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PERSPECTIVES

Optimizing the Treatment of Invasive Candidiasis—A Case for Combination Therapy

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Invasive candidiasis is a rising global health threat with increasing incidence, persistently high mortality, and diminishing treatment options. Antifungal resistance has rapidly emerged and spread, with multidrug-resistant species deemed an urgent and serious threat. While acknowledging the key role of antifungal stewardship and infection control in curbing spread, we examine the role of antifungal monotherapy in driving resistance and the potential for combination therapy to prevent stress adaptation and emergence of drug resistance. In addition to its role in mitigating resistance, combination treatment may improve drug penetration, expedite fungal clearance, and allow lower, less toxic doses of individual drugs to be used. A growing body of laboratory-based evidence suggests that antifungal combinations can yield synergistic activity against *Candida* spp., including against frequently multidrug-resistant *Candida auris*. It is imperative to test these combinations in clinical trials, incorporating resistance end points as a marker of success.

Keywords. *Candida auris*; candidiasis; combination; drug resistance; drug therapy.

An estimated 750 000 *Candida* bloodstream infections occur per year, mostly among critically ill patients, associated with death in 10%–47% of cases [1, 2]. There are only 3 drug classes routinely used for treatment, and resistance is rising against azoles and echinocandins, while the use of the polyene AmB (deoxycholate [AmB] and liposomal formulations [L-AmB]) is limited by toxicity and cost. New agents are on the horizon, but potential environmental use and single-agent clinical use threaten their longevity. Novel pharmaceutical approaches to optimize treatment and prevent antifungal resistance are essential to preserve the effectiveness of current and future antifungals.

THE SCOPE OF THE PROBLEM—ANTIFUNGAL-RESISTANT *CANDIDA* SPECIES

Widespread use of antifungals has driven a global epidemiological shift toward *Candida* spp. with reduced susceptibility to azoles, rising secondary resistance, and the emergence of multidrug-resistant *C. auris* and *C. glabrata* (*Nakaseomyces glabrata*) [3]. Non-*albicans* species, including intrinsically and frequently azole-resistant *C. krusei* (*Pichia kudriavzevii*) and *C. glabrata* are on the rise globally and are now responsible for more than half of invasive cases submitted to SENTRY surveillance (North America, Europe, Latin America, and Asia-Pacific regions) [4]. Azole-resistant *C. parapsilosis* has emerged as a global threat, following extensive spread of clonal strains harboring resistance mutations, with multiple outbreaks reported, including in Europe, the United States, and Brazil [5]. A recent meta-analysis of 79 studies revealed that fluconazole resistance has increased from 12% to 37% of *C. parapsilosis* isolates tested globally over the past 6 years [6]. *C. parapsilosis* is the most common cause of *Candida* bloodstream infections in South Africa, where around two-thirds are azole-resistant [7].

The rapid global spread of *C. auris* is particularly concerning. Although clade variations occur and breakpoints are tentative, *C. auris* is almost always (>90%) azole-resistant, with resistance to AmB reported in up to 30% [8, 9] and multidrug resistance (to azoles and polyenes) reported in 3%–17% [9]. Echinocandin resistance is currently rare (0%–3%) [8–10], but pan-resistance (to azoles, echinocandins, and AmB) has been reported [8, 11]. While outbreaks continue to be reported in the United States [10] and Europe [12, 13], *C. auris* has become a dominant pathogen elsewhere, now causing around a third of *Candida* bloodstream infections in South Africa [7].

With azole resistance rising, increasing reliance on echinocandins is threatening

Received 24 November 2023; editorial decision 29 January 2024; accepted 20 February 2024; published online 22 February 2024

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https://doi.org/10.1093/ofid/ofae072

the final useful drug class. Echinocandin resistance is reported in up to 10.6% of *C. glabrata* [4] and increasingly detected in *C. auris,* rising 3-fold in the United States in 2021 [10], including in patients without prior echinocandin exposure [8, 10].

THE ROLE OF ANTIFUNGAL MONOTHERAPY

Although nosocomial transmission is widely acknowledged [8], prior antifungal exposure is a known risk factor for azole and echinocandin resistance [14, 15]. Antifungal monotherapy is the recommended treatment for *Candida* bloodstream infections [2], initially with echinocandins, then azole stepdown if susceptible. Single antifungals are also used for treatment of noninvasive candidiasis and prophylaxis of yeast and mold infections in immune-suppressed populations, for example, second-generation azoles in hematological malignancies. Monotherapy promotes selection of intrinsic or acquired resistance mechanisms and induces stress adaptation (tolerance), which may facilitate yeast persistence and result in treatment failure [16]. Monotherapy may also result in suboptimal penetration of some body sites and biofilm, causing resistance compartmentalization, observed particularly in urine, the abdominal cavity, and on mucosal surfaces and biofilm-prone indwelling devices [9, 14].

Emergence of phenotypic and genotypic resistance has been observed in *Candida* spp. during antifungal exposure. Echinocandin resistance has been associated with FKS hotspot mutations developed during micafungin treatment in patients with recurrent *C. auris* bloodstream infections in the United States and South Africa [8, 17]. A prospective study of 193 patients treated for candidemia in Denmark demonstrated acquired resistance in 29.4% and 21.6% of *C. glabrata* isolated from oral swabs following ≥7 days of fluconazole or anidulafungin, respectively [20]. Serial colonizing *Candida* isolates from intensive care unit patients in the UK (CandiRes ISRCTN14165977) revealed a ≥4-fold minimal inhibitory concentration (MIC) increase to fluconazole in 6/39 (15%) and to anidulafungin in 8/88 (11%) following ≥7 days of exposure to the respective drug [18].

In addition to changes in MIC, antifungal monotherapy induces other adaptive mechanisms in *Candida* spp. Heteroresistance is an intrinsic phenomenon whereby a fraction of the total population (usually <1%) grows at drug concentrations above the MIC [16]. This resistant subpopulation is selected for, becoming predominant during drug exposure, leading to treatment failure [16, 19]. Size of heteroresistant populations in colonizing *Candida* spp. was correlated with duration of antifungal exposure in the CandiRes study [20]. Heteroresistance has been described in *C. glabrata* following azole monotherapy [19] and was associated with breakthrough *C. parapsilosis* infections in patients taking prophylactic echinocandin treatment [21]. Antifungal tolerance is also described in *Candida* spp., whereby phenotypically drug-susceptible subpopulations persist and grow slowly at supra-MIC concentrations. Tolerant cells adapt to drug via enhanced stress response pathway signaling, decreased drug accumulation, and cell wall remodeling [16], providing time for evolution of resistance-associated mutations.

THE ROLE OF COMBINATION ANTIFUNGAL THERAPY

While stringent antifungal stewardship and infection, prevention, and control measures are key, combination antifungal regimens have the potential to prevent resistance while optimizing the treatment of invasive candidiasis. Combination antifungals may enhance fungal clearance, potentially allow lower

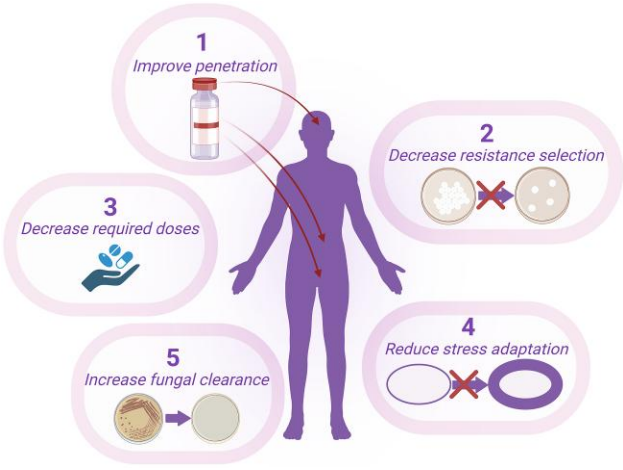
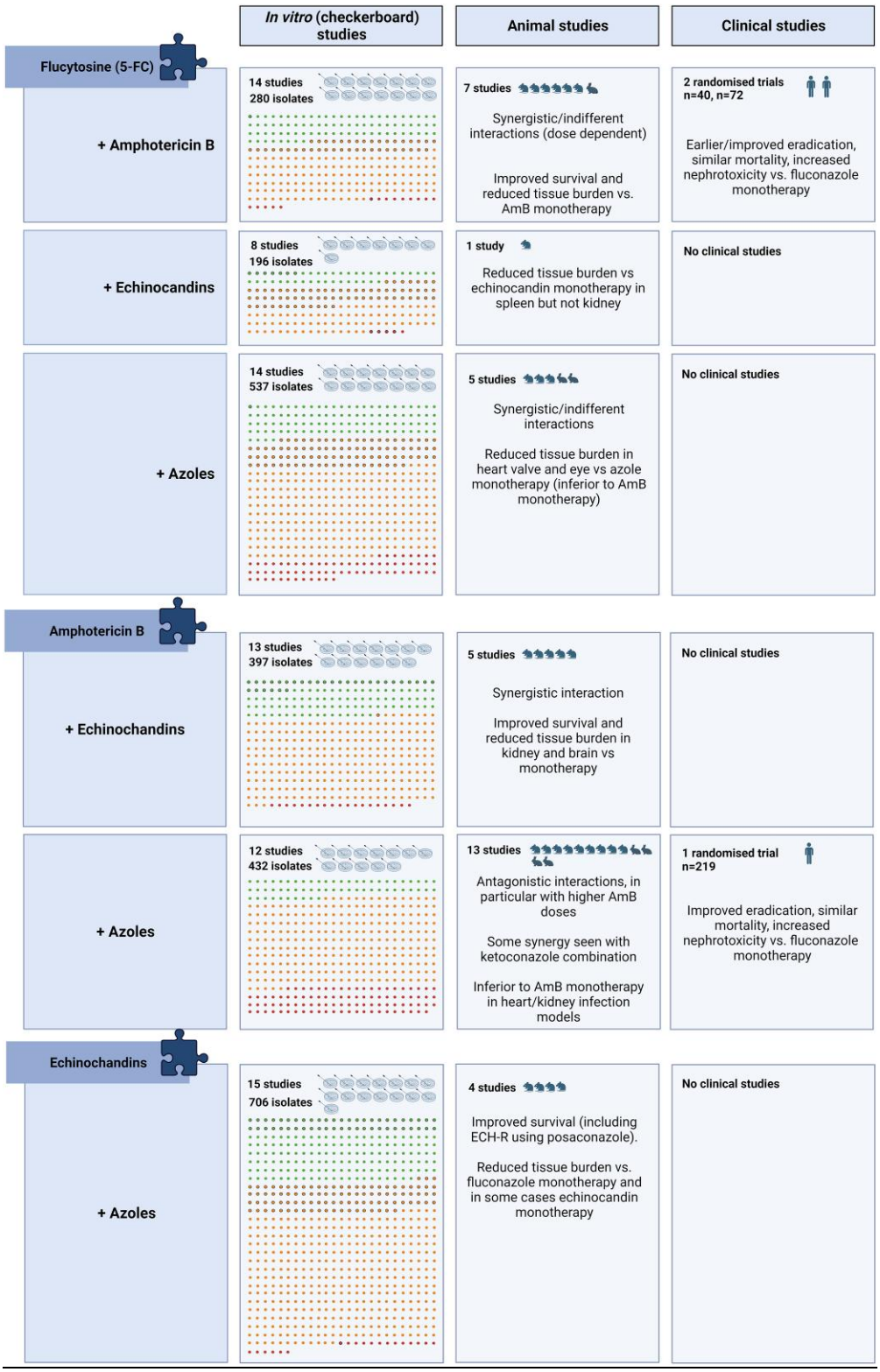


Figure 1. Mechanisms whereby combination therapy might optimize antifungal treatment of invasive candidiasis.

Table 1. Summary of Evidence From In Vitro, Animal and Clinical Studies of Dual Combination Antifungals Against *Candida* spp.



Studies were included (see full table in Supplementary Appendix 1) if they used antifungal drugs currently in use. *In vitro* studies were included if they used checkerboard techniques and reported fractional inhibitory concentration indices (FICI) defining synergy as FICI ≤0.5, additive/indifferent between 0.5 and 4.0, and antagonistic ≥4. Clinical studies were included if randomized trials. For in vitro studies, combinations are represented as synergistic, indifferent, or antagonistic for each isolate by green, orange, and red dots, respectively. Black bordered dots represent *C. auris* isolates.

Abbreviations: 5FC, flucytosine; AmB, amphotericin B; ECH, echinocandin; -S, -R, sensitive, resistant.

doses with reduced toxicity risk, optimize antifungal penetration to sanctuary sites where *Candida* persist, and mitigate the evolution of resistance and stress responses observed during antifungal monotherapy (Figure 1). Although theoretically lower doses could be used, the risk of cumulative toxicity and increased cost of drug combinations must be weighed against potential benefit.

Current Research

Most research has focused on the nature of interactions of drug combinations against *Candida* using *in vitro* or animal experiments, with very limited study of resistance mitigation. Antifungal interactions vary between synergy, indifference, and antagonism depending on the combination, concentration species/isolate (*C. albicans* predominant), and model used. Findings are not always consistent across *in vitro–in vivo* studies (Table 1): the single randomized trial of fluconazole +/− AmB reported improved mycological clearance and higher success rates with combination, despite *in vitro* and murine studies frequently reporting antagonism (Table 1; Supplementary Appendix).

Flucytosine Combination Therapy

Flucytosine (5-FC) is perhaps the most promising agent currently available for combination treatment of invasive candidiasis. In vitro studies report a range of interactions including synergy with every combination, including against *C. auris* [22–24]. Murine and rabbit studies also report synergy, improved survival, and reduced tissue burden for AmB-5FC combinations vs monotherapy. A single study of micafungin-5FC combination in a *C. glabrata* immunocompromised mouse model showed an indifferent effect (Table 1; Supplementary Appendix) [25]. Flucytosine has excellent oral bioavailability and penetration into peritoneal fluid, a common site of yeast persistence [26]. As an old, licensed drug and an essential component of cryptococcal meningitis treatment, flucytosine is becoming globally accessible.

Although limited clinical data exist, flucytosine combination therapy is recommended for complicated candidiasis, including meningitis and endocarditis, in clinical guidelines [2]. However, recent experience demonstrating rapidly evolving flucytosine resistance [11] must heed caution in cases of already multidrug-resistant *C. auris* where flucytosine may be the only active drug; flucytosine monotherapy is known to predispose to rapid emergence of resistance. This case, as well as reports of rising MICs to flucytosine in the context of occasional use in New York (personal communication, V. Chaturvedi), emphasizes that upfront combination treatment, to *mitigate* the evolution of resistance (as has been demonstrated in cryptococcal meningitis [27]), may be appropriate, rather than reserving combinations for salvage therapy. It also demonstrates the importance of meticulous preclinical and clinical evaluation of drug combinations to guide future management guidelines.

Novel Antifungal Agents

There are promising new antifungal drugs in the pipeline for candidiasis including fosmanogepix, ibrexafungerp, and the long-acting echinocandin rezafungin [28]. The concern, as new agents reach clinical use, is that exposure to similar molecules both in the environment and as monotherapy in patients will see similar resistance emerge, limiting future utility. Our in vitro study revealed manogepix (the active compound of fosmanogepix) to be the most synergistic of 4 agents tested in combination with anidulafungin against 15 *C. auris* isolates (clades I and III) [24]. Future drug development research must investigate combinations to optimize and future-proof novel agents.

Future Research

Although evidence that particular drug combinations can enhance *Candida* clearance is mounting, very little is known regarding impact on antifungal resistance. Future research will be enhanced by collaborations to survey and study antifungal resistance, collate diverse isolate banks, and build clinical networks. *In vitro* and animal model studies must investigate whether exposure to 2 drugs can suppress resistance. However, even if resistance mitigation is observed in the laboratory, differential tissue penetration may drive resistance compartmentalization in patients; clinical studies must follow. Drug development at both preclinical and clinical trial stages must consider these factors, incorporating resistance end points (including compartmentalization) and pharmacometrics, as well as mycological clearance, to define sustainable treatment approaches.

CONCLUSIONS

Invasive candidiasis is a rising global health threat. Emergence and spread of resistant *Candida* spp. demands an urgent and innovative response. Combination treatment must be explored for combatting on-treatment resistance evolution while enhancing efficacy. Preclinical studies have identified promising combinations. It is now time to take these approaches to the bedside to evaluate whether combination treatment can improve clinical and resistance outcomes for patients, particularly in settings harboring a high burden of antifungal resistance.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

Figure 1 was created with Biorender.com.

Patient consent. This is a perspective piece and does not include factors necessitating patient consent.

Financial support. R.M.W. is recipient of an NIHR Clinical Lectureship (CL-2019-16-001). This work was also supported by the NIHR through a Global Research Professorship (NIHR303140) using UK aid from the UK

Government to support global health research (to N.P.G.). P.A.S is supported by a Gilead UK & Ireland grant (17437) awarded to T.B., and B.A.C. is supported by an NIHR BRC grant 203320 awarded to the University of Exeter.

Potential conflicts of interest. R.B. has participated in educational activities (lectures, webinars) for Gilead and Teva and advisory boards for MSD and GSK. T.B. has participated in advisory boards for and received speaker fees from Gilead, Pfizer, and Mundipharma and received research funding from MSD, Gilead, and Pfizer. All other authors report no potential conflicts.

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