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Azole-resistant Aspergillus and Echinocandin-resistant Candida – What are the treatment options?

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Abstract

Purpose of Review—This review summarizes current treatment options for echinocandin-resistant *Candida* spp. (ERC) and azole-resistant *Aspergillus fumigatus* (ARAF), emphasizing recent *in vitro/in vivo* data, clinical reports, and consensus statements.

Recent Findings—Advances in ERC and ARAF treatment are limited to specific antifungal combinations and dose optimization but remain reliant on amphotericin products. Although novel antifungals may provide breakthroughs in the treatment of resistant fungi, these agents are not yet available. Early identification and appropriate treatment remain a paramount, albeit elusive, task.

Summary—When either ERC or ARAF are suspected or proven, amphotericin products remain the cornerstone of initial therapy. For ERC, azoles are de-escalation options for susceptible isolates in stable patients to avoid amphotericin toxicities. Although combination echinocandin with high-dose salvage posaconazole or isavuconazole may be attempted in ARAF, it requires careful consideration following patient stabilization. Future research defining optimal therapies and early identification of ERC and ARAF is of extreme importance.

Keywords

Antifungal; Resistance; Echinocandin-Resistant Candida; Azole-Resistant Aspergillus; Invasive Fungal Infection; Amphotericin; Posaconazole; Isavuconazole; Voriconazole; Caspofungin; Micafungin; Anidulafungin

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Conflict of Interest

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Introduction

The incidence of invasive fungal infections due to *Candida* spp. and *Aspergillus* spp. continues to increase as the population of immunocompromised patients increases [1, 2, 3, 4••, 5]. Available treatment options remain limited due to the lack of new antifungal class approvals. Mainstay options for the treatment of invasive fungal infections include polyenes, azoles, echinocandins, and flucytosine (5-FC). Although these agents are effective in treating the majority of fungal infections, the lack of advancement in alternative therapies poses a problem when fungal pathogens acquire resistance to preferred agents, which leads to extensive patient morbidity and mortality [2, 3, 4••].

Emerging resistance is anticipated to intensify in the years to come due to a variety of driving factors. Some *Candida* spp. can breed inherent resistance by changes in the DNA sequencing. Acquired resistance, noted in both *Candida* spp. and *Aspergillus* spp., occurs following drug exposure giving the potential for the pathogen to adapt over time [5]. Recent concerning trends of resistance to generally preferred antifungal agents are on the rise, including but not limited to, echinocandin-resistant *Candida* spp. (ERC) and azole-resistant *Aspergillus fumigatus* (ARAF) [5].

Much of the literature available describes resistance patterns of ERC and ARAF but lacks robust evidence to support treatment recommendations for these infections. This review will explore the mechanisms of resistance for ERC and ARAF and provide a treatment approaches to combat these invasive infections.

Echinocandin-Resistant Candida

Background/Epidemiology

Candida infections account for the majority of the fungal infections in immunocompromised patients and are recognized as the 4th leading cause of hospital-acquired bloodstream infections in the United States [1, 2, 6, 7]. Among these invasive Candida infections, > 90% are caused by the most common pathogens of *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei*. Each species may vary by regional ecology and produce unique virulence factors and resistance patterns, especially to echinocandins (Table 1) [7, 8]. Currently, the IDSA guidelines recommend echinocandins as preferred first-line agents for patients with invasive candidiasis, with recommendations to step down to azole antifungals in patients who are clinically stable within 5–7 days or who have azole-susceptible isolates. Echinocandins are also recommended as options in patients with prior azole exposures [7].

Echinocandins act as fungicidal agents to yeast by inhibiting the biosynthesis of glucan polymers specifically at 1,3-β-d-glucan synthase, an enzyme not found in mammalian cells [2, 5, 8]. The enzyme consists of two subunits: Rho1p, which is a guanosine triphosphate binding protein, and FKS, which is the catalytic component further classified into FKS1, FKS2, and FKS3 [5, 9]. Table 1 outlines the echinocandin resistance patterns of common *Candida* spp. adopted from 20 years of SENTRY data evaluating 15,308 isolates worldwide [10•]. Although echinocandin-resistant *Candida* (ERC) prevalence is low overall at this time, *C. glabrata*, *C. krusei, and C. auris* have the highest probability of resistance [5, 10•].

Comparatively, *C. parapsilosis* and *C. guilliermondii* exhibit the highest intrinsic echinocandin MICs [1, 10•].

Notably, the United States Centers for Disease Control and Prevention (CDC) and Public Health England (PHE) have issued recent warnings about the emergence of *C. auris*, a multidrug-resistant *Candida* spp. It is imperative to quickly identify *C. auris* in the clinical setting due to its vast ability to cause outbreaks; however, it is often misidentified leading to inappropriate management [8, 11, 12]. Rapid genotyping has been recommended in patients who are infected or colonized with C. auris to detect for ERC genes to help guide therapy [13]. Although *C. auris* is usually susceptible to echinocandins *in vitro*, there have been concerning cases of high-level echinocandin resistance in the clinical setting with resistance rates of up to 10% based on European Committee on Antimicrobial Susceptibility Testing (EUCAST)/Clinical Laboratory and Standards Institute (CLSI) methodology (Table 1) [14, 15••].

Mechanisms of Resistance

Two main mechanisms have been reported among *Candida* spp.: intrinsic point mutations and adaptive stress response [1]. The main resistance mechanism described in the literature relates to point mutations, specifically in *FKS1* or *FKS2* genes on highly selective “hot spot” regions, reducing susceptibility to echinocandins by up to 3000-fold [1, 2, 16, 17, 18]. However, it is apparent that not all FKS mutations are predictive of the degree of echinocandin resistance [10•, 18, 19]. As described above, these mutations can occur intrinsically in some *Candida* spp. (e.g., *C. parapsilosis* increasing MIC) or adaptively, which often occurs after being exposed to echinocandins over time.

Even *C. albicans*, normally susceptible to many antifungals, can develop resistance to echinocandins when exposed over a long period of time. *C. albicans* clinical isolates exposed to ~ 6 weeks of caspofungin and anidulafungin developed echinocandin resistance. ERC was conferred by a mutation in *FKS1* gene leading to amino acid change S645P. This study also noted that the acquired resistance may change fitness and virulence of the organism in an insect model [20].

A series of clinical isolates of *C. kefyr* from patients with hematologic malignancies that displayed reduced echinocandin susceptibilities were assessed for mechanisms of resistance. The isolates did not display genetic relatedness but did highlight that *FKS1* mutations may result in differential echinocandin sensitivities. Specifically, even though the S645P mutation showed resistance to anidulafungin, micafungin, and caspofungin, caspofungin retained some activity (MIC = 2 mg/L) compared to anidulafungin or micafungin (each MIC > 8 mg/L) [21].

Although the majority of resistance is associated with *FKS* mutations, not all ERC is directly linked to *FKS* mutations in the hot spot region. A study screened 1380 *C. glabrata* clinical isolates and assessed resistance mechanisms of 77 echinocandin-resistant isolates. Investigators were able to identify *FKS* mutations in 51 isolates. Some *C. glabrata* isolates considered ERC by MIC did not have *FKS* mutations identified. Investigators observed that micafungin MIC values in candidemia were the strongest predictor of isolates possessing

FKS mutations, with 91% expressing MICs in the resistant range. Of these 1380 *C. glabrata* strains, 10.3% were fluconazole resistant [17]. Alarmingly, 36.2% of echinocandin-resistant *C. glabrata* strains were resistant to fluconazole. This multidrug resistance relationship has been noted in other studies as well [6, 22, 23]. The cause of the co-resistance in isolates is unknown, due to the azoles and echinocandins having differences in their mechanisms of action and resistance patterns. One proposed mechanism has been reported to be a disrupted *MSH2* mismatch repair gene that can potentially produce multidrug-resistant strains, although still often susceptible to amphotericin (AMB) [22]. Alternatively, this co-resistance may be a result of decades of selective pressure.

Current Treatment Options

Suspicion of ERC should arise if clinical worsening or breakthrough Candida infection occurs despite appropriate source control and dosing of the echinocandin or if an *FKS* mutation is detected in the isolate. Risk factors associated with ERC include hospitalization in the previous 90 days, receipt of total parenteral nutrition in the previous 14 days, a previous episode of candidemia, prior echinocandin exposure, and known colonization with fluconazole-resistant *Candida* spp. [9]. These factors, including local susceptibility patterns and species-specific risk (i.e., *C. glabrata* and *C. auris* more likely than *C. albicans*), should be considered in the level of suspicion for ERC. Beyond standard phenotypic susceptibility testing, novel methods (e.g., molecular detection of *FKS* mutations) to identify resistance may be validated in the future. When ERC is suspected, a change of therapy while awaiting culture and susceptibility results may provide earlier optimal antifungal therapy (Table 2) [8, 10•].

Candidemia

Suspected or Known Resistance—When treating candidemia that is either suspected or known to have echinocandin resistance (prior echinocandin exposure, inability to clear blood cultures, clinically worsening), initiation of lipid formulation amphotericin-B (LF-AMB) at a dose of 3–5 mg/kg daily is recommended [7]. LF-AMB is currently a mainstay initial option for ERC since it binds ergosterol, is rapidly fungicidal, and is effective for invasive Candida infections. AMB products remain the only monotherapy option for ERC resistant to all echinocandins and azoles (Table 2). Liposomal amphotericin (LAMB) has been shown to have similar effects as compared to echinocandins on the reduction of cell viability in the biofilms and in antibiotic lock therapies of *C. albicans* strains [24]. A case series described poor outcomes in patients with ERC candidemia with mixed azole susceptibility patterns and full susceptibility to AMB. Authors concluded that delay in identification of resistance led to a delay in appropriate therapy contributing to death [25]. Although LF-AMB is recommended as the backbone of therapy for confirmed ERC, it has an extensive toxicity profile and may be intolerable for many patients. Therefore, alternative monotherapy or combination therapies may be considered as options in both early and late treatment of ERC.

Mixed Echinocandin Susceptibility—Some isolates of *Candida* may be able to be treated with an echinocandin depending on which specific mutations are expressed since not

all echinocandin resistance displays a class effect [8, 10•]. It is reasonable to switch echinocandin monotherapy in patients with a known susceptibility to another echinocandin, especially with concomitant azole resistance when LF-AMB cannot be tolerated. However, it is important to note that many isolates with *FKS* mutations are resistant to two or more echinocandins [10•].

Azole Susceptible—Not all ERC strains are also resistant to azoles, leaving azoles as an option for candidemia if the isolate is susceptible. Using fluconazole in patients who have ERC poses some concern since it is suspected that many isolates may be intrinsically resistant (*C. krusei*), display lesser susceptibility (*C. glabrata*), or aforementioned fluconazole-echinocandin co-resistance. Voriconazole, posaconazole, and isavuconazole may retain activity against many ERC isolates but susceptibility should be confirmed prior to step-down therapy, especially for *C. glabrata*. Voriconazole has been compared to AMB for 3 to 7 days followed by fluconazole and has been shown to be equally as effective in clearing bloodstream infections in non-ERC isolates [26]. Although susceptibility to voriconazole was not noted, successful use of voriconazole was reported in a retrospective analysis of echinocandin-resistant C. glabrata infections over a 10-year period [27].

Combination Therapies—In vitro activity of echinocandins combined with other antifungals against echinocandin-resistant and fluconazole-echinocandin-resistant isolates of *C. glabrata* has been tested via checkerboard method to evaluate the fractional inhibitory concentration index of antifungal combinations. Synergism was noted with the combination of caspofungin plus posaconazole, anidulafungin plus posaconazole, and anidulafungin plus voriconazole in 85%, 70%, and 70% of the multidrug-resistant isolates, respectively. Though all studied isolates were sensitive to AMB by MIC, a lack of synergy was noted with the combination of an echinocandin plus AMB [28••]. Although these results are promising for synergistic effects and provide a future direction for research, these combinations have not been well studied in humans. Though we caution a broad application to clinical use, the aforementioned echinocandin-azole combinations may provide a therapeutic option when LF-AMB cannot be used. Successful use of posaconazole plus LF-AMB has also been noted in a retrospective analysis of *C. glabrata* infections over a period of 10 years [27].

Other Sites of Infection

Guidelines recommend treating patients with native and prosthetic valve *Candida* endocarditis or implantable cardiac device candidiasis with LF-AMB with or without 5-FC as an initial therapy option, which remains appropriate for ERC. This regimen may be eventually stepped down to an azole in clinically stable patients with azole-susceptible isolates that have cleared blood cultures. Utilizing an azole, when appropriate criteria are met, allows for an oral option to prolong therapy or provide suppressive therapy as may be required in cases of prosthetic valve *Candida* endocarditis [7]. Although this step-down approach may remain viable when azole susceptibility is confirmed in ERC, specific consideration to the azole choice and *Candida* spp. is required. Differential susceptibility can occur in biofilms as highlighted by a case describing a poor outcome of a patient with ERC endocarditis. ERC was not confirmed until after failing micafungin plus fluconazole and surgical resection allowed for a direct valve culture. This case highlights the potential for

ERC subtypes to exist in biofilms, the importance of isolate susceptibility testing and source control [29].

In patients with ERC osteomyelitis, septic arthritis, and suppurative thrombophlebitis, source control should be evaluated and therapy with fluconazole or an alternative azole considered (if susceptible). LF-AMB remains a viable option for ERC and may be preferred as initial therapy. Step-down or suppressive therapy with an azole should be considered after appropriate response and proven susceptibility [7]. Case reports have described ERC septic arthritis and osteomyelitis successfully treated with LF-AMB for 4 weeks followed by voriconazole for 6 months [30]. Based on this case report and guideline recommendations, it is reasonable to do an extended course of LF-AMB followed by a long suppressive course of voriconazole for treatment of septic arthritis and osteomyelitis caused by ERC susceptible to voriconazole.

Candida auris

Especially concerning is the emergence of *C. auris*, a rare but commonly fluconazole resistant, frequently itraconazole, voriconazole, isavuconazole resistant, and variable AMB susceptible pathogen. Echinocandins remain the empiric treatment of choice; however, reports of echinocandin resistance and non-responsiveness are rising. Furthermore, the site of infection may reduce the activity of an echinocandin. Reports of persistent echinocandin-resistant *C. auris* urinary tract infections are emerging [11, 14]. Although it is rare for urinary tract infections to convert to candidemia, treatment options are limited if this were to occur. Echinocandins do not penetrate the urine well and thus 5-FC has been recommended as combination therapy for *C. auris* urinary tract infections due to its high bladder penetration [31]. AMB plus 5- FC has also been suggested but no clinical outcomes were reported [32]. Combination therapy such as conventional AMB with or without 5-FC may be used in urinary tract azole-resistant ERC infections that require antifungal therapy [7, 14, 32].

Echinocandin-resistant *C. auris* leaves the clinician with minimal options. LF-AMB remains the alternative therapy although 30% of isolates in the United States may be resistant [31, 33]. 5-FC may have variable activity and should be used in combination therapy only. Other reported combinations, such as micafungin plus voriconazole, may provide synergy though there is minimal clinical reports of successfully using this combination in echinocandin-resistant *C. auris* [34]. Pan-resistant C. auris has been reported following treatment [31]. In addition to aggressive source control for echinocandin- resistant *C. auris*, LF-AMB or a combination of active antifungals (micafungin plus voriconazole) may be reasonable.

Azole-Resistant Aspergillosis

Background/Epidemiology

Aspergillus spp. are the most common cause of invasive mold infections worldwide [35•]. Cases of azole-resistant *Aspergillus fumigatus* (ARAF) have been reported since the 1990s and have steadily increased over the past two decades [36]. Large epidemiologic studies report overall ARAF rates ranging from 3.2 to 27.6% of isolates, with higher resistance rates

in Europe [37, 38]. The United States remains largely unaffected by resistance [39], but clinical occurrences have been documented since the mid-2000s [40]. A recent comprehensive review of ARAF epidemiology has been published elsewhere [35•, 40].

Two primary risks of developing ARAF include environmental exposure to azole-based fungicides and long-term use of azole antifungal therapy [40, 41]. Azole fungicides are commonly used in agriculture internationally and may pose a public health treat by introducing resistance mechanisms in *Aspergillus* spp. [42]. Previous studies have reported that 64% of patients with invasive ARAF never received an azole, suggesting an important role of environmental exposures [42, 43]. Additionally, clinical use of long-term or suppressive azole therapy exerts pressure on the organism which may lead to the development of resistance within a given patient. Azoles (voriconazole and isavuconazole) are a preferred mainstay of therapy for most invasive aspergillosis due to proven efficacy, tolerability, and because they offer an option for oral therapy [44, 45]. Therefore, the management of ARAF is especially concerning and requires thoughtful regimen selection based on patient evaluation.

Mechanism of Resistance

Triazoles work by inhibiting the fungal cytochrome lanosterol 14-α-demethylase, which is encoded by the *CYP51A* gene. 14-α-demethylase inhibition leads to an accumulation of 14-α-methyl sterols, a lack of functional ergosterol, and subsequently destabilizes the fungal cell membrane. The most common mechanisms of ARAF are mediated by mutations in the *CYP51A* gene (Table 1).

Oftentimes, mutations in the *CYP51A* gene are seen in combination. The most common mutation combination is the presence of a 34-base pair tandem repeat (TR34) in the promoter region of the *CYP51A* gene, which is seen with a substitution of leucine 98 to histidine (L98H), denoted as TR34/L98H. The TR34/L98H mutation leads to an overexpression of *CYP51A* [35•, 46]. Another frequent mutation combination is a 46-base pair tandem repeat in the *CYP51A* promoter region (TR46) with a substitution at tyrosine 121 for phenylalanine (Y121) and threonine 289 for alanine (T289A), referred to as TR46/Y121/ T289A [36]. The TR34/L98H and TR46/Y121/T289A mutations confer pan-azole resistance and yield difficult to treat infections. Point mutations, like substitutions at M220 and G448S, also lead to resistance and can propagate resistance to one or more azole antifungal [47]. For example, the G54 and G138 point mutation confer resistance primarily to itraconazole and posaconazole, while point mutations at G448 lead to voriconazole resistance, with possible resistance to itraconazole and posaconazole [35•, 48, 49, 50] (Table 1).

Non-CYP-mediated mechanisms of resistance include two efflux pumps, ATP-binding cassette (ABC) and major facilitator superfamily (MFS) transporters, and *hmg1* and *erg6* enzyme dysregulation [46, 51]. Mechanistically, overexpression of transmembrane pumps may not allow for appropriate intracellular drug concentrations to be obtained [52]. Comparatively, mutations in hmg1 and erg6 enzymes increase ergosterol synthesis and ultimately lead to a need for an increased amount of azole in order to inhibit ergosterol synthesis [51] (Table 1).

Identification

One barrier to successful treatment of ARAF is the irregularity of positive culture data for many clinical infections. ARAF is often identified late in the treatment course, if at all, and amplifies a poor prognosis as mortality rates of 88% in patients are documented [35•]. Means of identification are limited to EUCAST and CLSI microdilution, azole-based agar plates, and commercially available (but not clinically validated) polymerase chain reaction (PCR) tests. Some experts recommend following biomarkers (e.g., galactomannan) to infer persistence of infection in patients with documented aspergillosis [40], or assessing isolates for resistance and drug susceptibility in areas of known resistance [4••]. In most situations, the above information will not be immediately available to the clinician. This leads the clinician to work off of assumptions of geographic resistance patterns, prior triazole exposure, or persistence and worsening of infection despite optimized therapy.

Treatment

Invasive Pulmonary Aspergillosis

Empiric Treatment—The treatment of invasive pulmonary aspergillosis (IPA) due to ARAF is complicated and requires host-specific considerations such as immunosuppression, drug tolerability, and severity of infection [53, 54, 55]. In 2015, an expert consensus statement was published on ARAF management [4••]. Opinion-based recommendations were made for initial therapy based on known regional resistance rates to azoles considering the site of infection. Initiation of guideline-directed therapy, specifically voriconazole for IPA, in regions with documented ARAF rates less than 5% was recommended. In regions with ARAF rates of 5–10%, expert opinion suggested initial regimens of voriconazole alone, voriconazole plus an echinocandin, or LAMB alone. In contrast, LAMB or the combination of an echinocandin plus voriconazole should be initiated in areas of ARAF > 10%. These opinions reflect a pragmatic approach weighing the risk of resistance with the risk of toxicities associated with LAMB. Though we agree with this approach, we suggest isavuconazole as a reasonable alternative where voriconazole would otherwise be utilized.

Known Resistance or Failing Current Azole Therapy—Both American and European practice guidelines for the treatment of aspergillosis recommend voriconazole (or isavuconazole) as first-line therapy in patients with IPA [44, 45]. In cases of suspected or known ARAF, clinicians should consider a stepwise approach to treatment based on the clinical scenario. This includes source control, and optimizing current azole therapy (if ARAF not confirmed), switching treatment to a LF-AMB product, or exploring combination regimens (Table 2). In all cases, choosing an antifungal (alone or in combination) that ARAF is susceptible to is recommended by the aforementioned practice guidelines.

Dose Optimization of Azole Therapy

Therapeutic Drug Monitoring: Many azoles have known pharmacokinetic variability and are prone to drug interactions. Optimizing therapy through therapeutic drug monitoring (TDM), agent selection with consideration to the site of infection, and evaluating patient-specific administration factors should be assessed in all patients. Appropriate treatment trough concentration targets for azoles may vary based on the infection but are generally

considered: itraconazole 1–4 mg/L, voriconazole 2–5.5 mg/L, and posaconazole 1–3.75 mg/L [45, 56]. Trough concentrations should be drawn after an appropriate time period for accuracy: voriconazole trough levels after 2–5 days and itraconazole and posaconazole trough levels after 5–7 days of therapy. Although not currently utilized in clinical practice, isavuconazole may have assigned TDM in the future [45, 57, 58]. In otherwise stable patients where ARAF is not confirmed but response to therapy is slow, optimizing azole therapy should be considered prior to switching therapy. We recommend TDM to optimize therapy in all patients to ensure subclinical drug exposure does not lead to treatment failure.

***High-Dose Posaconazole or Isavuconazole* Therapy:** Although not recommended in the expert consensus statement, dose escalation of posaconazole or isavuconazole, based on MIC, may be a treatment option. Investigators have evaluated MIC distribution of clinical *A. fumigatus* isolates to determine susceptibility profiles of wild-type (WT), T34/L98H and TR46/ Y121F/T289A *CYP51A* mutations [59]. Of 363 isolates, 141 presented as non-WT. Of those non-WT isolates, the majority (88/141) had the T34/L98H mutation. Notably, 10% of the non-WT isolates presented with no *CYP51A* mutation. The MIC distributions varied based on type of mutation. The T34/L98H mutation conferred the following MICs when tested by CLSI and EUCAST methods: itraconazole > 8 mg/L, voriconazole 2–16 mg/L, and posaconazole 0.25–2 mg/L. The TR46/Y121F/T289A mutation had the following MIC distribution: itraconazole > 16 mg/L, voriconazole > 8 mg/L, and posaconazole 0.25–4 mg/L. The CLSI epidemiologic cut-off values are 1 mg/L for itraconazole and voriconazole, and 0.25 mg/L for posaconazole. The lesser MIC variability and distribution observed for posaconazole suggests it could potentially be used to treat ARAF at high doses (and TDM targets). Some in vivo models have shown promise with this practice [60]. Furthermore, a case series of 7 patients with ARAF intentionally treated with high-dose posaconazole targeting trough concentrations >3 mg/L reported survival of 4 patients and 3 that died from their underlying disease [61••].

An in vitro study assessed high-dose isavuconazole in ARAF isolates using the EUCAST clinical breakpoints [62, 63, 64]. Using a Monte Carlo simulation, investigators found doubling the dose of isavuconazole (400 mg of active drug three times daily, followed by 400 mg once daily [isavuconazonium 744 mg = 400 mg isavuconazole]) had a 90% chance of achieving target attainment of the assigned epidemiologic cut-off of 2 mg/L [62, 63]. The EUCAST clinical breakpoints published for Aspergillus spp. define susceptibility for isavuconazole ≤ 1 mg/L [64].

Clinicians must understand that the use of higher azole exposure to overcome MICs is an interesting option and may be reasonable in some situations but must also consider that not all laboratory assumptions fully or successfully translate into clinical practice [65]. Though reports show promise, careful consideration of patient response, infection site, and pathogen/ antifungal MIC is required before utilizing this approach as a step-down option. At this time, high-dose azole therapy, if used, should be used in combination with another antifungal, especially in patients who are critically ill.

Pharmacotherapy Changes—Monotherapy—In patients with ARAF who are unstable/critically ill, LF-AMB remains a recommended therapy (Table 2) [4••, 45]. An

experimental murine model of ARAF concluded that LAMB retains activity in the presence of azole resistance mechanisms [4••, 66]. These investigators also assessed the impact of host factors on the efficacy of LAMB in a murine model [67]. Regardless of immunosuppression status, LAMB remained efficacious in the treatment of ARAF but they observed that higher daily doses of LAMB may be required. The practical implications of these studies support the use of LAMB in the treatment of ARAF though higher dosing/exposure has not been clinically validated. The timely switch to LF-AMB in cases of suspected or known ARAF is warranted in unstable/critically ill patients. Special consideration should be made in cases of azole-resistant *A. terreus* and other species intrinsically resistant to AMB, especially as documented pathogenic clinical isolates are beginning to emerge [68].

Consideration may be given to echinocandin monotherapy, specifically caspofungin, as activity may be retained. However, documented failure rates of 50% or higher in azole-susceptible *A. fumigatus* is unacceptably high [69, 70, 71]. We recommend combination antifungal therapy if an echinocandin is used. Overall, there is a paucity of clinical evidence and more data is needed to support the appropriate combination treatments for ARAF.

Pharmacotherapy Changes—Combination Therapies—A panel of experts recommend the combination of voriconazole and an echinocandin as an option for ARAF [4••]. This recommendation is based on preclinical data that demonstrated increased susceptibility in ARAF strains [72, 73]. The use of combination therapy with voriconazole and anidulafungin has shown a trend towards mortality benefit in patients with hematologic malignancies or hematopoietic stem cell transplant with invasive *Aspergillus* [74]. However, ARAF was not specifically addressed.

Since the publication of the expert consensus statement, *in vitro* data has questioned echinocandin-azole combination therapy. Isavuconazole in combination with anidulafungin, caspofungin, or micafungin in 30 *Aspergillus* strains, including 5 ARAF isolates, ultimately showed no synergism (5/5 indifference) with combination for ARAF isolates [75]. An ongoing clinical trial evaluating the use of a PCR assay for identifying resistance mechanisms in invasive aspergillosis is advising a therapeutic change to LAMB 3 mg/kg or the combination of an echinocandin plus posaconazole, with higher goal posaconazole trough concentrations of 3–4 mg/L (see “High Dose Posaconazole or Isavuconazole Therapy” above) when toxicity precludes the use of LAMB, if resistance is detected [76]. This study may provide much needed data on the use of combination and higher trough-driven posaconazole therapy for the management of ARAF.

Central Nervous System Infections

Central nervous system (CNS) aspergillosis has a dismal prognosis and very limited efficacy data to draw a best treatment approach for ARAF [77]. Current guidelines recommend voriconazole as the treatment of choice in susceptible isolates [44]. LAMB at a dose of 5 mg/kg/day is the mainstay of therapy for CNS ARAF due to its ability to penetrate brain parenchymal tissue. All experts in the consensus statement agreed that a second agent should be added, including 5-FC, voriconazole, or an echinocandin. While credence was given

specifically to 5-FC because of adequate CSF penetration, it has minimal *A. fumigatus* activity at a pH of 7 [4••, 78]. Optimal voriconazole dosing for CNS ARAF is unknown, but one anticipates dose-limiting toxicities with higher drug exposures even with close TDM. Other agents, like posaconazole or isavuconazole, have limited clinical data to support their use in CNS aspergillosis [79, 80, 81]. Although cerebral spinal fluid concentrations are low for these agents, parenchymal tissue concentrations are noted to be adequate [73, 75, 82]. Echinocandins are an adjunct treatment option, but these agents have poor CNS penetration and are not recommended as monotherapy [4••].

Other Sites of Infection

Other types of infection, like endophthalmitis, endocarditis, osteomyelitis, peritonitis, or pyelonephritis/cystitis, have minimal specific clinical data to update in terms of ARAF. It can be inferred from the aforementioned discussion that published expert consensus recommendations can be followed for these sites of infection in regard to dose and formulation of AMB product or adjunctive agents as reviewed elsewhere [44].

Expert Opinion

Although positive clinical outcomes with the treatment regimens outlined above have been noted in literature, robust prospective studies are needed to determine the optimal therapy for treatment of ERC and ARAF. Delaying appropriate therapy can lead to patient mortality and morbidity; therefore, prompt recognition and optimization are critical.

Currently, LF-AMB serves as a backbone initial therapy for the treatment of known or suspected ERC, especially in clinically unstable patients. Other treatment options are outlined in Table 2. Although combination therapy for ERC is promising, data is limited outside of specific clinical scenarios.

ARAF has clear documentation of high mortality rates and is rarely recognized early in the treatment course. In patients with known ARAF that are unstable or acutely ill, LF-AMB is the preferred therapy. Frequently, treatment-limiting toxicities and sub-optimal response with LF-AMB necessitate exploring alternative and combination therapies, respectively, as outlined in Table 2. Patient-specific factors, source control, and azole-specific MICs should be considered prior to therapy modification. An ongoing clinical trial may help with unanswered questions on optimal treatment approaches, including step-down therapy options [76].

Conclusion

Fungal resistance is an emerging health crisis requiring prompt evaluation and advanced knowledge of antifungal pharmacology to optimally manage infections. Current treatment options are limited and rely on LAMB for both ERC and ARAF. As the newer therapies emerge, treatment recommendations for ERC and ARAF are likely to shift based on safety and efficacy profile of the new agents. Future research defining optimal therapies and early identification of ERC and ARAF is of extreme importance.

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Table 1:

Resistance Profiles for Echinocandin-Resistant *Candida* and Azole-Resistant *Aspergillus*

Species	Resistance Rates (%)	Mechanism of Resistance****
Echinocandin Resistant *Candida* spp.*		
C. albicans	0.0 (A) <0.1–0.2 (C) 0.0–0.2(M)	***FKS1/2* HS1 alterations** *FKS1:* L662W, S629P, F625S, R631S; S645P, F641S, F641I, S629P, S654P, F650S, F641S, F641L *FKS2:* S663P, L662W, F659S/V/Y, D666E/K753Q, F659_del
C. glabrata	1.5–2.8 (A) 1.3–6.9 (C) 1.0–2.8 (M)	
C. parapsilosis	0.0–0.4 (A) 0.0 (C) 0.0 (M)	
C. tropicalis	0.0–1.3 (A) 0.0–2.0 (C) 0.0–2.0 (M)	
C. krusei	0.0–3.6 (A) 0.0–7.3 (C) 0.0 (M)	
*C. auris***	7–10 (A, M)	
Azole Resistant *Aspergillus* spp.***		
A. fumigatus	0–3.2	**Point mutations:** G54, G138, G448S, M220 **Tandem repeats/Combination mutations:** TR_{34}/L98H, TR_{46}/Y121/T289A **Non-CYP mediated mutations:** Efflux pumps: ABC and MFS transporters HMG-CoA mutations: *hmg1*, *erg6*, HMG-CoA point mutations
Non-fumigatus spp.	Not extensively documented	

* From SENTRY Antifungal Surveillance program 1997 – 2016 gathered from 135 medical centers in 39 countries [10•].

** No current clinical breakpoints reported for C. auris. Resistance rates estimated using EUCAST and CLSI *Candida* spp. breakpoints [15••].

*** Extrapolated from a study evaluating isolates from 22 centers in 19 countries (range: 0–26.1%). A surveillance study published in 2014 reported a 0% incidence of TR34/L98H mutations in 1,026 clinical isolates in the United States, but case reports of clinical case of azole-resistant *A. fumigatus* in the United States have been published [35•, 37–39]

**** not a comprehensive list of point mutations associated with echinocandin-resistant *Candida* spp. or azole-resistant *Aspergillus* spp.

A, anidulafungin; C, caspofungin; M, micafungin; HS, hot spot; ABC, ATP-binding cassette; MFS, major facilitator superfamily

Table 2:

Treatment Options for Echinocandin-resistant *Candida* spp. and Azole-resistant *Aspergillus* spp.

Antifungal Strategy	Site of Infection	Patient Population	Comments
Echinocandin Resistant *Candida* spp.			
First-line therapy			
Lipid Formulation Amphotericin-B Monotherapy	All sites	Clinically Stable, Clinically Unstable	Recommended first-line treatment for echinocandin-resistant *Candida* spp. Amphotericin products can be used in combination with other agents based on site of infection, but should serve as the backbone for therapeutic plans. Recommended Lipid Formulation Amphotericin-B dosing strategy is 3–5 mg/kg/day. Amphotericin is associated with significant toxicity
Alternative therapy options			
Susceptible Echinocandin Monotherapy	Candidemia	Clinically Stable, Clinically Unstable	Option for isolates with known variable echinocandin susceptibility in patients who cannot tolerate amphotericin products. Combination of a susceptible echinocandin with a synergistic azole may be considered. Echinocandin switch may be hindered by single echinocandin formularies.
Azole Monotherapy	All sites	Clinically Stable	Option should be used as a step down agent when azole susceptibility is known. It is reasonable to start with an amphotericin product then de-escalate to an azole to complete the duration of treatment after the patient is clinically improving.
Azole Resistant *Aspergillus* spp.*			
First-line therapy			
Lipid Formulation Amphotericin-B Monotherapy	All sites	Clinically Stable, Clinically Unstable	Recommended first-line treatment for azole-resistant *A. fumigatus*. Amphotericin products should serve as the backbone of therapeutic regimens and can be used in combination with other agents based on site of infection. Lipid Formulation Amphotericin-B is commonly recommended for IPA 3–5mg/kg/day. CNS aspergillosis is liposomal product at 5 mg/kg/day. Amphotericin is associated with significant toxicity.
Alternative therapy options			
Liposomal Amphotericin-B **plus** High-Dose Azole Combination Regimen	All sites	Clinically Stable, Clinically Unstable	High-dose posaconazole and isavuconazole are potential additions to amphotericin products for combination therapy. Data suggests viability of high-dose azole monotherapy in select populations, but currently should be avoided in clinically unstable patients.
Azole **plus** Echinocandin Combination Regimen	IPA	Clinically Stable	Voriconazole (standard dose) and an echinocandin is recommended as an empiric option in geographic regions with known resistance over 5%. If used in patients with known azole-resistant *A. fumigatus* high-dose posaconazole or high-dose isavuconazole should be considered.
Echinocandin Monotherapy	IPA	Not recommended	Echinocandin monotherapy is not recommended based on failure rates of 50% in non-azole resistant *A. fumigatus*.
Liposomal Amphotericin-B **plus** High-Dose Azole **Or** Flucytosine **Or** Echinocandin Combination Regimen	CNS	Clinically Stable, Clinically Unstable	In CNS aspergillosis, an amphotericin product should serve as the core agent for treatment. Experts recommend adding flucytosine, a high-dose azole, or an echinocandin as adjunctive agents. Flucytosine requires an acidic pH for activity against *Aspergillus* spp. It is unlikely that voriconazole can be dosed to overcome MICs in azole-resistant *A. fumigatus* therefore reasonable to utilize isavuconazole or posaconazole. Echinocandins do not have appreciable penetration into the CNS.

* In all patients being treated with itraconazole, voriconazole, or posaconazole, therapeutic drug monitoring is recommended to optimize drug exposure and minimize side-effects. High-dose azole therapy may target trough concentrations above standard published recommendations (e.g. posaconazole).

Abbreviations: CNS, central nervous system; IPA, invasive pulmonary aspergillosis; TDM, therapeutic drug monitoring