The Role of Resistance in *Candida* Infections: Epidemiology and Treatment

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1 Introduction

During the last three decades, there was a marked increase in the population of immunocompromised and severely ill individuals at risk of developing opportunistic fungal infections [1]. In particular, the increased use of immunosuppressive agents particularly in organ transplant patients, chemotherapy, and lifesaving medical technology resulted in this increase of both superficial and serious invasive fungal infections [2–4]. The initial increase in fungal infections occurred at a time when there were few available, effective, systemic antifungal agents. Parenteral and systemically active oral azoles only became available in the 1980s. Accompanying the introduction of these newer azoles, an explosion in numbers of patients with AIDS at high risk of developing oropharyngeal and esophageal candidiasis was encountered.

It was during the 1990s that drug resistance became an important problem in virtually all populations of patients at risk, but predominantly in patients with AIDS [5, 6]. Reports of resistance to antifungal drugs have appeared with increased frequency. Confusion abounds as to how common *Candida* resistance is and whether fungal isolates should routinely be sent for susceptibility testing. Simultaneously, both clinical resistance and the increased incidence of fungal infections drove the development of new generations and classes of antifungal agents. Although extremely rare prior to the 1990s, antifungal drug resistance has now rapidly become a major problem in certain populations. The highest-risk population has been the most vulnerable, viz., patients with HIV infection. Thus, in the decade of the 1990s, up to a third of advanced-stage AIDS patients had drug-resistant strains of *Candida albicans* isolated from the oral cavities. However, it is no longer HIV-infected patients that demonstrate major clinical problems with antifungal resistance [7]. Nevertheless, occasional cases of clinical and in vitro resistant mucosal candidiasis due to *C. albicans* continue to be reported; however, the availability of new azoles, e.g., posaconazole and parenteral echinocandins, usually resolves the therapeutic challenge. Unfortunately, highly immunocompromised patients following both bone marrow and solid organ transplants have become a focus of rising antifungal resistance to both azole and echinocandin antifungal drugs. The purpose of this chapter is to review the epidemiology, pathogenesis, risk factors, and treatment of resistant candidiasis. Understanding cellular and molecular mechanisms of antifungal drug resistance and associated risk factors is crucial to developing successful prophylactic and treatment strategies to prevent emergence of resistant fungi and is discussed in the subsequent chapters. Management of refractory fungal disease caused by resistant *Candida* species will be reviewed together with methods available to prevent further development of antifungal drug resistance in candidiasis.

2 Epidemiology of Candidiasis

Oropharyngeal candidiasis (OPC) is most prevalent in infants, the elderly, and compromised hosts and also associated with serious underlying conditions including diabetes, leukemia, neoplasia, steroid use, antimicrobial therapy, radiation therapy, and chemotherapy. At least a quarter of cancer patients not receiving antifungal prophylaxis develop OPC, whereas other investigators have observed OPC in more than half of all immunocompromised patients. Prolonged neutropenia appears to be the single most important risk factor for both oropharyngeal colonization with a *Candida* species and subsequent symptomatic disease [8]. Approximately 80–90 % of patients with HIV infection will develop OPC at some stage of the disease [6], and 60 % of untreated patients

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develop AIDS-related infection within 2 years of appearance of OPC [9]. *Candida albicans* remains the most common species responsible for OPC [10]. A small unique population at high risk for developing azole antifungal resistance are individuals with immunodeficiency-related chronic mucocutaneous candidiasis [11, 12].

Vulvovaginal candidiasis (VVC) is considered to be the second most common form of vaginitis worldwide affecting millions of immunocompetent women. More than 90 % of infections are caused by *Candida albicans* [13]. The high prevalence of this infection in otherwise healthy females is responsible for significant morbidity and use of antifungal therapy.

During the 1980s, data from the National Center for Health Statistics (NCHS) reported that bloodstream infections (BSIs) were the 13th leading cause of death in the USA. *Candida* bloodstream infection has an attributable mortality of approximately 35 % [1]. Fungal infections, particularly due to *Candida* species, increased dramatically and accounted for 8–15 % of all nosocomial bloodstream infections [14–17]. The National Hospital Discharge Survey (NHDS) reported rates of oropharyngeal and disseminated candidiasis to have increased fourfold and 11-fold, respectively, between 1980 and 1989, a trend that continued over the next two decades [18]. Bloodstream *Candida* infections previously predominantly seen in cancer patients became common in ICUs and pediatric wards [19]. The SCOPE study reported that for the 3-year period ending in 1998, *Candida* species remained the fourth most common cause of nosocomial bloodstream infection [16, 20]. Risk factors for the increased incidence of candidemia have been reviewed [21, 22]. Moreover, candidemia has the highest crude mortality (40–50 %) of all nosocomial bloodstream infections [20, 23, 24]. Autopsy studies have also confirmed the increase in the incidence of disseminated candidiasis. Candidemia is associated with prolongation of hospital stay 70 vs. 40 days compared to matched nonfungemic patients as well as considerable increase in costs of therapy [25].

At present, *C. albicans* accounts for ~40–60 % of all nosocomial invasive *Candida* infections, reflecting a continued shift toward *Candida* species other than *C. albicans* has occurred, and of relevance because of intrinsic or acquired antifungal resistance in several of these species [2, 15, 23, 26–28]. Within the hospital setting, areas with the highest rates of candidemia include intensive care units, surgical units, trauma units, and neonatal ICUs. In fact, 25–50 % of all nosocomial candidemia occurs in critical care units. Neutropenic patients, formerly the highest-risk group, are no longer the most vulnerable subpopulation, likely as a result of the use of fluconazole prophylaxis during neutropenia [29]. In some tertiary care centers, *C. albicans* is no longer the most frequent bloodstream isolate, having been replaced by *C. glabrata*, which in turn replaced *C. tropicalis* as the most prevalent non-*albicans* species, causing 3–50 % of all candidemias. The increased frequency of *C. glabrata* in ICUs is also attributed to fluconazole exposure in ICU patients [30, 31]. There is a wide global variation in the predominance of particular species with *C. tropicalis* common in South America and *C. parapsilosis* common in Europe [32].

3 Mechanism of Action of Antifungal Drugs [33]

3.1 Polyenes [34]

The most important polyenes include amphotericin B and nystatin. Amphotericin B binds to sterol, the primary fungal cell membrane altering membrane permeability and ultimately cell death. Amphotericin B also causes oxidative damage to fungal cells (Vol. 1, Chapter 26).

3.2 Fluoropyrimidines

Flucytosine, or 5-fluorocytosine (5-FC), is a synthetic fluorinated pyrimidine. It is transported into susceptible fungal cells by the action of an enzyme cytosine permease and then converted by cytosine deaminase to fluorouracil. The latter molecule is incorporated into RNA in place of uracil. In addition, flucytosine blocks thymidylate synthetase, an essential enzyme for DNA synthesis (Vol. 1, Chapter 27).

3.3 Azoles

The azole antifungal agents in clinical use contain either two or three nitrogens in the azole rank and are therefore classified as imidazoles (ketoconazole, miconazole, clotrimazole, econazole, and butoconazole) or triazoles (itraconazole, fluconazole, terconazole). The newer azole agents include voriconazole, posaconazole, ravuconazole, and albaconazole. The azoles inhibit ergosterol synthesis in the fungal cell membrane through their action on the cytochrome P450-dependent enzyme lanosterol 14α-demethylase. Differences among various azoles relate primarily to their pharmacokinetics as well as their affinity for the target enzymes. There are also some differences in antifungal spectrum. Voriconazole and posaconazole have activity against many yeasts and filamentous fungi as well (Vol. 1, Chapter 27).

3.4 Echinocandins

This new class consists of parenteral caspofungin, micafungin, and anidulafungin. These agents inhibit fungal cell wall synthesis of an enzyme 1,3-β-D-glucan synthase, preventing the

formation of 1,3-β-D-glucan, an essential component of the fungal cell wall. These agents result in a weakened cell wall resulting in fungal cell lysis and are considered candidacidal [35] (Vol. 1, Chapter 29).

4 Definition of Resistance

4.1 Refractory Candidiasis

This by no means uncommon condition refers to treatment failure of symptomatic patients with antifungal agents. Only one of the many causes of therapeutic failure is due to the presence of in vitro confirmed resistant *Candida* spp. (Box 66.1) (Fig. 66.1). Treatment failure can also be the result of failure of the antifungal agent to reach the target site of infection in sufficient concentrations due to inadequate dosing, impaired absorption (food, gastric pH), poor compliance, and drug interactions. Other causes of treatment failure include local factors that either interfere with drug action, e.g., purulent material in an undrained abscess, or prevent access to organisms seeking refuge in a biofilm, e.g., prosthesis both intravascular and intra-articular [32]. A profoundly depressed immune system may also be responsible for failure. Both adequate numbers of functioning polymorphonuclear leukocytes and cell-mediated immunity are also essential in eradicating *Candida* infection. Clinical resistance refers to treatment failure despite microbial susceptibility in vitro.

Box 66.1. Causes of Treatment Failure Resulting in Refractory Candidiasis

1. In vitro antifungal resistance
   (a) Primary (intrinsic)
   (b) Secondary
2. Failure of drug to reach the site of infection in effective concentration
   (a) Poor adherence
   (b) Inadequate dosing
   (c) Impaired oral absorption
   (d) Drug interactions
3. Failure to drain abscess
4. Local protective mechanisms, e.g., biofilm (catheter, prosthetic valve, device, foreign body)
5. Impaired host immune/defense mechanism
   (a) PMNs
   (b) CMI

*Mechanisms 2–5 result in clinical resistance with failure associated with susceptible microorganisms.

4.2 Primary or Secondary Resistance

An organism that is resistant to a drug prior to exposure is defined as having intrinsic or primary resistance. Examples of primary resistance include *C. krusei* to fluconazole and *C. krusei* and *C. lusitaniae* to flucytosine. Acquired or

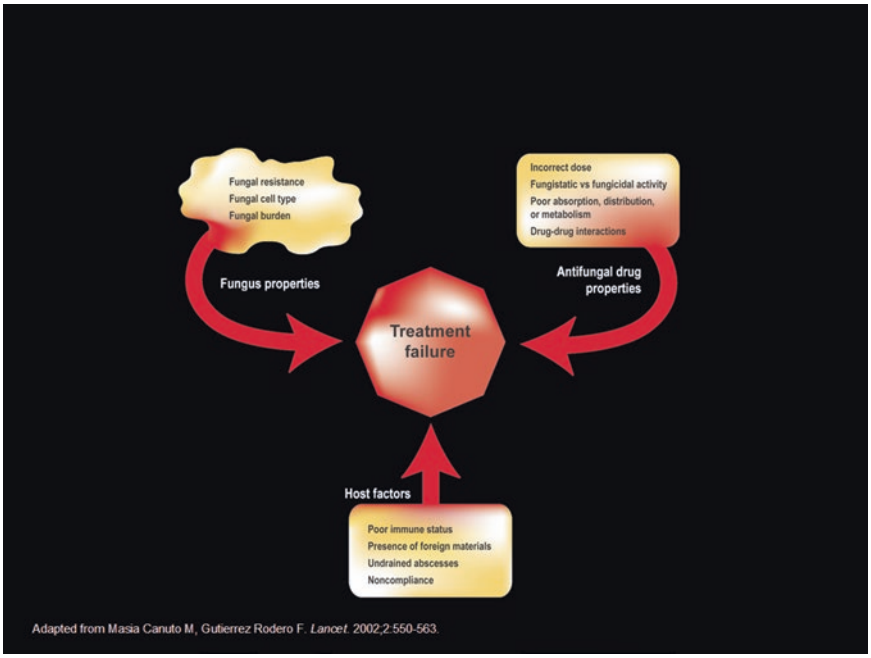

Fig. 66.1 Principal causes of failure of antifungal therapy

secondary resistance develops during or after exposure to an antifungal agent, e.g., HIV-infected patients with fluconazole-resistant OPC and esophageal candidiasis due to *C. albicans*. Cross-resistance refers to multidrug resistance either within the same class or multiple classes. Heteroresistance refers to variable in vitro susceptibility of different colonies of the same isolate obtained from the same agar plate. All forms of in vitro resistance may be temporary, transient, or irreversible.

5 Antifungal Susceptibility Tests

5.1 Methods

Testing methods and breakpoints for antifungal drugs were first suggested by Rex [36–39]. However, considerable change in methods followed to produce standardized, reproducible susceptibility methods for fungi resulting in the Clinical and Laboratory Standards Institute (CLSI) M27-A3 and the EUCAST methodology [40–47]. Accordingly, interpretive breakpoints determined by these methods are available for testing *Candida* species to fluconazole, itraconazole, voriconazole, flucytosine, amphotericin B, caspofungin, anidulafungin, and micafungin [42, 48–53] (Table 66.1). Recently, new interpretative standards have been introduced which profoundly impact upon determination and definition of susceptible and resistance isolates, potentially causing confusion for uninformed clinicians. Firstly, a new epidemiologic cutoff value (ECV) is now available and represents a more sensitive measure of change in susceptibility breakpoints [17, 43, 44]. The ECV method statistically determines the distribution of MICs within a given microbial species and is defined as the MIC value that excludes non-wild-type strains, specifically an isolate likely to contain a resistant mutation. Reliance upon the ECV results in variable breakpoints for different *Candida* species and in many cases a severalfold lowering of the susceptibility breakpoint, e.g., the previous *C. albicans* breakpoint for susceptibility to fluconazole was ≤8 mg/L, but with the new interpretation, this value is reduced to ≤2 mg/L and elevated to ≤16 mg/L for *C. glabrata*. The ECV method is valuable for detecting emergence of resistance in a *Candida* species in an institution. Using this method, most breakpoints have declined, and results of M27-A3 (CLSI) and EUCAST match more frequently. Moreover, as breakpoints decrease more isolates are deemed resistant, but no increased risk of treatment failure has been reported. This conclusion applies to both the triazoles and echinocandins with the new CLSI guidelines (Table 66.1). In the final analysis, therapeutic decisions are always individualized based upon the patient's response to therapy at the time susceptibility results become available.

In general, the susceptibility of the *Candida* isolate to the currently available antifungal agents is generally predictable if the species of the infecting isolate is known. However, individual isolates may not follow this general pattern [17].

In the past susceptibility testing of *Candida* isolates, even blood isolates, was not recommended on a routine basis. Testing was recommended only for persistent disease and failure of organism eradication in symptomatic patients with appropriate antifungal therapy. This principle was based upon the cost and lack of testing facilities available, but also driven by the rarity of in vitro resistance. However, recent surveillance suggests the emergence of reduced susceptibility of some *Candida* species in relation to azoles and echinocandins. Triazole resistance among *C. glabrata* isolates has increased to an extent that it is difficult to rely upon triazoles for therapy without performing susceptibility testing [32]. Unfortunately more recently, a similar trend has begun to emerge for a smaller proportion of *C. glabrata* isolates and the echinocandins [1, 13]. Accordingly, susceptibility testing is now required and recommended to guide the management of candidiasis. It is now recommended the laboratories perform routine antifungal susceptibility testing against both the triazole and echinocandins for *C. glabrata* isolates from blood and sterile sites and for other *Candida* species that have failed to respond to antifungal therapy. Although controversial, based upon the overall infrequency of antifungal resistance in *C. albicans*, routine testing for this species is not indicated in the absence of treatment failure. The value of testing for other *Candida* species is less clear, although occasional resistance among *C. tropicalis* and *C. parapsilosis* has been reported in certain hospitals with high use of antifungals. Hence, some authorities recommend triazole susceptibility testing for all bloodstream and clinically relevant *Candida* isolates, whereas testing for echinocandin susceptibility should be considered in patients who have had prior treatment with an echinocandin.

The objective of susceptibility testing is to differentiate infecting strains that are susceptible and hence likely to respond to a given antifungal drug from those strains resistant and hence more likely to fail therapy. With regard to echinocandins, it is essential that susceptibility tests capture high-MIC strains containing *FKS* mutations. To date the CLSI has used limited clinical data but also microbiologic data to define clinical breakpoint for all three echinocandins against *Candida* spp. [54]. Unfortunately, some resistant *Candida* strains were often misclassified by this breakpoint [55, 56]. As a result, new breakpoints were determined by CLSI that better accounted for *FKS* mutations [32, 47] (see Table 66.1). EUCAST established *Candida* species-specific and echinocandin-specific clinical breakpoints (Vol. 2, Chapter 18).

NCCLS M27-A methodology has only a limited ability to measure MICs of *Candida* isolates to amphotericin B. Rex et al. recommended the use of antibiotic medium 3 broth to measure

Table 66.1 In vitro susceptibility of *Candida albicans* and interpretative breakpoints

	Clinical breakpoints (in μg/mL)			
Organism	Susceptible	Susceptible dose dependent	Intermediate	Resistant
C. albicans				
Caspofungin	≤0.25	–	0.5	≥1
Anidulafungin	≤0.25	–	0.5	≥1
Micafungin	≤0.25	–	0.5	≥1
Fluconazole	≤2.0	4.0	–	≥8
Itraconazole	≤0.12	0.25–0.5	–	≥1
Voriconazole	≤0.12	–	0.25–0.5	≥1
C. parapsilosis				
Caspofungin	≤2	–	4	≥8
Anidulafungin	≤2	–	4	≥8
Micafungin	≤2	–	4	≥8
Fluconazole	≤2	4.0	–	≥8
Voriconazole	≤0.12	–	0.25–0.5	≥1
C. tropicalis				
Caspofungin	≤0.25	–	0.5	≥1
Anidulafungin	≤0.25	–	0.5	≥1
Micafungin	≤0.25	–	0.5	≥1
Fluconazole	≤2	4.0	–	≥8
Voriconazole	≤0.12	–	0.25 – 0.5	≥1
C. glabrata				
Caspofungin	≤0.12	–	0.25	≥0.5
Anidulafungin	≤0.12	–	0.25	≥0.5
Micafungin	≤0.06	–	0.12	≥0.25
Fluconazole		≤32	–	≥64
C. krusei				
Caspofungin	≤0.25	–	0.5	≥1
Anidulafungin	≤0.25	–	0.5	≥1
Micafungin	≤0.25	–	0.5	≥1
Fluconazole[a]	–	–	–	–
Voriconazole	≤0.5	–	1	≥2
C. guilliermondii				
Caspofungin	≤2	–	4	≥8
Anidulafungin	≤2	–	4	≥8
Micafungin	≤2	–	4	≥8

24 h 100 %, MIC end points read as 100 % inhibition at 24 h incubation; 24 h 50 %, MIC end points read as 50 % inhibition at 24 h incubation
[a]Fluconazole breakpoints are not available for *C. krusei* since this species is considered intrinsically resistant to this compound. All strains should be reported as resistant

resistance [48, 57]. In general, current methods are limited to identifying *Candida* isolates associated with clinical failure, although breakpoint minimal lethal concentrations (MLCs) and MICs of ≥1 μg/mL at 48 h have been recommended to more accurately predict mycologic *Candida* spp. failure with amphotericin B [58]. In a multicenter study of candidemia in non-neutropenic patients, all blood isolates demonstrated amphotericin B MICs less than 1.0 μg/mL. As with fluconazole, clinical failures (10–15 %) were all associated with in vitro susceptible isolates with low amphotericin B MICs [50].

The Etest is often used as an alternate to broth dilution methodology and certainly is useful in the setting of refractory clinical disease, and there is no other testing method available. The Etest is considered suitable for testing *Candida* spp. against amphotericin B or flucytosine, but less reliable for azole susceptibility [49, 51–53, 57].

5.2 In Vitro Susceptibility and Resistance of *Candida* Species (Table 66.2)

5.2.1 Azoles

The triazole, voriconazole, posaconazole, and ravuconazole exhibit greater potency and spectrum than either fluconazole or itraconazole but are still essentially fungistatic.

Table 66.2 Susceptibility of *Candida* spp. to antifungal agents

MIC_{50}	Amphotericin B	Fluconazole	Itraconazole	Voriconazole	Flucytosine	Caspofungin
C. albicans	0.5	0.5	0.12	0.03	≤0.25	0.12
C. tropicalis	0.25	1	0.06	0.06	≤0.25	0.25
C. glabrata	0.5	16	0.25	0.25	≤0.25	0.12
C. parapsilosis	0.25	1	0.12	0.03	≤0.25	1.0
C. krusei	0.25	64	0.5	0.5	16	0.5
C. lusitaniae	≥1	2	0.25	0.03	≤0.25	1.0

Shown are typical species-specific MIC_{50}s (μg/mL) adapted from reports describing collections of clinical isolates [50, 59–61]. MICs were obtained by the NCCLS M27 methodology (National Committee for Clinical Laboratory Standards, 1995) for all drugs but amphotericin B. If this method fails to detect amphotericin B-resistant *Candida* [50], then reported amphotericin B MICs were obtained by a more sensitive method based on the use of antibiotic medium 3 in an agar-based testing format

The activity of this broad-spectrum triazole extends to some fluconazole-resistant strains of *Candida*.

Primary and secondary azole resistance is species dependent and also shows marked geographic variation [59, 62]. There is no clear evidence for a correlation between the agricultural use of azoles and an increase in antimycotic resistance in *Candida* species. Primary resistance to azoles remains uncommon in candidiasis, with the exception of *Candida glabrata* and *Candida krusei*. Most acquired azole resistance emerged in AIDS patients with OPC and EC following prolonged azole therapy in the presence of advanced immunodeficiency. Azole resistance in other settings is uncommon [32, 63].

1. *C. albicans*. Primary resistance to fluconazole and itraconazole is extremely rare. Moreover, outside the realm of AIDS, acquired or secondary resistance has likewise remained uncommon especially with regard to bloodstream isolates. Each year, thousands of randomly obtained BSIs isolated from all over the world are tested in a single site (SENTRY), and over several years fluconazole-resistant *C. albicans* remains <5 % and shows no evidence of changing [60, 63, 64]. In contrast, Antoniadou et al. reported that 9 % of bloodstream isolates of *C. albicans* were resistant to fluconazole (MIC > 64 μg/mL) [65]. Spontaneous fluconazole resistance in the absence of prior azole therapy is rare but has been reported in otherwise healthy adults [66]. Based upon molecular modeling studies, it has been reported that certain mutations in *ERG* II result in significant levels of resistance to fluconazole and voriconazole but have less effect on the susceptibility of the organisms to itraconazole and posaconazole, possibly due to the more extensive binding of the latter agents to the target enzymes [67].
2. *C. tropicalis*. Occasional strains of *C. tropicalis* demonstrate azole resistance although MIC_{90} values indicate continued susceptibility. This species has a proclivity to produce trailing grown in vitro often misinterpreted as resistance.
3. *C. parapsilosis* strains are usually highly susceptible to all azoles [67].
4. *C. krusei*. This species is intrinsically resistant to fluconazole and has higher MICs to itraconazole in the S-DD range. Voriconazole is, however, very active against *C. krusei* [60, 68]. *C. krusei* incidence has remained stable over the last decade.
5. *C. dubliniensis*. This species has been increasingly identified and implicated in OPC in HIV-infected agents and is usually identified as *C. albicans*. Most *C. dubliniensis* strains are susceptible to fluconazole although in vitro resistance can be induced. Acquired resistance develops much more rapidly than in *C. albicans*.
6. *C. glabrata*. Among pathogenic yeast species, *Candida glabrata*, which accounts for 5–40 % of all yeast isolates, ranks second in all clinical forms of candidiasis today and in some studies of nosocomial candidemia is more common than *C. albicans* [32, 69]. This opportunistic pathogen is particularly relevant in immunocompromised patients including those receiving cytotoxic chemotherapy, undergoing transplantation, and infected with HIV. This critical *Candida* species represents the Achilles heel of the azole class [70, 71]. *C. glabrata* isolates exhibit bimodal susceptibility to azoles with 10–15 % of bloodstream isolates demonstrating fluconazole resistance (≥64 μg/mL) [60, 64]. Patterns of fluconazole susceptibility vary by geographic area, patient population, risk factors, and azole exposure [72]. In particular, clinical isolates obtained from patients with AIDS and OPC/EC and those with underlying malignancy show reduced susceptibility to fluconazole and itraconazole. Fluconazole resistance is lowest in Asia-Pacific and Latin-American regions (3–4 %) and highest in North America (10–15 %). Both the frequency of *C. glabrata* occurrence and azole susceptibility are profoundly affected by azole exposure, with 30–40 % of isolates being S-DD. International surveillance reveals that recently submitted bloodstream isolates (2001–2005) of *C. parapsilosis* and *C. tropicalis* in contrast to *C. albicans* did reveal a slight increase in fluconazole resistance. A similar increase in resistance was

observed for *C. glabrata* with sustained high rates of fluconazole resistance (14.3–18.3 %) over this period [63]. In general, whereas most *C. glabrata* isolates are still susceptible to voriconazole, most fluconazole-resistant *C. glabrata* isolates are resistant to itraconazole, and half are also resistant to voriconazole and posaconazole [60, 69, 73]. Not surprisingly, several reports of voriconazole-resistant *C. glabrata* breakthrough fungemia in bone marrow transplant recipients receiving long-term voriconazole prophylaxis have been reported [74].

5.2.2 Flucytosine

Intrinsic resistance among *C. albicans* has been described in 6.5–33 % of isolates and is invariably associated with serotype B isolates [75]. More recent studies have shown lower resistance frequency possibly due to infrequent use. Pfaller et al. studying 8803 clinical isolates of *Candida* spp. reported susceptibility as follows: *C. albicans* (97 %), *C. tropicalis* (92 %), *C. guilliermondii* (100 %), *C. dubliniensis* (100 %), *C. parapsilosis* (99 %), and *C. glabrata* (99 %) [64]. The least susceptible species was *C. krusei* (5 % susceptible, 67 % intermediate, and 28 % resistant). A smaller study reported that 82 % of *C. glabrata* were susceptible to flucytosine. The pharmacokinetics and in vitro activity of flucytosine make the agent particularly useful for azole-resistant *Candida* infections in relatively inaccessible sites such as CSF and the genitourinary tract.

Unfortunately, secondary acquired resistance is common (30 %) and acquired rapidly to flucytosine when used as monotherapy. Accordingly, flucytosine is almost always used in combination with other antifungals.

5.2.3 Polyenes

Resistance to amphotericin B may be intrinsic or acquired [76]. *C. albicans* resistance is extremely rare, although the NCCLS M27-A methodology may be underestimating its occurrence. For amphotericin B, NCCLS methodology generates a narrow MIC range limiting its ability to identify isolates likely to cause therapeutic failure [58]. Moreover, more important than resistance is the phenomenon of reduced susceptibility without frank resistance. Powderly et al. reported reduced amphotericin B sensitivity of blood isolates of *C. albicans* in neutropenic patients and correlating higher MICs with poor outcome [77]. Fortunately, such strains are rare and secondary resistance is uncommon [78]. Resistance in *C. parapsilosis* and *C. dubliniensis* but not *C. tropicalis* is rare [79]. Although *C. glabrata* and *C. krusei* isolates are usually considered susceptible to amphotericin B, they tend to have higher MICs, justifying initial empiric use of amphotericin B at a higher dose of 1.0 mg/kg/day. Sterling reported the emergence of resistance to amphotericin B during therapy for *C. glabrata* infection in an immunocompetent host [80]. Many but not all *C. lusitaniae* and some *C. guilliermondii* isolates demonstrate intrinsic resistance to amphotericin B [81]. Acquisition of secondary polyene resistance in species, in addition to *C. albicans*, includes *C. lusitaniae* and *C. guilliermondii* during amphotericin B therapy especially in myelosuppressed patients [78, 82–84]. Rare cases of fatal septicemia reported of amphotericin B-resistant *C. lusitaniae* [85]. Resistance to amphotericin B desoxycholate implies that the organism will be resistant to the various lipid formulations of amphotericin B.

5.2.4 Echinocandins

Early reports of clinical and/or in vitro resistance to any of the echinocandin agents were rare. In 2003, the in vitro activities of caspofungin against 3959 isolates of *Candida* spp. from 95 different medical centers were determined and compared with fluconazole and itraconazole [61]. No resistant strains of *C. albicans* were detected. Against all *Candida* species, 96 % of MICs were ≤2 μg/mL. *C. albicans*, *C. dubliniensis*, *C. tropicalis*, and *C. glabrata* were the most susceptible species, and *C. guilliermondii* was the least susceptible (MIC_{90} > 80 μg/mL). *C. parapsilosis* MIC_{90} 2–4 μg/mL was significantly increased versus *C. albicans* 0.25 μg/mL [61]. Echinocandins remain very active against azole-resistant isolates of *C. albicans* and *C. glabrata* (99 % of MICs were ≤1 μg/mL). There is no evidence of a significant impact of azole resistance mediated by CDR pumps on echinocandin resistance in clinical *Candida* isolates.

Similarly, large multinational *Candida* isolate collections have been used to evaluate in vitro resistance to micafungin and anidulafungin, and identical almost universal susceptibility has been reported and once more higher MICs of *C. parapsilosis* emerged [63]. Interestingly, caspofungin is not fungicidal for isolates of *C. parapsilosis* or *C. guilliermondii* [86].

Breakpoints for the echinocandin class of agents were delayed in appearance since in vitro and in vivo analyses were hampered by a dearth of resistant isolates. As a result Kartsonis et al. failed to establish any relationship between baseline caspofungin MICs and clinical outcome with isolates from both mucosal and invasive *Candida* infections [87]. An echinocandin MIC of ≤2.0 μg/mL, a blood concentration easily achievable in vivo under normal dosing, would encompass 99.7 % of all clinical isolates of *Candida* species [63].

While clinical failure due to echinocandin-resistant *Candida* isolates has been rare, acquired in vivo resistance following echinocandin exposure undoubtedly occurs, and resistant isolates have increasingly been reported. All the resistant isolates were shown to have homozygous mutations in the *FKS*1 gene. Clinical failure with all *Candida* species has also increasingly been reported [88–90].

Hernandez et al. in 2004 reported a patient with azole-refractory OPC/EC which in spite of initial improvement

eventually failed on caspofungin [91]. Initial isolates exhibited low caspofungin MICs, whereas a late isolate had higher MIC. The clinical response was reproduced in a murine model correlating MIC with the clinical response to caspofungin. Similarly, a case of progressive loss of echinocandin activity following prolonged use for treatment of *C. albicans* esophagitis was reported [92].

Moudgal et al. in 2005 described a patient with aortic valve endocarditis due to *C. parapsilosis* [93]. After initially responding to combination therapy with caspofungin (MIC 2 μg/mL) and fluconazole, he cleared his fungemia and was discharged on fluconazole only. He returned three months later with recurrent *C. parapsilosis*, now resistant to both fluconazole and caspofungin (MIC > 16 μg/mL) and also voriconazole and micafungin but not anidulafungin. Similar case reports regarding acquired echinocandin resistance in *C. glabrata* are reported more than a decade ago predicting a future likelihood of increased resistance in this species (see Chapter. 29, Volume 1) [94].

5.3 Correlation of In Vitro Susceptibility Testing and Clinical Outcome of Treatment with Antifungal Agents

In vitro susceptibility is only one of the many factors that influence the outcome of therapy of fungal infections [37]. A variety of pharmacokinetic and pharmacodynamic drug factors as well as a multitude of host factors (neutropenia, compliance, catheter presence, APACHE scores, abscess drainage) all interact to impact upon clinical outcome(s). Even the definition of clinical outcome is controversial, ranging from clinical improvement to mycologic evaluation (short or long term) on patient survival (days or weeks). Nevertheless, in vitro susceptibility determination may serve as an objective, reproducible measure that can profoundly influence drug selection with physicians recognizing the limitations of in vitro susceptibility testing.

Establishing that an isolate is resistant to an antifungal agent in vitro is an immensely useful step in selecting therapy. Determining that the isolate is susceptible to antifungal agents in no way predicts survival or fungal eradication. Clinicians should recall the old 90-60 rule in which a clinical response of 90 % or more can be expected when an in vitro sensitive strain is treated with an appropriate antibiotic in comparison to a 60 % response when a resistant strain is treated with drugs showing reduced or no activity in vitro.

With regard to candidiasis, in vitro and clinical outcome correlations have mainly been applied to OPC/EC and candidemia, where the 90-60 rule appears to have been met, recognizing this is merely a minimal standard. The most important principle applied is that organisms deemed resistant in vitro are much less likely to respond in vivo. Yet within the candidemia RCTs involving hundreds of patients, almost all patients failing did so with highly susceptible strains. This emphasizes the principle that susceptibility in vitro does not guarantee successful therapy. Most studies evaluating the 90-60 rule have applied to azoles, specifically fluconazole, and the best correlation was in OPC/EC in AIDS patients. The clinical predictability of amphotericin B susceptibility is less well established. Moreover, Sobel et al. found poor correlation between in vitro MICs and response to fluconazole therapy for VVC [95]. Finally, any discussion of clinical correlation must distinguish resistance developing in a given strain of the same species from the problem of acquiring less susceptible strains from the same or different species.

5.4 Indications for Antifungal Susceptibility Testing in *Candida* Infections

Apart for reasons of periodic epidemiologic surveillance and resistance monitoring, routine susceptibility testing by any of the aforementioned methods is not indicated. Testing is justified for all bloodstream *Candida* isolates, especially if associated with persistent, breakthrough, and recurrent candidemia and also refractory mucosal candidiasis, anticipated prolonged or critical therapy, e.g., endocarditis, osteomyelitis especially with non-*albicans Candida* invasive infections. Also, testing is essential with selected non-*albicans Candida* species, e.g., *C. glabrata*, initially treated by non-azole regimens anticipating a switch to oral therapy with either fluconazole or voriconazole to complete therapy. Given the increase of parenteral echinocandins as first-line therapy for candidemia only to have the remainder of the therapeutic course completed by oral triazoles, the Infectious Society of America now recommends that all first bloodstream isolates should be tested for antifungal susceptibility [96].

6 Epidemiology and Risk Factors for Resistant Candidiasis

Does azole use select for antifungal drug resistance? In this context, clinical resistance is encountered with (a) the presence of organisms with intrinsic, de novo resistance to antifungals usually seen with non-*albicans Candida* and rarely *C. albicans*, (b) alternately, evolution may occur of the initially sensitive strain to an identical strain that has undergone genetic and molecular changes, or (c) there is replacement of the strain with a new resistant strain of the same species or finally replacement with a new strain of a different species.

Evidence links empirical, prophylactic, and therapeutic use of azoles and selection for yeasts other than *C. albicans* that exhibit decreased susceptibility to azoles, e.g., *C. glabrata* and *C. krusei* infections in patients receiving fluconazole prophylaxis [97–99]. Most of the early data came from AIDS patients. The emergence of antifungal-resistant *C. albicans* fungemia has increasingly been reported in bone marrow transplant recipients being administered with long-term fluconazole prophylaxis [100]. Similarly, isolated reports of fluconazole-resistant fungi in surgical ICUs are emerging [101].

While molecular changes in a single strain invariably reflect a single or more usually multiple genetic mutations, the dynamics of acquisition of a new strain or species is less well understood. New more resistant *Candida* strains or species may be acquired during hospitalization from medical staff carriers. This process has been well documented with *C. albicans* and *C. parapsilosis*, but *C. glabrata* is rarely identified on the hands of carriers or in hospital environment. It is hypothesized that patients may be colonized in the gastrointestinal tract simultaneously by multiple strains of *Candida*, including the possibility of multiple species. Routine culture only captures the dominant strain or species. After antifungal drug ingestion or pressure, more susceptible strains are eliminated or so reduced in number so as to allow growth and emergence and recognition of more resistant strains or species that have coexisted long term but were previously not recognized.

6.1 HIV/AIDS

AIDS patients have been the focal point of much of the scientific inquiry into fluconazole resistance. On the one hand, oral and esophageal candidiasis became extremely common as a clinical manifestation of AIDS in the 1980s. The availability of fluconazole as both treatment and subsequently prophylaxis in patients with recurrent disease was an enormous boon to care. Within a few short years, clinical and in vitro fluconazole resistance was widespread and caused major alarm among AIDS practitioners [6, 32, 102]. Several studies, mainly retrospective, identified risk factors for acquisition of fluconazole resistance (Box 66.2). In addition to the status of the immune system (CMI), i.e., CD4 lymphocyte count, most studies concluded that patterns of fluconazole use particularly drug dose were the dominant factors associated with resistance acquisition [103–106]. In the majority of patients, mutation of a previously susceptible strain of *C. albicans* to a resistant strain is likely to have occurred, together with coinfection with *Candida* species resistant to fluconazole, e.g., *C. glabrata* [107].

Box 66.2. Risk Factors for Azole Resistance in Candidiasis

1. HIV/AIDS
   (a) Advanced immunosuppression (low CD4 cells)
   (b) High viral load
   (c) Fluconazole administration
      - Poor compliance
      - Past fluconazole exposure
         – Total dose
         – Intermittent therapy
         – Prophylaxis versus therapeutic
         – Low dose
2. Hematologic malignancy/BM transplantation
   (a) Azole exposure (prophylactic)
3. Prosthetic devices—foreign bodies
   (a) Biofilm

In a prospective, randomized, controlled trial conducted by the Mycoses Study Group, episodic treatment versus continuous prophylaxis with fluconazole was studied. The first conclusion was that overall resistance acquisition was uncommon in this HAART-compliant study population. Secondly, the use of episodic compared to continuous fluconazole prophylaxis was not shown to be protective in preventing emergence of resistance [108]. In general, no pattern of fluconazole prescription or ingestion has been consistently identified as contributing to azole resistance selection, although both dosing and duration have been widely implicated in emergence of resistance. Most importantly, it has not been established whether lower doses used for longer periods of time lead to antifungal resistance and whether intermittent therapy, especially using higher doses for shorter periods, prevents resistance [109]. In contrast to the above, occasionally resistant species were isolated in patients with HIV infection and no prior exposure to fluconazole [110].

It is noteworthy that in the last decade, because of the availability of potent and better tolerated ART, the occurrence of fluconazole-resistant OPC and *Candida* esophagitis has become infrequent.

6.2 Hematologic Malignancies and Transplant Patients

This growing population is the second focus of resistant candidiasis. Empiric systemic antifungals are widely used as empiric therapy for antibiotic-resistant fever in addition to azole prophylaxis both in neutropenic patients (usually fairly short term) and non-neutropenic high-risk posttransplant patients (often long term). Once more, azole exposure both

oral and systemic is recognized as (a) infrequent cause of azole-resistant *C. albicans* and (b) a more frequent and important cause of selection of non-*albicans Candida* species, both colonizing the gastrointestinal tract and as a cause of the ensuing infrequent invasive candidiasis [99, 111]. Primary fluconazole resistance has been reported in patients with severe neutropenia [112, 113]. Candidemia due to *C. krusei* has been associated with prior exposure to fluconazole [94, 114, 115].

6.3 Prosthetic Devices/In Vivo Biofilm

Evidence has been presented based upon in vitro, animal models and clinical studies that *Candida* organisms found in biofilm may show significant reduced susceptibility to azole drugs [116]. The implications are self-evident, since infections involving intravascular catheters and prosthetic valves and devices invariably fail intensive antifungal therapy and require surgical removal for cure. Clinical failure may also be due to failure of the antifungal drug to penetrate the biofilm access of yeast cells found within the biofilm [117]. The most important explanation for biofilm-related resistance appears to be the phenotypic and genotypic changes that are reported in biofilm containing yeast cells demonstrating in vitro antifungal resistance when compared to planktonic isotype cells. Nett et al. reported increased β-1,3-glucan content in *C. albicans* cell walls from biofilm compared to planktonic organisms thought to be responsible for polyene resistance and fluconazole resulting in limited intracellular penetration [118]. Biofilm-associated yeast cells are more susceptible to β-glucan inhibitors, i.e., echinocandins [119].

6.4 Antifungal Drugs

While most of the information available on drug-induced resistance followed the use of fluconazole and ketoconazole, usually as oral agents, little is known about the potential for broader-spectrum (itraconazole, voriconazole, posaconazole, caspofungin, micafungin, anidulafungin) or more active/potent in vitro drugs (voriconazole, posaconazole, echinocandins) or Candidacidal drugs (echinocandin) to select for less susceptible *C. albicans* or non-*albicans Candida* isolates.

Invasive infections due to amphotericin B-resistant *Candida* isolates have infrequently been reported in association with the use of this agent [58, 77, 120]. Many *C. lusitaniae* and some *C. guilliermondii* isolates demonstrated primary resistance to amphotericin B, but secondary resistance to amphotericin B appears to be uncommon. Acquired resistance associated with disseminated infections due to *C. glabrata*, *C. krusei*, and *C. albicans* that developed during therapy is described but is uncommon [121]. Resistance appears to be due to alteration or a decrease in the amount of ergosterol in the cell membrane. Yoon demonstrated in vitro reversible switching of *C. lusitaniae* with acquired amphotericin B resistance [122]. Nystatin-resistant *C. rugosa* was reported a burn unit following extended use of prophylactic topical nystatin [123].

A growing mass of data indicates that frequent and prolonged exposure to azole may influence the emergence of non-*albicans Candida* species especially *C. glabrata* but may also select for acquired resistance in *C. albicans* strains particularly following prolonged exposure to subinhibitory azole concentrations [32, 100, 111, 115]. However, the overall effect of azoles on *Candida* species distribution and resistance development is incompletely understood [124, 125]. Blott et al. reported that over an 11-year period in a single institution, the volume of fluconazole consumption did not correlate with *Candida* sp. distribution [124].

6.5 *Candida* Vaginitis

In spite of widespread use and abuse of over-the-counter (OTC) imidazole antifungals, little evidence has emerged of azole resistance in *C. albicans* or selection of non-*albicans Candida* spp. [126, 127]. However, prolonged use of long-term, low-dose (150 mg/week) fluconazole maintenance prophylaxis, in women with recurrent vulvovaginal candidiasis (RVVC), has recently been reported to contribute to both fluconazole and azole class resistance resulting in refractory vaginitis caused by in vitro resistant *C. albicans* [128, 129]. Moreover, in a study of HIV-positive women with RVVC receiving fluconazole, some evidence did surface of emergence of *C. glabrata* as a more frequent pathogen [130, 131].

6.6 Azole Cross-Resistance

Given that the azole class of antifungal agents share a common mechanism of action and in most cases of resistance, development of cross-resistance is common.

When selecting antifungal treatment, it is essential to establish whether the patient has received previous antifungal therapy because patients may harbor *Candida* species resistant to multiple azole agents [132–134]. Both in vitro and clinical studies have clearly demonstrated high frequency of azole cross-resistance [135]. Several studies indicated cross-resistance to itraconazole, ketoconazole, and other imidazoles in isolates resistant to fluconazole [32, 136]. Most of the strains concerned were fluconazole-resistant isolates of *C. albicans* obtained from patients with advanced AIDS and refractory OPC [137, 138], but others have reported cross-resistance in virtually all species of *Candida* exposed to non-fluconazole azoles, e.g., itraconazole and ketoconazole [132, 133, 139, 140]. Moreover, resistance found to first- and second-generation azoles may

extend, even in the absence of exposure, to newer triazoles, voriconazole and posaconazole, either as absolute resistance or more frequently as higher MIC values [136, 141–144]. In general, fluconazole-resistant strains had higher MICs to voriconazole and posaconazole. Nevertheless, cross-resistance varies considerably among species; hence, some but not all *C. parapsilosis* and *C. albicans* isolates maintain susceptibility to itraconazole, posaconazole, and voriconazole despite fluconazole resistance. Cross-resistance is often more predictable for *C. tropicalis* isolates, and lack of cross-resistance is seen with *C. krusei*. The development of resistance to azoles invariably requires more than one mutation; hence, isolates with resistance to both fluconazole and itraconazole exhibit multiple mechanisms or types of resistance and therefore are more likely to demonstrate resistance or reduced susceptibility to newer azole agents. Cross-resistance is a very common if not universal feature in azole-resistant *C. glabrata* isolates, especially in those that are capable of expressing multiple mechanisms of resistance [145, 146].

Susceptibility testing of 6970 *Candida* isolates from 200 centers worldwide by Pfaller et al. revealed that *C. albicans* and *C. glabrata* strains resistant to both fluconazole and itraconazole were less susceptible to posaconazole, ravuconazole, and voriconazole [60]. Slightly less than 50 % of *Candida* species isolates resistant to fluconazole maintained susceptibility to newer triazole agents [147]. In a study of azole cross-resistance, fluconazole MICs of ≤32 μg/mL predicted susceptibility, and MICs of ≥64 μg/mL predicted resistance of *Candida* spp. to voriconazole and posaconazole [147]. Voriconazole was active against *C. krusei* regardless of azole susceptibility. While much has been written of fluconazole prophylaxis leading to widespread azole resistance, similarly itraconazole prophylaxis was shown to be associated with cross-resistance to fluconazole [133, 148]. While much of the literature on azole cross-resistance has focused on mucosal candidiasis, similarly, large surveillance surveys of *Candida* spp. causing invasive infection including candidemia have shown evidence of cross-resistance.

6.7 Drug Pharmacokinetics, Pharmacodynamics, and Resistance in Candidiasis

Andes et al. reported the impact of fluconazole dosing regimens and pharmacodynamics on resistance development in *C. albicans* [149, 150]. Fluconazole regimens that produced prolonged sub-MIC concentrations were associated with resistance development. The emergence of the resistant phenotype was associated with increased expression of *CDR1*- and *CDR2*-encoded efflux pumps but not *MDR1*-encoded pumps or *ERG II* [149, 150]. In a murine systemic candidiasis model, the more frequently administered dosing regimens prevented the emergence of a resistant cell phenotype.

A correlation between in vitro susceptibility and response to therapy of non-mucosal candidiasis has been demonstrated in some studies [151, 152] but not others. Clancy et al. in 2003 evaluated 32 bloodstream *Candida* isolates and concluded that geometric mean MIC and fluconazole dose/MIC ratio predicted clinical failure [153]. Inadequate dosing of fluconazole (≤200 mg/day) and ratio <50 correlated with therapeutic failure, but not necessarily with resistance development.

6.8 Echinocandin Resistance

Candida sp. isolates resistant to echinocandins were first reported in 2005 [88], but reports of resistance were rare, at <2–3 % with *C. albicans* and most *Candida* species [43, 62, 154, 155]. However with time, reports of clinical failure with isolates demonstrating high MIC were increasingly but still not frequently seen [92, 156–165]. Overall, echinocandin resistance among most *Candida* species has been largely unchanged in the past few years [32]. However, this does not apply to *C. glabrata*, where echinocandin resistance is increasing and there is justifiable concern especially since many isolates also demonstrate azole resistance [166–168]. The SENTRY Antimicrobial Surveillance Program revealed that 8.0–9.3 % of blood isolates of *C. glabrata* from 2006 to 2010 were echinocandin drug resistant [154]. Of concern, Alexander et al. reported an increase in echinocandin-resistant *C. glabrata* bloodstream isolates, in Duke Hospital from 2–3 % in 2001–2006 to more than 13 % in 2009–2010 [166]. This is not widespread throughout the USA in that one recent study showed 3.1–5.7 % resistance in *Candida* isolates [62, 168]. Nevertheless, echinocandin resistance was similarly linked to azole resistance in *C. glabrata*. In this large Pham study, nearly all isolates containing an *FKS* mutation were resistant to at least one echinocandin, and 36 % were also resistant to fluconazole [168].

6.8.1 Mechanism of Acquired Echinocandin Resistance

Echinocandin resistance results from modification of glucan synthase, which is encoded by genes *FKS1* and *FKS2*. Unlike azole drugs, echinocandins are not substitutes for multidrug transporters [169]. Echinocandin resistance is nevertheless well characterized, conferred by restricted mutations in two highly conserved "hot spot" regions of the *FKS* genes [167]. The *FKS* mutations result in amino acid mutations that induce MIC values from 20- to 100-fold and reduced sensitivity of glucan synthase to drug by 50–30,000-fold [170]. These less susceptible fks mutant strains respond poorly to echinocandin drugs in pharmacodynamic models of infection [171, 172] and are associated with reduced clinical response [173, 174]. The *FKS* resistance mechanisms have been observed in many *Candida* species [175]. In all *Candida* species, except *C. glabrata*, mutations occur within two "hot spot" regions of *FKS1* [55] (see Chapter

29, Volume 1). In *C. glabrata* mutations occur in the homologous hot spot regions of *FKS1* and *FKS2* [55, 155, 170].

The echinocandin drugs are highly serum protein bound potentially reducing susceptibility testing. Serum is considered to reduce the fungicidal characters of the echinocandins, resulting in fungistatic activity against certain *Candida* species [175].

Biofilms also play a role in antifungal resistance [176]. Decreasing glucan production, accompanying echinocandin use increases susceptibility of yeast organisms contained within the biofilm to the effects of these drugs [177].

7 Refractory Candidiasis: Clinical Resistance Syndromes and Their Management

7.1 Oropharyngeal and Esophageal Candidiasis

Refractory OPC and EC represent the commonest manifestation of clinical azole resistance and failure that is supported by concomitant in vitro azole resistance. Most patients present with highly symptomatic episodes with oropharyngeal pain and debilitating dysphagia and odynophagia requiring hospitalization. The majority of patients with refractory upper gastrointestinal candidiasis have AIDS and advanced immunodeficiency. In the 1990s, the annual incidence of clinical failure of fluconazole in OPC was approximately 5 % [104–107]. Accordingly, refractory superficial candidiasis peaked and became a major clinical problem during the decades of the 1990s prior to the availability of highly active antiretroviral therapy (HAART) [110, 178, 179]. The majority of these patients have refractory disease caused by *C. albicans* [180]. Only a minority have non-*albicans Candida* spp. usually *C. glabrata*, strains of which are usually resistant in vitro to fluconazole. Resistant strains of *C. albicans* and *C. glabrata* are frequently, but not invariably, cross-resistant to itraconazole and ketoconazole [181]. Refractory mucosal candidiasis has also been reportedly associated with *C. tropicalis* and *C. krusei* [5]. In the absence of coinfection with non-*albicans Candida* species, refractory candidiasis is seen with both in vitro resistant and sensitive *C. albicans*. The reason for treatment failure caused by azole-sensitive *C. albicans* is usually the result of noncompliance with ART therapy, drug underdosing, or drug interactions. Another major factor is simply advanced immunodeficiency. With refractory esophagitis, it is important to exclude concomitant pathology such as CMV or HSV esophagitis. Other explanations for the in vitro-in vivo discrepancy in compliant patients relate to heteroresistance in individual colonies of *Candida*, with chance selection of a "susceptible" colony. Most patients with refractory OPC and EC almost always have usual *Candida* spp. isolates with in vitro resistance.

Finally, some experts have questioned the virulence capacity of non-*albicans Candida* species to induce OPC and EC, let alone refractory disease [5, 182]. It is true that refractory candidiasis in patients with AIDS, from whom NAC strains are isolated, usually represents mixed infections with coexistent *C. albicans*; however, resistant disease due to *C. glabrata* in the absence of *C. albicans* is now widely accepted.

The availability of HAART was rapidly followed by a marked decline in the frequency of refractory OPC and EC [183]. It was assumed that enhanced mucosal immune function was responsible for this phenomenon. However, this issue is more complex in that refractory disease resolved within days and weeks of initiation of HAART, preceding demonstrable improvement or change in CD4 lymphocyte cell count or any other marker of CMI, suggesting that some other beneficial effects might be responsible [184]. Another observation included the disappearance of azole-resistant strains of *C. albicans* and *C. glabrata* with the reappearance of azole-sensitive strains. How was improved mucosal CMI selecting susceptible strains of *Candida*? Another more recent hypothesis relates to a direct effect of HIV structural components in directly influencing genes carried by *Candida* responsible for virulence expression including development of azole resistance. Accordingly, HIV gp 160 and gp 41 may influence *Candida* in vitro, selecting for azole resistance [185]. According to this hypothesis, the mucosal viral load (HIV RNA) would enhance *Candida* virulence in situ and finally induce or select for azole resistance. Introduction of HAART and rapid decrease in viral load, before immune recovery, would explain early resolution of refractory mucosal candidiasis and reemergence of azole-susceptible strains. Therapeutic protease inhibitors may further reduce *Candida* virulence by inhibiting fungal secretory aspartyl proteinases [186].

It follows that in the post-HAART era, the frequency of refractory disease as well as in vitro azole resistance declined substantially. The majority of patients with chronic and refractory disease are usually noncompliant AIDS patients infected with susceptible *C. albicans*. In a study of in vitro susceptibility of oral isolates in the HAART era, Tacconelli showed a reduction in azole resistance from 37 to 7 % [187]. The explanation for the reduced or diminished at-risk population is thought to relate to reduced fluconazole exposure, i.e., fewer low-dose regimens and less continuous long-term therapy; however, this hypothesis is unproven. Barchiesi et al. reported that most patients on HAART are colonized by strains of *C. albicans* susceptible to fluconazole (93 % sensitive) [188]. Most cases of OPC in the HAART era are caused by fluconazole-sensitive *C. albicans*.

A high prevalence of non-*albicans Candida* species (*C. albicans* 49 %, *C. glabrata* 24 %) with frequent resistance to fluconazole and itraconazole has also been reported in patients with advanced cancer, especially head and neck malignancy [189, 190]. Another small but critically important patient population includes patients with the various genetic

forms of chronic mucocutaneous candidiasis such as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) patients [191]. Frequent decreased susceptibility of *C. albicans* to fluconazole is a common complication of prolonged fluconazole use in this population.

Clinical management of refractory OPC requires evaluation and determination of etiological mechanisms responsible for clinical resistance, including CD4 count, compliance with HAART therapy, previous OPC, and exposure to azoles, usually fluconazole [192]. Finally, clinical resistance implies failure to respond despite adequate delivery of a tolerable therapeutic concentration of the drug. Once in vitro resistance is suspected, cultures are obtained and susceptibility determined of the responsible organisms. Most commonly, *C. albicans* is present, sometimes together with a second species usually *C. glabrata*. While awaiting microbiology and susceptibility results, treatment is initiated. Therapeutic strategies are listed in Box 66.3. Initial options include progressive increasing doses of oral fluconazole from 100 to 400 mg/day, including fluconazole suspension [193] or swish-and-swallow amphotericin B suspension (100 mg/mL, taken as 1 mL qid) [194]. Although cross-resistance with other triazoles is common, in the event of retained itraconazole sensitivity, itraconazole suspension (10 mg/mL, taken as 10 mL bid) is often effective, although usually on a temporary basis only [195]. However, the most important advance in therapy of fluconazole-refractory OPC is oral posaconazole. Although initially available only as an oral suspension, it is now prescribed as posaconazole tablet 400 mg bid for 14 days. Given its safety profile, posaconazole is used preferentially to oral voriconazole.

Box 66.3. Therapy of Fluconazole-Refractory Oropharyngeal (OPC) and Esophageal Candidiasis (EC)

OPC

- High doses of fluconazole tablets
- Fluconazole suspension
- Itraconazole capsules/suspension
- Amphotericin B oral suspension
- IV amphotericin B/lipid formulation
- Posaconazole oral/IV
- Voriconazole oral/IV
- IV echinocandin
- Immunomodulation
  - G-CSF
  - GM-CSF
  - α-Interferon

EC

- IV echinocandin
- Fluconazole
- IV lipid formulation of amphotericin B
- IV voriconazole*

*If susceptible in vitro

Parenteral antifungals have become the last resort employing intravenous amphotericin B, echinocandin, or voriconazole [196]. All these options may successfully control and eradicate acute symptomatic infection; however, unless immune reconstitution follows, relapse is inevitable. Potentially, the aforementioned parenteral antifungals could be given on an intermittent maintenance basis; however, maintenance suppressive therapy with oral posaconazole 400 mg per day is effective [197].

While HAART therapy offers a definite solution in AIDS patients, the same cannot be said from CMC patients with progressive azole resistance starting with fluconazole and extending sequentially to itraconazole and then voriconazole with either *C. albicans* or *C. glabrata*. Intermittent parenteral echinocandins or lipid formulation of amphotericin B will be necessary, although the use of oral posaconazole is preferred [198].

7.2 Refractory Esophageal Candidiasis (Box 66.3)

As for refractory OPC, clinically resistant EC is mainly seen in untreated AIDS patients with advanced immunodeficiency, with a history of sporadic previous treatment with fluconazole. Refractory, especially chronic, EC is associated with a profound impact on general health leading to weight loss, malnutrition, and overall reduced general health status. Oral cultures usually reveal the *Candida* species responsible for esophageal disease, recognizing that more than one resistant species may coexist. Most cases of fluconazole-resistant EC are similarly resistant to itraconazole [199]. In a minority of patients still capable of swallowing, oral posaconazole is still a therapeutic possibility. If swallowing is not possible, therapeutic options now include amphotericin B deoxycholate or lipid formulations used parenterally in hospitalized patients, and while widely recognized as efficacious, there are little published data documenting efficacy. Cost with the use of lipid formulations and toxicity associated with conventional AmB remain issues. Regardless of which formulation is chosen, low-dose regimens frequently fail in patients with azole-resistant *C. albicans* and/or *C. glabrata*. Response to IV therapy is frequently slow, and >0.8 mg/kg AmB or 5 mg/kg of lipid AmB should be used.

Fortunately, the drugs of choice are IV echinocandins. Studies confirm similar efficacy with daily IV caspofungins, anidulafungin, and micafungin. Accordingly, caspofungin was found to have ~70 % efficacy rate in treating patients with fluconazole-refractory EC [6, 198, 200–202]. No cross-resistance exists between azoles and echinocandins. Similar efficacy for EC has been observed with parenteral voriconazole, also achieving ~70 % response rates but with little experience published with fluconazole-resistant species [203]. Table 66.1 shows the impact of fluconazole-resistant

C. albicans on susceptibility to voriconazole; hence, higher doses of voriconazole may well be indicated [87, 144]. The recent availability of parenteral posaconazole increases therapeutic options, and oral posaconazole is recommended as de-escalation therapy to complete parenteral echinocandin treatment.

Regardless of the parenteral regimen selected, the dominant issue remains the maintenance antifungal prophylaxis in these severely immunocompromised individuals. It cannot be emphasized sufficiently that the key to preventing further recurrences or inevitable relapses of refractory EC lies with successful initiation of HAART therapy. Noteworthy several studies indicated that relapse rates of EC are higher following initially successful echinocandin treatment [204]. Until HAART therapy reverses susceptibility, maintenance prophylaxis is best afforded with oral posaconazole.

7.3 Refractory *Candida* Vaginitis (VVC)

Two forms of vulvovaginal candidiasis (VVC) exist. In the first place, an individual episode of symptomatic vaginitis may not respond to conventional topical or oral antifungal therapy. The other form of refractory disease is found in a larger population of women with frequently recurring episodes of relapsing symptomatic vaginitis although each individual episode of VVC responds to conventional therapy (RVVC).

Failure to achieve clinical improvement and symptom resolution, i.e., azole-resistant vaginal *C. albicans*, is still uncommon but has increasingly been reported in both HIV-positive and HIV-negative women [205]. It is actually remarkable that resistance is not more frequent given the widespread use of low-dosage fluconazole as single-dose therapy or once-weekly maintenance prophylaxis for RVVC. Nevertheless, any patient with acute *Candida* vaginitis, failing to improve with a standard regimen of oral or topical azoles, with persistent symptoms, positive microscopy, and culture, should be treated with topical vaginal boric acid 600 mg daily for 14 days. At the same time, the *C. albicans* isolate should be sent for azole susceptibility testing. The same cannot be said for acute *C. glabrata* vaginitis which responds to azole agents with a 50 % rate only [206]. Acute *C. glabrata* vaginitis should be treated with topical boric acid 600 mg suppositories daily for 11–21 days with an anticipated clinical and mycological response rate of ~70 % [179]. Higher cure rates (>90 %) can be obtained with topical 17 % flucytosine intravaginal cream, 5 g nightly for 14 days, although the cream must be compounded and is not widely available and hence is expensive [206, 207]. High cure rates also follow daily intravaginal amphotericin B 50 mg suppository for 14 days or in combination with topical flucytosine [208].

Acute vaginitis due to *C. krusei*, although rare, will not surprisingly fail to respond to oral fluconazole, due to innate or primary resistance [209]. Occasionally, patients may respond to oral itraconazole or topical miconazole or clotrimazole prescribed for 14 days. *C. krusei* is also resistant to flucytosine, and hence vaginitis due to this species is often extremely difficult to control.

It should be emphasized that refractory acute vaginitis is extremely rare, although busy practitioners might not agree. This is because of incorrect diagnosis on the part of practitioners who treat vaginitis on an empiric basis, invariably failing to measure vaginal pH, perform microscopy, and obtain a vaginal culture. Several studies have confirmed the poor diagnostic acumen of practitioners. Self-diagnosis by women is no better. Other species of *Candida* can cause vaginitis, but tend to rapidly respond to azole therapy.

Much more common and affecting millions of women, in their childbearing decades worldwide, is recurrent vulvovaginal candidiasis (RVVC) thought to affect 6–8 million women in the USA. Under these circumstances recurring episodes of vaginitis respond appropriately to antifungal therapy regardless of route, only for symptoms and signs to recur within a month or two but rarely monthly [210]. RVVC is mostly caused by azole-sensitive *C. albicans* (>90 %) and less commonly by *C. glabrata* (5 %). RVVC is rarely a manifestation of drug resistance but of host factors that predispose to genital tract yeast colonization and host immune response hyperreactivity to *Candida* antigens [210]. RVVC is best controlled by once-weekly fluconazole maintenance prophylaxis administered for 6 or more months [128], although other forms of suppressive azole therapy are effective but less convenient [211, 212]. Boric acid has also been used effectively [213].

The management of azole-refractory vaginitis due to in vitro confirmed fluconazole-resistant *C. albicans* is initially managed with daily vaginal boric acid for 2 weeks, while in vitro susceptibility tests become available. Acute, nonrecurrent vaginitis may require no additional therapy; however, women suffering from RVVC will of necessity require a maintenance antifungal regimen. Possible alternatives to weekly fluconazole are daily ketoconazole or itraconazole 100–200 mg, provided that susceptibility is confirmed in vitro. As per standard protocols, the maintenance daily regimens are continued for at least 6 months. In the event of frequently reported azole cross-resistance, no oral azoles are likely reasonable safe alternative agents. In this scenario, long-term maintenance therapy can be achieved with topical boric acid or nystatin for the same long-term duration, but little published data are available. Similarly, daily combination therapy with boric acid and nystatin is effective for symptomatic recurrent VVC due to *C. glabrata* although such cases are rare.

Box 66.4. First-Line Antifungal Drug Therapy of Candidemia (Parenteral)

1. Amphotericin B (conventional deoxycholate)
2. Lipid formulation AmB
3. Fluconazole (400 mg/day)
4. Fluconazole (800 mg/day)
5. Itraconazole
6. Voriconazole
7. Caspofungin
8. Amphotericin B + flucytosine
9. Amphotericin B + fluconazole

7.4 Refractory Candidemia and Disseminated Candidiasis

The incidence of bloodstream infections (BSIs) due to *Candida* spp. has increased worldwide, with accompanying significant mortality. Fortunately, in parallel with this increase has been an increase in the therapeutic armamentarium for candidemia (Box 66.4). The purpose of this chapter is not to review management of candidemia (see reviews [96, 114, 214]). Drug resistance is monitored by a variety of study organizations in multiple countries. Perhaps the most comprehensive antifungal susceptibility monitoring organization is the SENTRY system receiving in excess of 2000 bloodstream *Candida* isolates annually from all over the world [63]. Compiled data are shown in Table 66.3. Nevertheless, given the proportional and occasionally found absolute increase in cases of invasive candidiasis and candidemia due to non-*albicans Candida* species especially *C. glabrata*, together with the availability of safe and in the past predictable effective echinocandins, guidelines from national and international infectious disease societies have recently been issued which acknowledge the reduced azole susceptibility of non-*albicans Candida* species. Hence, until information of the identity of the *Candida* species responsible for the bloodstream infection is available, echinocandins are considered drugs of first choice to be prescribed [96].

Table 66.3 Species distribution of *Candida* from cases of invasive candidiasis[a]

	% of total no. of cases[b]					
Species	1997–1998	1999	2000	2001	2002	2003
C. albicans	73.3	69.8	68.1	65.4	61.4	62.3
C. glabrata	11.0	9.7	9.5	11.1	10.7	12.0
C. tropicalis	4.6	5.3	7.2	7.5	7.4	7.5
C. parapsilosis	4.2	4.9	5.6	6.9	6.6	7.3
C. krusei	1.7	2.2	3.2	2.5	2.6	2.7
C. guilliermondii	0.5	0.8	0.8	0.7	1.0	0.8
C. lusitaniae	0.5	0.5	0.5	0.6	0.5	0.6
C. kefyr	0.2	0.4	0.5	0.4	0.4	0.5
C. rugosa	0.03	0.03	0.2	0.7	0.6	0.4
C. famata	0.08	0.2	0.5	0.2	0.4	0.3
C. inconspicua			0.08	0.1	0.2	0.3
C. norvegensis			0.08	0.1	0.07	0.1
C. dubliniensis			0.001	0.08	0.1	0.05
C. lipolytica			0.06	0.06	0.06	0.08
C. zeylanoides			0.03	0.08	0.02	0.04
C. pelliculosa				0.06	0.05	0.04
Candida spp.[c]	3.9	6.0	3.7	3.3	7.9	4.9
Total no. of cases	22,533	20,998	11,698	21,804	24,680	33,002

[a]Data compiled from the ARTEMIS DISK Surveillance Program, 1997–2003
[b]Includes all specimen types and all hospitals from a total of 127 different institutions in 39 countries
[c]*Candida* species not otherwise identified

7.4.1 *C. albicans*

Despite the widespread use of fluconazole over the last 15 years, fluconazole resistance in *C. albicans* blood isolates remains below 5 %, with no evidence of a progressive increased resistance with time or associated with a specific geographic area [60]. It is not fear of an azole-resistant strain of *C. albicans* that drives principles of antifungal drug selection. Candidemia due to drug-resistant *C. albicans* is rare, but has been rarely reported in patients with hematologic malignancy [100]. However, *C. albicans* is no longer the most prevalent *Candida* species responsible for BSI, and rarely is drug resistance a management issue. Should an azole-resistant *C. albicans* isolate be responsible for the candidemia, the clinical manifestations include persistent candidemia on fluconazole therapy, relapsing candidemia or possibly increased mortality, and finally breakthrough candidemia. In the last decade, results of at least five randomized prospective controlled studies have been published involving fluconazole and other antifungal drugs [215–220]. Attempts have been made to correlate clinical outcome with in vitro MICs. In none of these studies has *C. albicans* antifungal resistance, specifically fluconazole resistance emerged as a cause of drug failure [216, 217]. The lack of fluconazole resistance in *C. albicans* BSI isolates after all these years remains reassuring, but the altered epidemiology is less so. In contrast to other studies, correlation between in vitro susceptibility and response to fluconazole therapy has been demonstrated, but rarely is persistent fungemia due to azole-resistant *C. albicans* but rather non-*albicans Candida* species [221].

7.4.2 *C. glabrata*

As evident in Table 66.2, candidemia due to *C. glabrata* has increased especially in North America and Europe. Fluconazole resistance in bloodstream *C. glabrata* isolates is evident in 7–10 % of strains, with an addition of 27–30 % of isolates considered S-DD indicating reduced fluconazole susceptibility of *C. glabrata* isolates. Accordingly, only 50–70 % of *C. glabrata* bloodstream isolates are highly susceptible to fluconazole. Several studies involving *C. glabrata* have shown a similar susceptibility pattern [63, 64].

Documented failure or suboptimal response to fluconazole and other antifungals has been forthcoming in some studies and is impressively present in others [221]. When failure was always apparent, this may simply reflect small numbers of patients with *C. glabrata* fungemia, i.e., some published studies have lacked the power to show any differences in outcome by *Candida* species.

Supporting the in vitro data are numerous case reports of fluconazole failure to eradicate *C. glabrata* fungemia subsequently responsive to parenteral polyene or echinocandin therapy as well as retrospective analysis of patients with persistent candidemia [151, 221]. Accordingly, most experts would recommend avoiding any azoles, including voriconazole, initially in patients with candidemia caused by *C. glabrata* and initiate therapy with an echinocandin. Until the *Candida* isolate (species) is identified and species identity is becoming more and more rapidly established, then given the increased likelihood of *C. glabrata* and other reduced fluconazole susceptibilities, selection should include the possibility and commence with an echinocandin. In candidemia patients doing well on azoles, continued therapy with the azole would be perfectly reasonable.

8 Adjuvant Therapy for Resistant Candidiasis

The use of immune and nonimmune adjuvants to treat refractory candidiasis is almost exclusively seen in patients with AIDS or chronic mucocutaneous candidiasis (CMC). Even with the latest generation of azoles (voriconazole, posaconazole) and polyene and echinocandin use, refractory mucosal disease is still reported due to resistant *C. albicans*, *C. glabrata*, and rarely other *Candida* species. There have been anecdotal successes reported with immunostimulators mainly recombinant human granulocyte-macrophage colony-stimulating factor (rhu GM-CSF) [222, 223]. Also, interferon gamma has occasionally been given [196]. Unfortunately, investigators tend to publish only successful therapeutic endeavors and failures are more frequent [224, 225]. Even so, long-term use and success of these growth factors have not been forthcoming especially associated with CMC. The use of these agents, given these expenses, requires the performance of randomized controlled studies which are unlikely given the current infrequency of these refractory cases. The value of GM-CSF in invasive candidiasis has not been demonstrated but may have a role in persistently neutropenic patients. Monoclonal antibodies were shown to prevent disseminated candidiasis in a mouse model and have been the bases for vaccine development. Likewise, the administration of anti-*Candida* heat shock antibodies may have an adjuvant role together with antifungals for resistant or refractory candidemia.

9 Prevention of Antifungal Resistance in *Candida* Species

In general, standard principles of infection control that apply to all microorganisms and particularly nosocomial infections should be applied to prevent antifungal resistance.

Avoidance of prophylactic or suppressive therapy and a preference for repeated short course of azoles for OPC in the late stages of AIDS are an attractive but unproved measure for delaying the appearance of azole resistance. In a study conducted in patients with recurrent OPC and AIDS, episodic fluconazole therapy was compared to continuous fluconazole therapy aimed at evaluating likelihood of inducing fluconazole resistance and refractory oropharyngeal candidiasis [98]. The study failed to show a difference in the two arms with regard to selection or induction of azole resistance. This somewhat disappointing result may reflect the fact that the study was conducted during the HAART era with relatively few individuals presenting with refractory mucosal disease, with advanced immunodeficiency and unavailability of HAART therapy. The study outcome is in sharp contrast to clinical experience obtained in the pre-HAART era.

It goes without saying that all unnecessary use of azoles should be avoided, whether as prophylaxis or therapy. Many clinicians prescribe a lower than recommended prophylactic dose of oral fluconazole in neutropenic patients, i.e., 100 mg vs. 400 mg daily. To date, no evidence has emerged of increased fluconazole resistance as a specific consequence of this reduced daily dose. Nevertheless, many experts advise against the use of azole prophylaxis in neutropenia of short duration. Paterson suggested that combining oral amphotericin B with azoles may prevent the emergence of resistant *Candida* species in neutropenic patients; however, oral amphotericin B is poorly tolerated and noncompliance is common [226].

Studies have indicated that most *Candida* species are carried and readily transferred manually by nursing physicians and other medical personnel [227]. Accordingly, adherence to strict handwashing principles applies equally to *Candida* and specifically the transfer of resistant strains of *C. albicans* and other *Candida* species [228, 229]. In particular, *C. parapsilosis* is frequently isolated from the hands in contrast to *C. glabrata* which appears to be endogenously acquired from GIT carriage only. Isolation of patients with resistant strains of *Candida* is not indicated in this era of universal precautions. Perhaps, the most controversial is the use of antifungal prophylaxis in selected high-risk patients in intensive care units. Several studies suggest limited benefit and only in selected high-risk ICU patients [230, 231, 232].

10 Conclusion and Perspective

During the last two decades, enormous strides have been made in understanding the subcellular, molecular, and genetic basis of antifungal resistance. All in all, clinically refractory candidiasis is uncommon. The explosion in clinically resistant cases of OPC and EC early in the AIDS epidemic has not stood the test of time with the arrival of antiretroviral therapy. Of course, clinically resistant cases still occur and remain a therapeutic challenge, but the majority of cases of mucosal disease are caused by azole-sensitive *Candida albicans*. There has been an increase in non-*albicans Candida* species causing invasive candidiasis. Much, but not all, evidence points to widespread prophylactic, empirical, and therapeutic use of fluconazole. Nevertheless, blood isolates of *C. albicans* remain remarkably and predictably susceptible to fluconazole and other azoles, and this is a worldwide experience. There is no doubt that certain *Candida* species are less susceptible and/or resistant to fluconazole and show cross-resistance to all azoles. This species-specific (*C. krusei*, *C. glabrata*) azole resistance has a major influence in antifungal drug selection. These two species not only expose vulnerability of the azole class but require higher doses of polyenes. As such, fungal susceptibility tests in the past were rarely available and infrequently and selectively used. This however has changed with increased availability. The newer generations of azoles are often active against non-*albicans Candida* species (*C. glabrata* and *C. krusei*) and as such offer early confident broad-spectrum therapy. Moreover, they are frequently active against fluconazole-resistant *C. albicans*. The echinocandins have further eased the concern of azole resistance in candidiasis, but time has yet to determine the potential for echinocandin-acquired resistance in candidiasis.

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