susceptibility. InfectDrug Resist. 2019; 12: 2409-16.
23. Kaur R, Dhakad MS, Goyal R, Kumar R. Emergence of non-albicans Candida species and antifungal resistance in intensive care unit patients. Asian Pac J Trop Biomed. 2016; 6(5):455-60.
24. Kothiavade RJ, Kara MM, Valanad AG, Panthaki MH. Candida tropicalis: its prevalence, pathogenicity and increasing resistance to fluconazole. J Med Microbiol. 2010; 59(8):873-80.
25. Rechert PA, Samarayakej L, Samarayakee YH, Grote M, Pow E, Cheung B. High oral prevalence of Candida krusei in leprosy patients in Northern Thailand. J Clin Microbiol. 2002; 40(12):4479-85.
26. Hope W, Morton A, Eisen DP. Increase in prevalence of nosocomial non-Candida albicans candidaemia and the association of Candida krusei with fluconazole use. J Hosp Infect. 2002; 50(1):56-65.
27. Sandhya Rani T, Srikumar R, Reddy LP, Latha S. Prevalence and characterization of opportunistic candidial infection among patients with type II diabetes mellitus. Indian J Public Health. 2019; 10(7):448-58.
28. Sharma Y, Chumber SK, Kaur M. Studying the prevalence, species distribution, and detection of in vitro production of phospholipase from Candida isolated from cases of invasive candidiasis. J Global Infect Dis. 2017; 9(1):8-11.
29. Yang CW, Barkham TM, Chan FY, Wang Y. Prevalence of Candida species, including Candida dubliniensis, in Singapore. J Clin Microbiol. 2003; 41(1):472-4.
30. Esmat MM, Mohamed T, Abdellrahman AH. Species identification and antifungal susceptibility profile of Candida isolates from ICU patients in Sohag University Hospital, Upper Egypt. Egypt J Med Microbiol. 2015; 38(3676):1-9.
31. Ziesing S, Suerbaum S, Sedlacek L. Fungal epidemiology and diversity in cystic fibrosis patients over a 5-year period in a national reference center. Med Mycol. 2016; 54(8):781-6.
32. Al Shakhiri M, Klingspor L, Bergman P, Hjelte L, de Monestrol I. A 16-year retrospective study on fungal prevalence and diversity in patients with Cystic Fibrosis-Candida dubliniensis was associated with a decline in lung function. Int J Infect Dis. 2020; 96:663-70.
33. Vermitsky JP, Edlund TD. Azole resistance in Candida glabrata: coordinate upregulation of multidrug transporters and evidence for a Pdr1-like transcription factor. Antimicrob Agents Chemother. 2004; 48(10):3773-81.
34. Whaley SG, Berkow EL, Rybak JM, Nishimoto AT, Barker KS, Rodgers PD. Azole antifungal resistance in Candida albicans and emerging non-albicans Candida species. Front Microbiol. 2017; 7:2173.
35. Yoo JI, Choi CW, Lee KM, Kim YK, Kim TU, Kim EC, et al. National surveillance of antifungal susceptibility of Candida species in South Korean hospitals. Med Mycol. 2009; 47(5):554-8.
36. Berkow EL, Lockhart SR. Fluconazole resistance in Candida species: a current perspective. Infect Drug Resist. 2017; 10:237-45.
37. Perlin DS. Echinocandin resistance in Candida. Clin Infect Dis. 2015; 61(Suppl 6):S612-7.
38. Bitew A, Abebow Y. Vulvovaginal candidiasis: species distribution of Candida and their antifungal susceptibility pattern. BMC Womens Health. 2018; 18(1):94.
39. Kadry AA, El-Ganny AM, El-Baz AM. Relationship between Sap prevalence and biofilm formation among resistant clinical isolates of Candida albicans. Afr Health Sci. 2018; 18(4):1166-74.
40. Pristov KE, Ghanoum MA. Resistance of Candida to azoles and echinocandins worldwide. Clin Microbiol Infect. 2019; 25(7):792-8.
41. Khan Z, Ahmad S, Mokaddas E, Meis JF, Joseph L, Abdullah A, et al. Development of echinocandin resistance in Candida tropicalis following short-term exposure to caspofungin
for empiric therapy. Antimicrob Agents Chemother. 2018; 62(4):e01926-17.
42. Gallis HA, Drew RH, Pickard WW. Amphotericin B: 30 years of clinical experience. J Infect Dis. 1990; 12(2):308-29.
43. Vincent BM, Lancaster AK, Scherz-Shouval R, Whitesell L, Lindquist S. Fitness trade-offs restrict the evolution of resistance to amphotericin B. PLoS Biol. 2013; 11(10):e1001692.