Candiduria: Epidemiology, Resistance, Classical and Alternative Antifungal Drugs

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Received: February 22, 2013; Accepted: February 22, 2013; Published: April 25, 2014

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Abstract

Candiduria comprises the isolation of *Candida* species in urine of symptomatic and asymptomatic patients. Yeasts of the *Candida* genus can be found in various ecosystems such as soil, food, water, part of the microbiota of humans and animals. *Candida albicans*, the opportunistic pathogen, is one of the leading causes of fungal infections in humans, especially in immunocompromised patients. It is a major human fungal pathogen and one of the most common causes of nosocomial urinary tract infection. However, we should emphasize the relevance of non-*albicans* species that are considered emergent species. Currently, non-*Candida albicans* species have become a major problem in the hospital environment due to high resistance to antifungal agents available in market. The objective of this review is to demonstrate the prevalence of non-*albicans* species in urine of hospitalized patients and their characteristics regarding their resistance to classical antifungal agents available in market and alternative drugs that are emerging. The classic antifungals used to treat fungal diseases are the azoles, echinocandins, polyenes and fluocytosine, however, due to the presence of resistance mechanisms to each class of antifungal becomes necessary to find alternatives to treat these infections. Natural products may be a traditional medication and now these days in great demand, as they are perceived to have minimal side effect on humans. As example, pure polyphenol curcumin I (CUR-I) in combination with azoles and polyenes represent a novel therapeutic strategy to improve the activity of common antifungals. Additionally, routine use of a mixture of probiotics may be a useful strategy to reduce the prevalence of candidemia and candiduria. Cranberry juice-derived proanthocyanidins (PACs) features has an excellent in vitro activity against *C. albicans* biofilm formation, among others. Furthermore, the outlook of antifungal resistance in the hospital environment is very worrying, and many researchers have tirelessly sought other alternatives for the treatment of fungal infections.

Keywords: Candiduria; Resistance; Species non-*albicans*

Introduction

Candiduria refers to the isolation of *Candida* species in urine of symptomatic and asymptomatic patients [1]. Yeasts of the *Candida* genus can be found in various ecosystems such as soil, food, water, part of the microbiota of humans and animals. The commensal microorganisms that inhabit the gastrointestinal tract primarily take also part of the vaginal microflora, the urethra and lungs [2]. However, if an imbalance in their relationship

with the host occurs, these same yeasts can become pathogenic, thus are considered opportunistic. This imbalance may be due to impairment of host defense mechanisms (extremes of age, underlying disease, immunosuppression) or disruption of anatomical barriers (burns, catheters or invasive surgeries) [3,4] using molecular techniques suggested that the molecular profile of yeasts that colonize the host is similar to that found in cases of infection, which can cause candidemia and consequently candiduria.

The finding of *Candida spp* in urine may mean that the patient has cystitis or pyelonephritis, or more likely, may only reflect colonization of the perineum, indwelling catheters or bladder. Currently, the laboratory diagnostic tests available are neither sensitive nor specific to reliably distinguish infection from colonization [5]. The colonization is usually asymptomatic but adhesion of *Candida spp* on drainage catheter in the urinary tract can result in high concentration of *Candida* colonies in urine cultures. Confirmation of candiduria by a second examination of sterile urine after changing the catheter or suprapubic sample are necessary before further investigation and initiation of treatment [6,7]. Most patients with candiduria are asymptomatic and there are no associated signs or symptoms [7].

Currently, non-*C. albicans* species have become a major problem in the hospital environment due to high resistance to antifungal agents employed during treatment. The objective of this review is to demonstrate the prevalence of non-*albicans* species in urines of hospitalized patients and their characteristics regarding their resistance to classical antifungal agents available in market and alternative drugs that are emerging.

Epidemiology

Candida albicans, the opportunistic pathogen, is one of the leading causes of fungal infections in humans, especially in immunocompromised patients [8]. It is a major human fungal pathogen and one of the most common causes of nosocomial urinary tract infection [9]. However, we should emphasize the relevance of non-*albicans* species that are considered emergent species. Candiduria is very common event among patients exposed to risk factors, and up to 20% of hospitalized patients may have candiduria throughout its hospitalization, particularly

patients from intensive care unit [10,11]. The common risk factors include urinary tract devices, prior surgical procedures, and recent use of antibiotics, advanced age, female sex, *Diabetes mellitus*, immunosuppressive therapy and prolonged hospital stay [12-15]. Further, *C. albicans* has been shown to form biofilms in vivo murine model of catheter associated candiduria [16] and biofilms have been observed on infected urinary catheters in humans [17]. It should be noted, the formation of biofilms is also associated with increased drug resistance. Fungal urinary infections occurred more frequently in patients with urinary catheters than in those without urinary catheters (40% versus 22%) [18].

The general consensus is that candiduria is very common in hospitalized patients [14,18-20]. There is evidence that the incidence is related to the use of antibiotics [21]. The hospital environment also influences the incidence of candiduria, being more common in intensive care units (ICU) and among those in burn units [20,22].

In a study of *Candida* species isolated from urine of patients hospitalized in the United States, Kauffman et al. (2000) [14] found *C. albicans* in the first place (52%) of all yeasts, followed by *C. glabrata* (16%), *C. tropicalis* (8%), *C. parapsilosis* (4%) and *C. krusei* (1%). In Turkey, Ozhak-Baysan et al. (2012) [23] showed that *C. albicans* is more prevalent (44%) of all yeasts, followed by *C. tropicalis* (20%), *C. glabrata* (18%), *C. krusei* (6%), *C. famata* (5%), *C. parapsilosis* (4%), *C. kefir* (2%) and *C. guilliermondii* (1%). In the urine of diabetic patients hospitalized from Ethiopia, the most common species were *C. albicans* (42.0%), *C. glabrata* (34.2%) and *C. tropicalis* (15.8%) [24] (Table 1). It is possible to observe that the most frequent species in the urine of patients is *C. albicans*, followed by *C. tropicalis* or *C. glabrata*.

In Table 2 is observed the distribution of *Candida* species in different years in Brazil, being the three most prevalent species *C. albicans*, *C. tropicalis* and *C. glabrata*. Despite the prevalence of *C. albicans* in infections of the urinary tract, the increased of non-albicans species are presented.

Most microorganisms causing urinary vesical catheter related to infection originates in the gastrointestinal tract of patients. After use of indwelling catheter, over time, there is an increased

Table 1: Etiology of candiduria in some countries.

Etiological Agent	Kauffman et al. [14]	Ozhak-Baysan et al. [23]	Yisman et al. [24]
	U.S.	Turkey	Ethiopia
<i>C. albicans</i>	52%	44%	42%
<i>C. tropicalis</i>	8%	20%	15,8%
<i>C. glabrata</i>	16%	18%	34,2%
<i>C. parapsilosis</i>	4%	4%	-----
<i>C. kefir</i>	-----	2%	-----
<i>C. famata</i>	-----	5%	-----
<i>C. krusei</i>	1%	6%	-----
<i>C. guilliermondii</i>	-----	1%	-----

Table 2: Etiology of candiduria in Brazil.

Agente Etiológico	Kobayashi [12]	Passos [25]	Binelli [26]	Almeida et al. [27]
<i>C. albicans</i>	35,5%	70%	52,2%	36%
<i>C. tropicalis</i>	22,2%	2%	43,5%	42%
<i>C. glabrata</i>	8,8%	7%	-----	20%
<i>C. parapsilosis</i>	11,1%	4,6%	-----	-----
<i>C. kefir</i>	-----	4,6%	-----	-----
<i>C. famata</i>	-----	7%	-----	-----
<i>C. krusei</i>	6,6%	2,2%	-----	2%
<i>C. guilliermondii</i>	4,4%	-----	-----	-----
<i>Candidaspp</i>	11,1%	-----	4,3%	-----

In Table 2 is observed the distribution of *Candida* species in different years in Brazil, being the three most prevalent species *C. albicans*, *C. tropicalis* and *C. glabrata*. Despite the prevalence of *C. albicans* in infections of the urinary tract, the increased of non-albicans species are presented.

colonization of the periurethral region, particularly in catheter interface/mucosa, and microorganisms make use of the catheter surface to ascend into the bladder. In a smaller portion, these microorganisms can be from the hospital environment biotic or abiotic, acquired through the hands of health professionals who improperly handle the system vesical catheter or even through the infusion of contaminated products [28].

The main problem encountered in patients with candiduria by non-albicans species is the resistance of antifungal drugs. Therefore, many of these species are resistant to antifungal agents and there is the requirement to know the mechanisms of resistance involved, as the alternative therapy for these cases.

Treatment and Classical Antifungal Drugs

The classic antifungals used to treat fungal diseases are the azoles, echinocandins, polyenes and flucytosine. The largest family of antifungal drugs is the azole family. Azoles disrupt the cell membrane by inhibiting the activity of the lanosterol 14- β -demethylase [29], enzyme involved in the biosynthesis of ergosterol. The azole family includes imidazoles (econazole, miconazole, clotrimazole, and ketoconazole) and triazoles (fluconazole, itraconazole), and the latest agent voriconazole (second-generation, synthetic triazole derivative of fluconazole) and posaconazole (hydroxylated analogue of itraconazole) [29,30]. Echinocandins (caspofungin, micafungin, and anidulafungin) are lipopeptidic antifungal agents that inhibit the synthesis of fungal cell wall by noncompetitive blockage of the (1,3)- β -Dglucan synthase [31]. Polyenes such as nystatin and amphotericin B (both isolated from *Streptomyces spp.*) bind ergosterol and disrupt the major lipidic component of the fungal cell membrane resulting in the production of aqueous pores [32]. Nucleoside analogues act as inhibitors of DNA/RNA synthesis, flucytosine is a pyrimidine analogue [33].

The presence of yeast in the urine, when viewed under a microscope or culture, should be assessed in the clinical environment, thus is possible to determine their relevance and make the proper decision on the need for antifungal therapy.

Similar to the case of asymptomatic bacteriuria, there has been a debate about whether it is necessary and how to treat patients with candiduria [34]. Finally, asymptomatic candiduria can be resolved either spontaneously or with the removal of the catheter [35]. High-risk patients can be treated with antifungal prophylactic. In neonatal ICU patients, especially those with low birth weight, fluconazole prophylaxis can prevent invasive candidiasis [36,37]. Concern about the treatment is that the excess of prophylaxis and treatment may affect microbiome in general [9].

Three major classes of antifungal drugs are currently available for use against *Candida* infections, but each class has notable limitations, particularly against candiduria [38]. Azole antifungal agents are most commonly used for the treatment of infections with *C.albicans* but are fungistatic and weakly active against biofilms [39,40]. Echinocandins are highly active against *C.albicans*, but generally do not achieve clinically useful concentrations in the urinary tract [41]. Echinocandins have very few side effects, but are poorly excreted renally [42]. Although there are some studies in animals and humans, there are reports of successful treatment of persistent urinary tract infections with resistant *C.glabrata* [43,44]. IDSA guidelines, however, do not currently recommend echinocandins for treatment of non-*C. albicans* candiduria because of very limited clinical data [45]. Amphotericin B has significant toxicities and poor aqueous solubility [46]. Flucytosine, at a dosage of 25 mg/kg (rounded to the nearest 250 mg) give by orally four times daily, can also be used to treat cystitis [47]. Due to its suppressive effects of bone marrow, patients have to be monitored. Interest about the emerging development of resistance to treatment with flucytosine may be less relevant for candiduria because of the high concentrations of the drug in urine and relatively short duration of therapy [9]. Fluconazole and caspofungin is excreted primarily in the urine as active forms, being the agents of choice to treat fungal infections of the urinary tract. Unfortunately, some of these antifungal drugs have been used extensively and has led to greater selective pressure and development of antifungal resistance [48].

In neonates, spite of the need for aggressive treatment, few antifungal drugs are specifically indicated for the treatment of invasive *Candida*. Amphotericin compounds and fluconazole are frequently used, and several newer antifungal agents have become available [45]. All *Candida* species isolated from the

urine of children were susceptible to antifungal agents, except to fluconazole [49]. Almeida et al. [27] demonstrated through antifungal susceptibility testing, that *C.albicans* was susceptible to both antifungal agents, but 31.2% of non-*C. albicans* exhibit eddose-dependent susceptibility to fluconazole, and 3.1% were resistant to amphotericinB. Intertary care center in South India, 39 *Candida* strains were susceptible to amphotericinB, whereas 12 isolates (30.8%) were resistant to fluconazole [50].

It is important to observe the *Candida* species in question, since species differ in resistance standard (Table 3).

Several approaches have been proposed to increase the susceptibility of *C.albicans* to fluconazolin order to deal with failure of treatment, including the combination of different kinds of fluconazol with non-antifungal agents such as antibacterial, calcineurin inhibitors, heat shock protein 90 inhibitors, regulators of calcium homeostasis and traditional Chinese medicine drugs. Many of these combinations showed synergistic effects against *C.albicans*, in particular resistant strains. The main purpose of this synergism is to increase the membrane permeability, efflux reduce antifungal drugs that interfere with intracellular ion homeostasis, inhibiting the activity of proteins and enzymes necessary for the survival of fungi and inhibit the formation of biofilms [51]. Currently, given the observed yeasts resistance profile, the use of two or more drugs together becomes an alternative treatment to avoid resistance.

Mechanisms of resistance to antifungal

Microbiological resistance can be primary (intrinsic) or secondary (acquired). Primary resistance is found naturally among certain fungi without prior exposure to the drug and emphasizes the importance of identification of fungal species from clinical specimens. Examples include resistance of *C.krusei*to fluconazole. Secondary resistance develops among previously susceptible strains after exposure to the antifungal agent and is usually dependent on altered gene expression. The resistance cannot always be predicted, it highlights the importance of individualizing treatment strategies on the basis of the clinical situation [52]. The mechanism of resistance will be different depending on the mode of action of antifungal compounds.

Mechanisms of azole resistance

The resistance to azoles involves a reduced drug intracellular accumulation, decreased target affinity/processivity for the drug,

Table 3: General patterns of susceptibility of *Candida* species (modified from Pappas et al.) [45].

Species	Fluconazole	Itraconazole	Voriconazole	Posaconazole	Flucytosine	Amphotericin B	Candins
<i>C. albicans</i>	S	S	S	S	S	S	S
<i>C. tropicalis</i>	S	S	S	S	S	S	S
<i>C. parapsilosis</i>	S	S	S	S	S	S	S to R ^a
<i>C. glabrata</i>	S-DD to R	S-DD to R	S-DD to R	S-DD to R	S	S to I	S
<i>C. krusei</i>	R	S-DD to R	S	S	I to R	S to I	S
<i>C. lusitaniae</i>	S	S	S	S	S	S to R	S

NOTE: I: intermediately susceptible; R: resistant; S: susceptible; S-DD: susceptible dose-dependent.

^aEchinocandin resistance among *C. parapsilosis* isolates is uncommon.

and counteraction of the drug effect. The development of active efflux pumps results in decreased drug concentrations at the site of action. Efflux pumps are encoded in *Candida* species by 2 gene families of transporters: the *CDR* genes of the ATP binding cassette super family, and the *MDR* genes of the major facilitator's class [53,54]. Whereas *CDR* gene up-regulation confers resistance to almost all azoles, *MDR*-encoded efflux pumps have a narrower spectrum specific for fluconazole. It has been demonstrated that mutations in *ERG11*, the gene encoding for the target enzyme lanosterol C14a-demethylase, prevents binding of azoles to the enzymatic site [55]. Some *Candida* isolates with reduced susceptibility to azoles have higher intracellular concentrations of ERG11p than azole-susceptible strains [56].

Exposure to azole compounds results in depletion of ergosterol from the fungal membrane and accumulation of the toxic product 14a-methyl- 3,6-diol, leading to growth arrest. Mutation of the *ERG3* gene prevents the formation of 14a-methyl-3,6-diol from 14a-methylfecosterol [57]. Replacement of ergosterol with the later product leads to functional membranes and contradicts the action of azoles on the ergosterol biosynthetic pathway.

Real-time reverse transcription-PCR quantification showed that sessile cells over expressed ERG11 (coding for lanosterol 14 alpha-demethylase) and MDR1 (coding for an efflux protein belonging to the major facilitator superfamily), thus these mechanisms may contribute to the fluconazole resistance of the *C. tropicalis* biofilm [58].

Mechanisms of polyene

Resistance breakpoints for polyenes have not been determined. Most clinicians use a MIC of ≥ 1.0 mg/ml to indicate resistance to amphotericin B. Defects in the *ERG3* gene involved in ergosterol biosynthesis lead to accumulation of other sterols in the fungal membrane. Consequently, polyene-resistant *Candida* isolate have relatively low ergosterol content, compared with that of polyene-susceptible isolates [59]. Resistance to amphotericin B may also be mediated by increased catalase activity, with decreasing susceptibility to oxidative damage [60,61].

Amphotericin B, the main polyene antimycotic until now is considered as the golden standard for treatment, has antifungal activity against a wide spectrum of species, such as *Candida*, *Aspergillus* and *Cryptococcus* [62]. Amphotericin B has a broad spectrum and the occurrence of resistance is low, the use of amphotericin B is limited due to dose-dependent toxic side effects, such as nephrotoxicity [63].

Mechanisms of echinocandin

Echinocandins (caspofungin, micafungin, and anidulafungin) are lipopeptidic antifungal agents that inhibit the synthesis of fungal wall by noncompetitive blockage of the (1,3)- β -D-glucan synthase. The fungicidal activity of the three agents (caspofungin, micafungin and anidulafungin) is concentration dependent against most *Candida* species [64,65]. The mechanisms of echinocandin resistance are still being investigated. In *Candida* species, secondary resistance is associated with point mutations in the *Fks1* gene of the β -1,3-D-glucan synthase complex [66]. Within

Fks1 lies a highly conserved region where several mutations have been identified, mostly at the Ser645 position.

Lagrotteria et al. [67] reported that an echinocandin, a micafungin, is safe and effective drug for the treatment of urinary tract infections. Echinocandins, among antifungal agents, were the most effective against strains of *C. glabrata* fluconazole-sensitive and fluconazole-resistant and micafungin showed the lowest minimum inhibitory concentrations [68].

Mechanisms of flucytosine

Flucytosine is a pyrimidine base analogue that inhibits cellular synthesis of DNA and RNA. Flucytosine resistance is linked to a deficiency of enzymes involved in the absorption, transport and processing of flucytosine. Genes that encode enzymes involved in the metabolism of flucytosine also contribute to cross-resistance of *Candida* sp to fluconazole and flucytosine [69]. Some yeast strains are intrinsically resistant to flucytosine impaired because of the absorption cell due to mutations in the gene encoding cytosine permease FCY2 [70,71].

Therapeutic Alternative Antifungals

Natural products as traditional remedies are in great demand, as they are perceived to have minimal side effect on humans [72]. Various alternatives have been studied with the aim of improving the treatment of infections caused by *Candida* species.

Some substances has been reported such as maleic acid [73], aqueous extract of *Arctium minus*, exhibit antifungal effect against *Candida* species isolated from the oral cavity [74]. Sharma et al. [75] showed the first evidence that pure polyphenol curcumin I (CUR-I) in combination with azoles and polyenes represents a novel therapeutic strategy to improve the activity of common antifungals.

Other alternative are probiotics, for patients receiving broad-spectrum antibiotics, routine use of a mixture of probiotics may be a useful strategy to reduce the prevalence of candidemia and candiduria [76].

Kulikov et al. [77] demonstrated that oligo chitosans showed antifungal activity against *Candida* species and clinical isolates of *C. albicans*, which are resistant to a number of classic antifungals. Thus, oligochitosan can be regarded as a possible alternative or addition to the pharmaceutical composition of known antifungal agents.

Cranberry juice-derived proanthocyanidins (PACs) have an excellent in vitro activity against *C. albicans* biofilm formation in artificial urine, preliminary evidence showed that cranberry PAC activity against *C. albicans* biofilm formation is due to anti-adherence properties and/or iron chelation [78].

The fungistatic and growth inhibiting effects of *Brucea javanica* extract have shown that it has potential to be considered as a promising candidate for the development of antifungal agent in oral health products [79]. Nordinet al. [80] showed that the betel leaf extract has strong fungistatic activity against *Candida* species.

Despite the panorama of antifungal resistance in the hospital environment is very worrying, we observed that many researchers have tirelessly sought other alternatives for the treatment of fungal infections.

References

- Colombo A, Guimarães T (2007) Candidúria: uma abordagem clínica e terapêutica. *Rev Soc Bras Med Trop* 40(3): 332-337.
- Alonso-Valle H, Acha O, Garcia-Palomo JD, Fariñas-Alvarez C, Fernández-Mazarrasa C, et al. (2003) Candidemia in tertiary care hospital: epidemiology and factors influencing mortality. *Eur J Clin Microbiol Infect Dis* 22(4): 254-257.
- Dignani MC, Solomkin JS, Anaissie E (2003) *Candida*. In: Anaissie E, McGinnis MR, Pfaller MA (Eds.), *Med mycology*. (1st Ed), Churchill Livingstone Philadelphia, US, pp. 195-239.
- Bonfim-Mendonça Pde S, Fiorini A, Shinobu-Mesquita CS, Baeza LC, Fernandez MA, et al. (2013) Molecular Typing of *Candida albicans* isolates from hospitalized patients. *Rev Inst Med trop S Paulo* 55(6): 385-391.
- Bukhary ZA (2008) Candiduria: A Review of Clinical Significance and Management. *Saudi J Kidney Dis Transpl* 19(3): 350-360.
- Kauffman CA (2005) Candiduria. *Clin Infect Dis* 41(6): 371-376.
- Lundstorm T, Sobel JD (2001) Nosocomial candiduria: A review. *Clin Infect Dis* 32(11): 1602-1607.
- Zhang L, Yan K, Zhang Y, Huang R, Bian J, et al. (2007) High throughput synergy screening identifies microbial metabolites as combination agents for the treatment of fungal infections. *Proc Natl Acad Sci* 104(11): 4606-4611.
- Achkar JM, Fries BC (2010) *Candida* Infections of the Genitourinary Tract. *Clin Microbiol Rev* 23(2): 253-273.
- Jarvis WR (1995) Epidemiology of nosocomial fungal infections with emphasis on *Candida* species. *Clinical Infectious Diseases* 20(6): 1526-1530.
- Nucci M (2000) Candiduria in hospitalized patients: a review. *Braz J Infect Dis* 4(4): 168-172.
- Kobayashi CC, de Fernandes OF, Miranda KC, de Sousa ED, Silva Mdo R (2004) Candiduria in hospital patients: A study prospective. *Mycopathologia* 158(1): 49-52.
- Gubbins PO, McConnell SA, Penzak SR (1999) Current management of funguria. *Am J Health Syst Pharm* 56(19): 1929-1935.
- Kauffman CA, Vazquez JA, Sobel JD, Gallis HA, McKinsey DS, et al. (2000) Prospective multicenter surveillance study of funguria in hospitalized patients. *Clin Infect Dis* 30(1): 14-18.
- Guler S, Ural O, Findik D, Arslan U (2006) Risk factors for nosocomial candiduria. *Saudi Med J* 27(11): 1706-1710.
- Wang X, Fries BC (2011) A murine model for catheter-associated candiduria. *J Med Microbiol* 60(10): 1523-1529.
- Maki DG, Tambyah PA (2001) Engineering out the risk for infection with urinary catheters. *Emerg Infect Dis* 7(2): 342-347.
- Richards MJ, Edwards JR, Culver DH, Gaynes RP (2000) Nosocomial infections in combined medical-surgical intensive care units in the United States. *Infect. Control Hosp Epidemiol* 21(8): 510-515.
- Alvarez-Lerma F, Nolla-Salas J, Leon C, Palomar M, Jorda R, et al. (2003) Candiduria in critically ill patients admitted to intensive care medical units. *Intensive Care Med* 29(7): 1069-1076.
- Schaberg DR, Culver DH, Gaynes RP (1991) Major trends in the microbial etiology of nosocomial infection. *Am J Med* 91(3B): 72S-75S.
- Weinberger M, Sweet S, Leibovici L, Pitlik SD, Samra Z (2003) Correlation between candiduria and departmental antibiotic use. *J Hosp Infect* 53(3): 183-186.
- Bougnoux ME, Kac G, Aegerter P, d'Enfert C, Fagon JY (2008) Candidemia and candiduria in critically ill patients admitted to intensive care units in France: incidence, molecular diversity, management and outcome. *Intensive Care Med* 34(2): 292-299.
- Ozhak-Baysan B, Ogunc D, Colak D, Ongut G, Donmez L, et al. (2012) Distribution and antifungal susceptibility of *Candida* species causing nosocomial candiduria. *Med Mycol* 50(5): 529-532.
- Yismaw G, Asrat D, Woldeamanuel Y, Unakal C (2013) Prevalence of candiduria in diabetic patients attending Gondar University Hospital, Gondar, Ethiopia. *Iran J Kidney Dis* 7(2): 102-107.
- Passos XS, Sales WS, Maciel PJ, Costa CR, Miranda KC, et al. (2005) *Candida* colonization in intensive care unit patients' urine. *Mem Inst Oswaldo Cruz* 100(8): 925-928.
- Binelli CA, Moretti ML, Assis RS, Sauaia N, Menezes PR, et al. (2006) Investigation of the possible association between nosocomial candiduria and candidaemia. *Clinical Microbiology and Infection* 12(6): 538-543.
- Almeida AA, Mesquita CSS, Svidzinski TI, Oliveira KM (2013) Antifungal susceptibility and distribution of *Candida* spp. isolates from the University Hospital in the municipality of Dourados, State of Mato Grosso do Sul, Brazil. *Rev Soc Bras Med Trop* 46(3): 335-339.
- Pfaller MA (1996) Nosocomial candidiasis: emerging species, reservoirs and modes of transmission. *Clinical Infectious Diseases* 22(2): 89-94.
- Hof H (2006) A new, broad-spectrum azole antifungal: posaconazole—mechanisms of action and resistance, spectrum of activity. *Mycoses* 49(1): 2-6.
- Hay R (2003) Antifungal drugs. In: Katsambas A & Lotti T (Eds.), *European Handbook of Dermatological Treatments* (2nd Ed), Springer, Berlin, Germany, pp: 700-710
- Grover N (2010) Echinocandins: a ray of hope in antifungal drug Therapy. *Indian Journal of Pharmacology* 42(1): 9-11.
- Sanglard D, Odds FC (2002) Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. *The Lancet Infectious Diseases* 2(2): 73-85.
- Vermes A, Guchelaar HJ, Dankert J (2000) Flucytosine: a review of its pharmacology, clinical indications, pharmacokinetics, toxicity and drug interactions. *Journal of Antimicrobial Chemotherapy* 46(2): 171-179.
- Nicolle LE (2006) Asymptomatic bacteriuria: review and discussion of the IDSA guidelines. *Int J Antimicrob Agents* 28(1): S42-S48.
- Sobel JD, Kauffman CA, McKinsey DA, Zervos M, Vazquez JA, et al. (2000) Candiduria: a randomized, double-blind study of treatment with fluconazole and placebo. The National Institute of Allergy and Infectious Diseases (NIAID) Mycoses Study Group. *Clin Infect Dis* 30(1): 19-24.
- Healy CM, Baker CJ, Zaccaria E, Campbell JR (2005) Impact of fluconazole prophylaxis on incidence and outcome of invasive candidiasis in a neonatal intensive care unit. *J Pediatrics* 147(2): 166-171.

37. Healy CM, Campbell JR, Zaccaria E, Baker CJ (2008) Fluconazole prophylaxis in extremely low birth weight neonates reduces invasive candidiasis mortality rates without emergence of fluconazole-resistant *Candida* species. *Pediatrics* 121(4): 703–710.
38. Cowen LE, Steinbach WJ (2008) Stress, drugs, and evolution: the role of cellular signaling in fungal drug resistance. *Eukaryotic Cell* 7(5): 747–764.
39. Jain N, Kohli R, Cook E, Gialanella P, Chang T, et al. (2007) Biofilm formation by and antifungal susceptibility of *Candida* isolates from urine. *Appl Environ Microbiol* 73(6): 1697–1703.
40. Douglas LJ (2003) *Candida* biofilms and their role in infection. *Trends Microbiol* 11(1): 30–36.
41. Malani AN, Kauffman CA (2007) *Candida* urinary tract infections: treatment options. *Expert Rev Anti Infect Ther* 5(2): 277–284.
42. Boucher HW, Groll AH, Chiou CC, Walsh TJ (2004) Newer systemic antifungal agents: pharmacokinetics, safety and efficacy. *Drugs* 64(18): 1997–2020.
43. Abruzzo GK, Gill CJ, Flattery AM, Kong AL, Leighton C, et al. (2000) Efficacy of the echinocandin caspofungin against disseminated aspergillosis and candidiasis in cyclophosphamide-induced immunosuppressed mice. *Antimicrob Agents Chemother* 44(9): 2310–2318.
44. Sobel JD, Bradshaw SK, Lipka CJ, Kartsonis NA (2007) Caspofungin in the treatment of symptomatic candiduria. *Clin Infect Dis* 44(5): e46–e49.
45. Pappas PG, Kauffman CA, Andes D, Benjamin Jr DK, Calandra TF, et al. (2009) Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 48(5): 503–535.
46. Klepser M (2011) The value of amphotericin B in the treatment of invasive fungal infections. *J Crit Care* 26(2): 225.e1–10.
47. Tokunaga S, Ohkawa M, Nakashima T, Yamaguchi K, Nishikawa T, et al. (1992) Clinical evaluation of flucytosine in patients with urinary fungal infections. *Jpn J Antibiot* 45(8): 1060–1064.
48. Espinel-Ingroff A (2009) Novel antifungal agents, targets or therapeutic strategies for the treatment of invasive fungal diseases: a review of the literature (2005–2009). *Rev Iberoam Micologia* 26 (1): 15–22.
49. Seifi Z, Azish M, Salehi Z, Zarei Mahmoudabadi A, Shamsizadeh A (2013) Candiduria in children and susceptibility patterns of recovered *Candida* species to antifungal drugs in Ahvaz. *J Nephropathol* 2(2): 122–128.
50. Giri S, Kindo AJ, Kalyani J (2013) Candidemia in intensive care unit patients: a one year study from a tertiary care center in South India. *J Postgrad Med* 59(3): 190–195.
51. Liu S, Hou Y, Chen X, Gao Y, Li H, et al. (2014) Combination of fluconazole with non-antifungal agents: A promising approach to cope with resistant *Candida albicans* infections and insight into new antifungal agent discovery. *Int J Antimicrob Agents* S0924-8579(14)00002-8.
52. Kanafani ZA, Perfect JR (2008) Resistance to Antifungal Agents: Mechanisms and Clinical Impact. *Clin Infect Dis* 46(1): 120–128.
53. Albertson GD, Niimi M, Cannon RD, Jenkinson HF (1996) Multiple efflux mechanisms are involved in *Candida albicans* fluconazole resistance. *Antimicrob Agents Chemother* 40(12): 2835–2841.
54. Sanglard D, Ischer F, Monod M, Bille J (1997) Cloning of *Candida albicans* genes conferring resistance to azole antifungal agents: characterization of CDR2, a new multidrug ABC transporter gene. *Microbiology* 143(2): 405–416.
55. Löffler J, Kelly SL, Hebart H, Schumacher U, Lass-Flörl C, et al. (1997) Molecular analysis of cyp51 from fluconazole-resistant *Candida albicans* strains. *FEMS Microbiol Lett* 151(2): 263–268.
56. Lopez-Ribot JL, McAtee RK, Lee LN, et al. (1998) Distinct patterns of gene expression associated with development of fluconazole resistance in serial *Candida albicans* isolates from human immunodeficiency virus infected patients with oropharyngeal candidiasis. *Anti microb Agents Chemother* 42(11): 2932–2937.
57. Kelly SL, Lamb DC, Kelly DE, et al. (1997) Resistance to fluconazole and cross-resistance to amphotericin B in *Candida albicans* from AIDS patients caused by defective sterol delta5,6-desaturation. *FEBS Lett* 400(1): 80–82.
58. Bizerra FC, Nakamura CV, de Poersch C, Estivalet Svidzinski TI, Borsato Quesada RM, et al. (2008) Characteristics of biofilm formation by *Candida tropicalis* and antifungal resistance. *FEMS Yeast Res* 8(3): 442–450.
59. Dick JD, Merz WG, Saral R (1980) Incidence of polyene-resistant yeasts recovered from clinical specimens. *Antimicrob Agents Chemother* 18(1): 158–163.
60. Sokol-Anderson ML, Brajtburg J, Medoff G (1986) Amphotericin B-induced oxidative damage and killing of *Candida albicans*. *J Infect Dis* 154(1): 76–83.
61. Mesa-Arango AC, Scorzoni L, Zaragoza O (2012) It only takes one to do many jobs: Amphotericin B as antifungal and immunomodulatory drug. *Front Microbiol* 3: 286.
62. Ellis D (2002) Amphotericin B: spectrum and resistance. *J Anti microb Chemother* 49(1): 7–10.
63. Fanos V, Cataldi L (2000) Amphotericin B-induced nephrotoxicity: a review. *J Chemother* 12(6), 463–470.
64. Cappelletty D, Eiselstein-McKittrick K (2007) The echinocandins. *Pharmacotherapy* 27(3): 369–388.
65. Vazquez JA (2005) Anidulafungin: a new echinocandin with a novel Profile. *Clinical Therapeutics* 27(6): 657–673.
66. Balashov SV, Park S, Perlin DS (2006) Assessing resistance to the echinocandin antifungal drug caspofungin in *Candida albicans* by profiling mutations in FKS1. *Antimicrob Agents Chemother* 50(6): 2058–2063.
67. Lagrotteria D, Rotstein C, Lee CH (2007) Treatment of candiduria with micafungin: A case series. *Can J Infect Dis Med Microbiol* 18(2): 149–150.
68. Mario DAN, Denardi LB, Bandeira LA, Antunes MS, Santurio JM, et al. (2012) The activity of echinocandins, amphotericin B and voriconazole against fluconazole-susceptible and fluconazole-resistant Brazilian *Candida glabrata* isolates. *Mem Inst Oswaldo Cruz* 107(3): 433–436.
69. Papon N, Noël T, Florent M, Gibot-Leclerc S, Jean D, Chastin C, et al. (2007) Molecular Mechanism of Flucytosine Resistance in *Candida lusitanae*: Contribution of the FCY2, FCY1, and FUR1 Genes to 5-Fluorouracil and Fluconazole Cross-Resistance. *Antimicrob Agents Chemother* 51(1): 369–371.
70. Peman J, Canton E, Espinel-Ingroff A (2009) Antifungal drug resistance mechanisms. *Expert Review of Anti-Infective Therapy* 7(4): 453–460.

71. Espinel-Ingroff A (2008) Mechanisms of resistance to antifungal agents: yeasts and filamentous fungi. *Rev Iberoam Micol* 25(2): 101–106.
72. Maghrani M, Zeggwagh NA, Haloui Met, et al. (2005) Acute diuretic effect of aqueous extract of *Retamaraetam* in normal rats. *J Ethnopharmacol* 99(1): 31–35.
73. Mota AC, Lima EO, Castro RD, Batista AU (2010) Antifungal Activity of Maleic Acid against *Candida* spp Associated with Oral Infections. *Pesq Bras Odontoped ClinIntegr* 12(3):357-361.
74. Lubian CT, Teixeira JM, Lund RG, Nascente PS, Del Pino FAB, et al. (2010) Atividade antifúngica do extrato aquoso de *Arctiumminus*(Hill) Bernh. (Asteraceae) sobre espécies orais de *Candida*. *Rev Bras Pl Med* 12(2): 157-162.
75. Sharma M, Manoharlal R, Negi AS, Prasad R (2010) Synergistic anticandidal activity of pure polyphenol curcumin I in combination with azoles and polyenes generates reactive oxygen species leading to apoptosis. *FEMS Yeast Research* 10(5): 570–578.
76. Kumar S, Singhi S, Chakrabarti A, Bansal A, Jayashree M (2013) Probiotic use and prevalence of candidemia and candiduria in a PICU. *Pediatr Crit Care Med* 14(9): e409-415.
77. Kulikov SN, Lisovskaya SA, Zelenikhin PV, Bezrodnykh EA, Shakirova DR, et al. (2014) Antifungal activity of oligochitosans (short chain chitosans) against some *Candida* species and clinical isolates of *Candida albicans*: Molecular weight-activity relationship. *Eur J Med Chem* 74: 169-178.
78. Rane HS, Bernardo SM, Howell AB, Lee SA (2014) Cranberry-derived proanthocyanidins prevent formation of *Candida albicans* biofilms in artificial urine through biofilm- and adherence-specific mechanisms. *J Antimicrob Chemother* 69(2): 428-436.
79. Nordin MA, Wan Harun WH, Abdul Razak F (2013) Antifungal susceptibility and growth inhibitory response of oral *Candida* species to *Bruceajavanica* Linn. Extract. *BMC Complement Altern Med* 13: 342.
80. Nordin MA, Wan Harun WH, Abdul Razak F, Musa MY (2014) Growth inhibitory response and ultrastructural modification of oral-associated candidal reference strains (ATCC) by Piper betle L. extract. *Int J Oral Sci* 6(1): 15-21.