Candiduria: Epidemiology, Resistance, Classical and Alternative Antifungal Drugs

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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 emerging 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 an 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, Ozhan-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. kefyr (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 colonization of the periurethral region, particularly in catheter interface/mucosa, and microorganism makes 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 fluocytosine. 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 (hydroxy analogue of itraconazole) [29,30]. Echinocandins (caspofungin, micafungin, and anidulafungin) are lipopeptidic antifungal agents that inhibit the synthesis of fungal cell wall by noncompetitive blockade of the (1,3)-β-D-glucan 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, fluocytosine 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.

### Table 1: Etiology of candiduria in some countries.

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td>U.S.</td>
<td>Turkey</td>
<td>Ethiopia</td>
</tr>
<tr>
<td>C. albicans</td>
<td>52%</td>
<td>44%</td>
<td>42%</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>8%</td>
<td>20%</td>
<td>15,8%</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>16%</td>
<td>18%</td>
<td>34,2%</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>4%</td>
<td>4%</td>
<td>---</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>---</td>
<td>2%</td>
<td>---</td>
</tr>
<tr>
<td>C. famata</td>
<td>---</td>
<td>5%</td>
<td>---</td>
</tr>
<tr>
<td>C. krusei</td>
<td>1%</td>
<td>6%</td>
<td>---</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>---</td>
<td>1%</td>
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</tr>
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</table>

### Table 2: Etiology of candidurain Brazil.

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>35,5%</td>
<td>70%</td>
<td>52,2%</td>
<td>36%</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>22,2%</td>
<td>2%</td>
<td>43,5%</td>
<td>42%</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>8,8%</td>
<td>7%</td>
<td>---</td>
<td>20%</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>11,1%</td>
<td>4,6%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>---</td>
<td>4,6%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>C. famata</td>
<td>---</td>
<td>7%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>C. krusei</td>
<td>6,6%</td>
<td>2,2%</td>
<td>---</td>
<td>2%</td>
</tr>
<tr>
<td>C. guilliermondii</td>
<td>4,4%</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>11,1%</td>
<td>---</td>
<td>4,3%</td>
<td>---</td>
</tr>
</tbody>
</table>

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.
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 microbiotome 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 renal [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 for 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 ed dose-dependent susceptibility to fluconazole, and 3.1% were resistant to amphotericin B. Intercarey center in South India, 39 _Candida_ strains were susceptible to amphotericin B, 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, in deducing 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,

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Fluconazole</th>
<th>Itraconazole</th>
<th>Voriconazole</th>
<th>Posaconazole</th>
<th>Flucytosine</th>
<th>Amphotericin B</th>
<th>Candins</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>S-DD to R</td>
<td>S-DD to R</td>
<td>S-DD to R</td>
<td>S-DD to R</td>
<td>S</td>
<td>S</td>
<td>S to R*</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>R</td>
<td>S-DD to R</td>
<td>S</td>
<td>I to R</td>
<td>S</td>
<td>I to R</td>
<td>S</td>
</tr>
<tr>
<td><em>C. lusitaniae</em></td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S to R</td>
<td>S</td>
</tr>
</tbody>
</table>

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

*Candida* species (modified from Pappas et al.) [45].

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-methylfocolesterol [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 ERG1I (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 antmycotic 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 blockade of the (1,3)-beta-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 beta-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 fluconosine

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

Terapeutic 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 oligochitosan showed antifungal activity against Candida species and clinical isolates of Calbicans, which are resistant to a number of classic antifungals. Thus, oligochitosan can be regarded as a possible alternative or adition 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 in hibiting effects of Bruceajavanica 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


