Antifungal agents commonly used in the superficial and mucosal candidiasis treatment: mode of action and resistance development

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Abstract
Recent progress in medical sciences and therapy resulted in an increased number of immunocompromised individuals. Candida albicans is the leading opportunistic fungal pathogen causing infections in humans, ranging from superficial mucosal lesions to disseminated or bloodstream candidiasis. Superficial candidiasis not always presents a risk to the life of the infected host, however it significantly lowers the quality of life. Superficial Candida infections are difficult to treat and their frequency of occurrence is currently rising. To implement successful treatment doctors should be up to date with better understanding of C. albicans resistance mechanisms. Despite high frequency of Candida infections there is a limited number of antimycotics available for therapy. This review focuses on current understanding of the mode of action and resistance mechanisms to conventional and emerging antifungal agents for treatment of superficial and mucosal candidiasis.

Key words: antifungal agents, Candida albicans, mode of action, resistance mechanisms.

Introduction
Candida albicans infections are a problem of growing clinical importance worldwide. Literature data point out that this opportunistic pathogen is the leading cause of superficial and disseminated fungal infections in humans. Moreover, about 96% of all opportunistic mycoses are caused by Candida sp. [1–3]. In healthy individuals Candida colonizes mainly mucosal surfaces of the oral cavity, gastrointestinal and urogenital tracts without disease symptoms, where most frequently identified species are C. albicans (70%) and C. glabrata (7%) [3–7]. Moreover, an association between fungal colonization and candidiasis has been previously described. According to Nguyen et al. [8], colonization of the epithelial surfaces with pathogenic Candida strains is required for pathogenesis development. In immunocompromised humans, Candida frequently causes infections ranging from superficial mucosal lesions to disseminated or bloodstream infections [6]. More than 100 species of Candida have been identified, however only a few have been isolated from humans. Although C. albicans remains the most common cause of fungemia and hematogenously disseminated candidiasis, there has been an increase in infections caused by C. glabrata, C. tropicalis, C. parapsilosis, C. krusei, and C. lusitaniae [9]. Infections with these different species may require different therapeutic considerations [9]. Most human superficial and mucosal infections are caused by C. albicans, although other species such as C. glabrata, C. tropicalis, C. parapsilosis, C. kefyr, C. krusei, and C. guilliermondii may also be implicated in superficial as well as mucosal diseases. Although C. albicans can be cultured from the mouths of non-infected normal individuals, it does not cause oropharyngeal candidiasis unless predisposing factors exist to allow the infection to become established (Table 1) [3, 10, 11]. Candida albicans superficial infections include oral and vaginal thrush as well as chronic mucocutaneous candidiasis [12]. Being an important cause of morbidity and difficult to treat, superficial candidiasis of the mucosa, skin and nails have become a significant problem worldwide [11]. Although superficial candidiasis rarely presents a risk to the life of patients, it significantly lowers the quality of life [11, 13].

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The high frequency of occurrence of candidiasis combined with difficult treatment cause a tendency toward better understanding of C. albicans resistance mechanisms. Therefore, this review focuses on characterization of antifungal agents commonly used in treatment and fungal resistance mechanisms. We discuss here several topical and systemic options for the treatment of candidiasis.

### Antifungal agents

For the last three decades, fungal infections have become a major problem worldwide, especially among the immunocompromised individuals [3]. Despite that Candida is the leading cause of the opportunistic fungal infections, there is a limited number of antimycotics available for therapy [2, 3, 14]. Perea et al. [15] divided antifungal agents commonly used for candidiasis treatment in five major groups basing on their mode of action; group I: inhibition of RNA and/or DNA synthesis (fluorinated pyrimidine analogs 5-FC); group II: alteration of the membrane function (polycenes: nystatin, natamycin, amphotericin B AMB); group III: alteration of cell wall biosynthesis by inhibition of β(1,3)-glycan synthase (echinocandins: caspofungin, micafungin, anidulafungin); group IV: inhibition of ergosterol biosynthesis by inhibition of squalene epoxidase and/or accumulation of toxic sterol intermediates (allylamines: terbinafine, naftifine); and group V: inhibition of lanosterol demethylase in ergosterol biosynthesis (azoles) [14, 15].

#### Fluorinated pyrimidine analog 5-FC

Flucytosine (5-fluorocitosine, 5-FC) is a fluorinated derivative of the pyrimidine cytosine typically used in candidiasis treatment [16, 17]. The antifungal activity of 5-fluorocitosine results from its rapid conversion to 5-fluorouracil [17]. Flucytosine is metabolized via the pyrimidine salvage pathway [18]. The 5-FC is taken up by cytosine permease (encoded by FCY2) and transported into fungal cells [17, 19]. Next, this compound is enzymatically transferred into 5-fluorouracil (5-FU) by cytosine deaminase (encoded by FCY1) [19, 20]. Moreover, the uracil phosphoribosyltransferase (encoded by FUR1) transforms 5-FU into 5-fluorouridine monophosphate (5-fluoro-UMP) [19]. The 5-fluoro-UMP inhibits DNA synthesis by targeting thymidylate synthetase. Furthermore, after transformation to 5-fluorouridine triphosphate (5-fluoro-UTP), this antifungal agent disturbs protein synthesis by incorporating into RNA, which results in the cell death [16, 20]. Mammalian cells lack the cytosine deaminase enzyme, therefore they are not directly subject to the toxic effects of 5-FC [18]. By far, resistance to 5-FC is observed in vitro in 3–10% of C. albicans isolates. Moreover, 30% of isolates develop resistance during treatment with 5-FC [18, 19, 21]. According to literature data [17, 19], resistance to flucytosine is linked to the deficiency in enzymes involved in uptake, transport and transformations of 5-FC. Genes coding enzymes involved in 5-FC metabolism also contribute to cross resistance to flucytosine and fluconazole in Candida spp. [22]. Papon et al. [22] demonstrated that inactivation of FCY1, FCY2, FUR1 in C. lusitaniae produced two patterns of resistance, where mutant fur1 was resistant to 5-fluorouracil, while mutants fcy1 and fcy2 were resistant to fluconazole. Moreover, fungal resistance might also result from an increased synthesis of pyrimidines that compete with fluorinated antimetabolites of 5-FC and therefore decrease its antifungal activity [17, 23]. As mentioned above, rapid development of resistance in Candida spp. during treatment with 5-FC was observed.

Therefore, the 5-FC monotherapy with few exceptions is not recommended. Moreover, toxic effects of 5-FC such as skin rash, nausea, bone marrow suppression, liver dysfunction, vomiting and diarrhea have been confirmed [24]. Despite that, flucytosine remains useful in multi-drug therapy in hematogenous candidiasis treatment as an adjunct to amphotericin B or azoles [21]. Furthermore, this antifungal agent is currently used in treatment of life-threatening Candida infections such as endocarditis, meningitis and hepatosplenic disease [18, 24].

#### Polenes

Polyene antifungal agents are natural compounds derived from fermentation by Streptomyces [25]. Nystatin, natamycin and amphotericin B are three main polyene drugs used in mycoses treatment [26]. The parenteral administration of nystatin is followed by severe side effects [25]. Contrariwise, as this antifungal agent is not absorbed

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**Table 1. Risk factors for the development of oropharyngeal candidiasis**

| Category                              |
|---------------------------------------|
| Immunosuppression                     |
| HIV infection                         |
| Chronic mucocutaneous candidiasis     |
| Neutropenia                           |
| Drugs                                 |
| Cytotoxic chemotherapy                |
| Corticosteroids                       |
| Broad-spectrum antimicrobial agents   |
| Anticholinergics                      |
| Diabetes mellitus                     |
| Nutritional deficiencies              |
| Iron deficiency                       |
| Malnutrition                          |
| Prior or current local pathology      |
| Dentures                              |
| Xerostomia                            |
| Infancy                               |
either from the gastrointestinal tract or via skin, both oral and topical administration of this antifungal agent are not related to significant toxic side effects [25]. Thus, nystatin is currently used in superficial candidiasis treatment, such as oral and vaginal infections with topical or oral administration only [25, 27]. Superficial Candida infections of the skin can be treated topically with amphotericin B or nystatin. Congenital cutaneous candidiasis may resolve with topical and oral nystatin treatment. Nystatin creams used for treatment of an acute Candida vaginitis achieved mycological cure rates of approximately 75% to 80%. On the other hand, polyenes (nystatin) achieved slightly lower clinical and mycological cure rates than azole agents [28]. Natamycin is considered as a drug of choice in filamentous fungi topical treatment [29]. Moreover, this antifungal agent is currently used in fungal keratitis [26, 29].

Contrariwise to previous members of the polyene group, fungicidal in its nature amphotericin B (AMB) was long considered as the gold standard in antifungal therapy [30, 31]. Currently, this antifungal agent is used in treatment of infections caused by such pathogens as Candida spp., Aspergillus spp., Fusarium spp., Cryptococcus spp., Mucor spp., Rhizopus spp., Trichosporon spp., Scedosporium spp. and Malassezia spp. [26, 30]. Despite the broad spectrum of antifungal activity, AMB is also characterized by widespread tissue distribution, long elimination half-life and a significant toxicity profile [32]. The toxicity profile of all polyenes result from their affinity for cholesterol which is a human counterpart for ergosterol [26]. The toxic side effects of AMB include infusion-related events, such as chills, fever, headache, nausea and vomiting, and dose-limiting nephrotoxicity [24].

The primary target of polyenes is ergosterol [14]. The AMB binds ergosterol localized in the fungal cell membrane leading to the pore formation, increased permeability of the membrane, oxidative damage and in the end leaking of cellular contents [14, 16]. Although resistance to amphotericin B is uncommon in C. albicans, other Candida species are thought to be intrinsically resistant to AMB [16]. It has been estimated that decreased ergosterol content in the fungal cell membrane is associated with resistance to amphotericin B as AMB-resistant Candida strains have relatively low ergosterol content in comparison to susceptible isolates [14, 33]. Lowering ergosterol content is associated with defects in the ERG3 gene, which leads to the accumulation of other sterols instead of ergosterol [19, 33–35].

Management of Candida superficial infections including treatments with amphotericin B. Low-dose amphotericin B 0.4 mg/kg/day is effective in the treatment of oropharyngeal candidiasis and esophageal candidiasis (if endoscopy confirms the persistence). Lipid formulation is available if the patient is unable to tolerate conventional amphotericin B. Duration of therapy is at least 10 to 14 days. The management of ocular infections consists of high-dose amphotericin B (0.7 to 1 mg/kg/day) preferably in conjunction with fluconosine, because of the poor intraocular penetration achieved by amphotericin B. Therapy should be continued for at least 10 to 14 days after resolution of all signs and symptoms of infection. So far, three lipid products of amphotericin B have been marketed: amphotericin B colloidal dispersion (ABCD, Amphocil), amphotericin B lipid complex (ABLC, Abelcet), and liposomal amphotericin B (L-AMB, Ambisome). Large prospective randomized trials showed that ABLC at a dose of 5 mg/kg/day was as effective as and less nephrotoxic than conventional amphotericin B at a dose of 0.7 to 1 mg/kg/day in hematogenous candidiasis. Overall, published data suggest that the three lipid formulations of amphotericin B are associated with less renal toxicity than conventional amphotericin B. Among the three lipid formulations of amphotericin B, L-AMB (Ambisome) is the least nephrotoxic and appears to result in significantly fewer infusion-related infections [36]. There is no consensus about appropriate dosing of the lipid formulations of amphotericin B. However, doses of L-AMB as low as 1 to 3 mg/kg/day and doses of ABLC and ABCD of 5 mg/kg/day seem to be adequate for treatment of Candida infections. Duration of therapy depends on the extent and seriousness of the infection. In view of the recent European study [37], most Candida strains were sensitive to amphotericin B. This is also good news for treatment of oral candidiasis as amphotericin B can be used topically in the form of lozenges.

Echinocandins

The echinocandins are a class of semisynthetic lipopeptide antifungal compounds synthetically derived from natural lipopeptides produced by Aspergillus niger, Z. bailii, and S. cerevisiae. While being fungicidal against C. albicans and fungistatic against Aspergillus spp. and despite high activity against Pneumocystis carinii, they have no effect on such pathogens as Cryptococcus, Trichosporon, Scedosporium, and Fusarium species [33, 39, 40]. Despite the narrow spectrum of activity, this group of antifungals is broadly effective against azole-resistant Candida strains [41]. Currently three members of this class are licensed for mycoses treatment: caspofungin (CAS), micafungin (MFG) and anidulafungin (ANI) [16, 42]. Adverse events and toxic effects of echinocandins include headache, rash, fever, liver toxic effects, phlebitis, histamine release and hemolysis. However, their occurrence is rare [39]. The cell wall represents a perfect potential target for antifungal agents, as this structure is absent in mammalian cells [39]. Echinocandins disturb cell wall biosynthesis by inhibition of 1,3-β-glucan synthase [14]. This enzyme is responsible for synthesis of 1,3-β-glucan, which is a crucial component that strengthens the cell wall of C. albicans and S. cerevisiae [16]. Lack of the glucan component in the cell wall results in osmotic instability and ultimately in cell lysis [40]. Resistance to echinocandins is linked to a mutation in 1,3-β-glucan synthase complex [14]. The 1,3-β-glucan synthase consists of two units Rholp and Fksp,
where Fksp is the active site of this enzyme and Rho1 is the regulator [39, 42]. Mutation in the FKS gene results in resistance to echinocandins [14, 42]. It was previously described [43, 44] that mutations within the FKS gene of C. glabrata clinical isolate resulted in reduced susceptibility or resistance to echinocandins. Furthermore, fungal resistance might also result from lack of 1,3-{$\beta$}-glucan in the fungal cell wall. It was described that the cell wall of C. neoformans consists mainly of $\alpha$-(1,3)- or $\alpha$-(1,6)-glucan and therefore, this species is resistant to the echinocandins [45]. By far, no cross-resistance with azoles or polyenes has been described, however mechanisms of resistance to echinocandins are still being investigated [33–35, 38, 39]. Caspofungin is as effective as amphotericin B for treatment of oropharyngeal candidiasis in doses of 50 to 70 mg/day [36].

**Allylamines**

Terbinafine and naftifine are two main allylamines used in mycoses treatment. This class of antifungal compounds possess fungicidal activity against dermatophytes and fungistatic activity against C. albicans [14, 46]. Terbinafine can be applied both systematically and topically and is widely used in treatment of fungal infections of skin, nails and hair [47, 48]. The side effects of allylamines treatment are rare (2–3% of patients) and include itching, burning and redness at the application site [46]. The case study of Ghanoun and Elewski [48] demonstrated that terbinafine used together with fluconazole provided successful treatment of oropharyngeal candidiasis which was not responding to fluconazole. Moreover, terbinafine demonstrated high fungicidal activity on itraconazole resistant fungi during in vitro study [49]. Allylamines inhibit ergosterol biosynthesis independent of cytochrome P-450 enzymes, by binding to squalene epoxidase (Erg1p) resulting in accumulation of squalene in high amounts inside the cell [46, 47, 50]. This leads to increased membrane permeability, disturbance of cell organization and ultimately to cell death [46, 50]. According to Osborne et al. [51] and Cannon et al. [14], fungal resistance to terbinafine is due to a single amino acid substitution in Erg1p. In C. albicans, resistance to terbinafine might be also related to genes encoding membrane transport proteins CDR1, AFG2 and H0L3. Up-regulation of these genes serve to extrude the antifungal agent accumulated inside the cell out [52]. Moreover, according to Odds [53], resistance to terbinafine is linked to MAT locus homozygosity. The mentioned study [53] suggests that allylamines resistance is mediated by the same mechanism as efflux pump-mediated azole resistance. Other allylamines resistance mechanisms include induction of detoxification and stress tolerance [14]. Terbinafine seems to be more active against infections caused by C. parapsilosis and C. albicans. Topical antifungal agents may have a role in preventing relapse of the infections after successful oral therapy [36]. According to the latter authors [36], oral treatment options include terbinafine 250 mg/week $\times$ 9 to 18 months. Moreover, topical treatment onychomycosis due to Candida spp. are usually of little value.

**Azole antifungal agents**

Azole antifungal agents possess fungicidal activity against Aspergillus spp. and fungistatic activity against Candida spp. [54]. Several agents of this class including fluconazole (FLC), itraconazole (ITR), voriconazole (VRC) and posaconazole (POS) have been widely used in mycoses treatment [55]. Azoles target a lanosterol demethylase (Erg11p), a cytochrome P-450 enzyme mediating rate-limiting step in ergosterol biosynthesis [14, 19]. By binding the heme moiety in Erg11p, azoles inhibit activity of this enzyme and therefore disturb ergosterol biosynthesis [16]. Due to accumulated intermediates of ergosterol biosynthesis, a subsequent mechanism of sterol metabolism mediated by C5,6 desaturase enzyme (encoded by ERG3) is activated [34, 56]. The Erg3p mediates conversion of non-toxic 14{$\alpha$}-methylfecoesterol to 3,6-diol (14{$\alpha$}methylgergosta-8,24(28) -3{$\beta$},6{$\alpha$}-diol) [34, 56]. Conversion of Erg11p substrate into toxic methylated sterols leads to growth inhibition [14, 19]. Azole antifungal agents are mostly the first choice for antifungal therapy [57]. Yet, their fungistatic nature against Candida spp. caused a strong directional selection towards azole-resistant strains [54]. In a recent study, Ramesh et al. [58] tested the antifungal pattern of the C. albicans isolates from the oral cavity of HIV-infected patients. Results of the mentioned study [58] demonstrated growing resistance of C. albicans to tested azoles that varied from 11.9% to 41.1% depending on azole used. This causes a need for better understanding of fungal resistance mechanisms. The main azole resistance mechanisms in Candida and other pathogenic fungi include:

1) Mutations or overexpression of ERG11

Non-synonymous mutations in ERG11 cause amino acid substitutions resulting in alteration of lanosterol demethylase and decreased azole affinity to this enzyme [55]. Moreover, each copy of ERG11 contributes to azole resistance [59]. Overexpression of ERG11 also compels the need for a higher azole concentration in order to bind all of Erg11p molecules present in the fungal cell [60].

2) Reduced accumulation of the azole inside fungal cell

a) Reduced uptake of azole. The study of Mansfield et al. [57] had proven that in C. albicans and other fungal pathogens, azoles are up-taken via energy-independent facilitated diffusion. Decreased azole uptake might be linked to changes in composition of fungal cell membrane [50]. Moreover, azole import levels vary among resistant C. albicans clinical isolates suggesting a role of import in resistance to azoles [57]. The data acquired by Mansfield et al. [57] suggest that all azoles use the same import mechanism mediated by a transporter, therefore a mutation in putative transporter would result in azole cross-resistance.
b) Efflux via ABC transporters. Lowering intracellular accumulation of azoles is energy dependent [57]. Two types of azole transporters in *C. albicans* have been described: the major superfamily transporter encoded by *MDR1* and the ATP-binding cassette (ABC) transporters encoded by *CDR1* and *CDR2* [61]. The ABC transporters are ATP-dependent whereas Mdr1p depends on utilization of the proton motive force at the cellular membrane in order to transport azoles out of the cell [57]. Overexpression of these drug efflux pumps transport azoles out of the cell, therefore reducing intracellular drug accumulation [50, 62]. Upregulation of *MDR1* results in fluconazole resistance, contrariwise upregulation of ABC transporters leads to multi azole resistance [63]. The expression of drug efflux pumps is regulated by *TAC1* and *MRR1* [55]. According to Coste et al. [63], *Candida* strains that are homozygous at the mating-type locus and have hyperactive *TAC1* possess an increased resistance to fluconazole. Hyperactive *TAC1* causes a constitutive high expression of *CDR1* and *CDR2* [63]. Yet, the loss of heterozygosity (LOH) also fulfills an important role in the azole resistance mechanism as TAC hyperactive phenotype occurs only when it is homozygous [59, 63]. Moreover, according to Hoot et al. [64], in *C. albicans* LOH occurs through a recombination-mediated event via homologous recombination pathways. However, defects in homologous recombination might result in altered LOH events and therefore sensitivity to azoles [64].

3) Tolerance to methylated sterols via mutation in *ERG3* A defective Erg3p function resulting in changed membrane sterol content has been linked to azole resistance [34, 62]. It was documented [35] that azole-resistant *C. albicans* isolates exhibit defective sterol Δ5,6-desaturation. Mutation in sterol Δ5,6-desaturase results in production of 14α-methylfecosterol which is capable of cell growth [35]. Cells with defective sterol Δ5,6-desaturation lack ergosterol (as Erg1p is still inhibited by azole drug) and therefore are cross-resistant to polycenes such as amphotericin B [34, 35]. It was previously suggested [65] that mutations in filamentation regulator *EFG1* increase susceptibility to azoles as this gene participates in regulating the expression of *ERG3*.

4) Biofilm formation *Candida* biofilms are composed of cells embedded in an extracellular matrix [66]. Those structures are highly resistant to antifungal treatment, especially to azoles and AMB [54, 66]. Resistance of biofilms toazole drugs results from conjunction of several mechanisms acting in a time-dependent manner, including such phenomena as phenotype changes due to decreased growth or/nutrient limitation; surface-induced expression of the resistance genes; disabled drug penetration through biofilm matrix; high cell density; decreased ergosterol levels in mature biofilms; upregulation of genes coding drug efflux pumps during biofilm formation; presence of highly azole-resistant persister cells in the biofilm structure [66–68]. Glucan present in extracellular matrix sequesters azoles and prevents them from reaching the target enzyme [54]. Therefore, *Candida* biofilms remain sensitive to the newly introduced echinocandins that target cell wall β-glucan biosynthesis [66]. In *C. albicans* matrix, glucan levels are regulated by the molecular chaperone Hsp90 – the heat shock protein 90. By compromising its function, biofilm dispersal is blocked and its ability to serve as potential source of infection is reduced [54].

5) Import of host cholesterol

In the absence of ergosterol, some fungal pathogens such as *Candida glabrata* and *Aspergillus fumigatus* are able to import and utilize host cholesterol [69–71]. This phenomenon was also described in *Saccharomyces cerevisiae* [72, 73]. Fungi incorporate exogenous sterols from serum under aerobic (C. *glabrata* and A. *fumigatus*) or anaerobic (S. *cerevisiae*) conditions [70, 71, 73]. According to Xiong et al. [71], accelerated growth along with the extensive cholesterol import occurs in the presence of serum. Moreover, it was demonstrated [71] that import cholesterol uptake significantly increases in the presence of azoles and it appears to attenuate effects of these antifungal agents. The results of previous studies [70, 71, 73] suggested that incorporation of host cholesterol by fungi complements defects in ergosterol biosynthesis, therefore suppressing growth defects and azole toxicity.

Treatments for superficial candidiasis are fluconazole, itraconazole, and ketoconazole [74]. These are generally used for severe or chronic oral candidiasis and chronic mucocutaneous candidiasis. The daily doses used are ketoconazole 200 mg (400 mg in AIDS patients), itraconazole, and ketoconazole. According to Horgan and Powderly [10], treatment of oropharyngeal candidiasis is relatively simple, with most types responding well to therapy. Generally, the latter authors [10] discussed that clotrimazole, ketoconazole, fluconazole, and itraconazole are probably equivalent in the acute treatment of most cases of oropharyngeal candidiasis. The duration of therapy is also variable and in uncomplicated infections there has been tendency to shorten the course of therapy. As reported Horgan and Powderly [10], patients should receive itraconazole and fluconazole for at least 7 days. It was described that daily fluconazole was more effective than clotrimazole in preventing mucosal candidiasis. Ketoconazole and itraconazole are probably also useful but have not been extensively evaluated in controlled trials. Moreover, fluconazole at dosages 50 mg/day to 400 mg/day has been effective in preventing oropharyngeal candidiasis and decreasing colonization with *Candida*. On the other hand, because fluconazole is less active against *C. glabrata* and *C. krusei*, increased colonization and, at some centers, increased infections with these species has been reported when fluconazole was used routinely for prophylaxis. In patients with infection caused by organisms
with intermediate susceptibility, higher doses of fluconazole (up to 800 mg) may be tried. Moreover, other azoles may be efficacious because some fluconazole-resistant isolates retain sensitivity to itraconazole and ketoconazole. Resistance to fluconazole or ketoconazole may develop if the drug is used continuously in the face of clinically unresponsive infection. Vaginal infections respond to intensive topical therapy given for 3 to 5 days with either cream or vaginal tablets. In these cases, nystatin or an imidazole such as miconazole is used. Chronic or persistent infection is a clinical problem that does not have a reproducible solution. However, it is important in all such cases to ensure by way of cultures that symptoms are caused by Candida and not other infectious agents. Regimens that have been attempted include continuous fluconazole or itraconazole for 1 to 2 months followed by intermittent mid-cycle therapy with either itraconazole 400 mg daily for 2 to 3 days or fluconazole 200 mg daily for a similar period. Despite these measures, relapse is common. For infections of the skin surface, azole creams or ointments are usually successful [10]. In the case of patients with acute vaginal Candida infections, azole agents achieved clinical and mycological cure rates of approximately 85% to 90%. Contrariwise, there is little evidence that any azole agent is superior to others. Oral antimycotic agents i.e., ketoconazole (400 mg daily for 5 days), itraconazole (200 mg daily for 3 days or 400 mg for 1 day), and fluconazole (150 mg in a single daily dose) were all shown to be highly effective in achieving clinical mycological cure in acute Candida vaginitis. Management of Candida vulvovaginitis during pregnancy is more difficult, because clinical response tends to be slower and recurrences are more frequent. Sobel [28] reported that most topical antifungal agents are effective, especially when prescribed for longer periods of 1 to 2 weeks, however, single-dose therapy with clotrimazole was shown to be effective during pregnancy. The management of women with recurrent vulvovaginal candidiasis RVVC (defined as more than 4 epis-

**Table 2. Therapy for vaginal candidiasis**

| Drug      | Formulation | Dosage regimen |
|-----------|-------------|----------------|
| **Topical agents** | | |
| Butoconazole | 2% cream | 5 g for 3 days |
| Clotrimazole | 1% cream | 5 g for 7–14 days |
| | 100 mg vaginal tablets | 1 tablet for 7 days |
| | 100 mg vaginal tablets | 2 tablets for 3 days |
| | 500 mg vaginal tablets | 1 tablet – single dose |
| Miconazole | 2% cream | 5 g for 7 days |
| | 100 mg vaginal suppository | 1 suppository for 7 days |
| | 200 mg vaginal suppository | 1 suppository for 3 days |
| | 1200 mg vaginal suppository | 1 suppository – single dose |
| Econazole | 150 mg vaginal tablets | 1 tablet for 3 days |
| Fenticonazole | 2% cream | 5 g for 7 days |
| Tioconazole | 2% cream | 5 g for 3 days |
| | 6.5% cream | 5 g – single dose |
| Terconazole | 0.4% cream | 5 g for 7 days |
| | 0.8% cream | 5 g for 7 days |
| | 80 mg vaginal suppository | 80 mg for 3 days |
| Nystatin | 100 000 U vaginal tablets | 1 tablet for 14 days |
| **Oral agents** | | |
| Ketoconazole | 400 mg bid | for 5 days |
| Itraconazole | 200 mg bid | for 1 day |
| | 200 mg | for 3 days |
| Fluconazole | 150 mg | Single dose |

*bid (Latin: bis in die) – two times a day*
sodes per year) requires systemic azoles fluconazole or itraconazole for 14 days, followed by a maintenance regimen (fluconazole 150 or 100 mg weekly for 6 months or daily low-dose ketoconazole – 100 mg daily for 6 months is well tolerated and efficacious) [36, 75, 76]. As an alternative to daily ketoconazole, weekly therapy with oral fluconazole (100 mg) or topical clotrimazole (500 mg) can be used. Treating RVVC remains challenging; long-term prophylaxis with 150 mg fluconazole once weekly for 6 months resulted in 91% of relapse-free patients at the end of treatment, but symptomatic relapse occurred in 57% of patients within 6 months after the cessation of treatment [77]. As described by Sobel [28], in patients with frequent recurrence of C. albicans after an initial response to the aforementioned agents, a long-term regimen of topical nystatin in combination with ketoconazole or itraconazole can be prescribed after in vitro susceptibility tests indicate azole susceptibility. Reversely to acute Candida vaginitis, antifungotics available for local use as creams, lotions, aerosol sprays, vaginal tablets, suppositories, and coated tampons are presented in Table 2.

Conclusions

Rapidly growing resistance of fungal pathogens to commonly used antifungal agents remains a concern for modern medicine. Most of Candida resistance mechanisms result from point mutations of target enzymes or regulatory genes. Moreover, a broad use of antifungotics caused a directional selection among targeted pathogenic populations towards those with effective resistance mechanisms. Emergence of resistant strains resulted in an increased mortality rate and therefore compelled the need for search for novel antifungotics and new potential drug targets. When Candida strains show reduced susceptibility to antifungal drugs [78], it is imperative to keep in mind the need for careful screening of drug resistance of Candida isolates among non- and hospitalized patients and this should be considered carefully by clinicians. In the treatment of mycotic diseases, detailed in vitro and in vivo studies are needed to specify the extent of their effectiveness. Furthermore, viewpoints of the most recent data [79, 80] on oral and vaginal candidiasis therapy explored the treatment with probiotic bacteria that may be an effective alternative to prevent it. In this context, further studies are needed to evaluate the promising colonization results of these studies.

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