Review

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Combination antifungal therapy: A strategy for the management of invasive fungal infections

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INTRODUCTION

Invasive mycoses have become an important public health problem as their incidence has increased dramatically in the last decades, while the discovery of the ideal antifungal agent has not been yet obtained[1-4]. The population at risk for these deadly mycoses includes patients with AIDS, transplant recipients, patients with haematological malignancies and other immunocompromised individuals, exposed to fungal pathogens4. Aetiology and epidemiological patterns of these invasive mycoses are changing due to advances in medical management or healthcare practices, such as the introduction of newer modalities for hematopoietic stem cell transplantation, the improvements of organ transplantation practices, the use of novel immunosuppressive agents and current antimicrobial prophylactic strategies.

Although *Candida albicans* is the predominant causative agent of invasive mycoses, other species of *Candida*, and different *Aspergillus* and *Cryptococcus* species are frequently involved in infections affecting immunocompromised patients. The role of other yeast-like organisms, such as *Trichosporon*, *Saprochaete* or *Malassezia* and filamentous fungi, such as *Fusarium*, *Acremonium*, *Mucor*, *Rhizopus*, *Paecilomyces*, *Scedosporium*, *Scopulariopsis brevicaulis*, dermatophytes, dematiaceous and dimorphic fungi as emerging pathogens in human diseases is also important[4-18]. Moreover, classic species such as *C. parapsilosis, C. glabrata* or *A. fumigatus* currently known are complex of new cryptic species[19]. The use of highly active antiretroviral therapy has resulted in a significant decrease in the incidence of fungal opportunistic infections among persons with AIDS who live in developed countries. However, since the availability of highly active antiretroviral therapy is quite limited in many developing countries with widely spread HIV epidemics, fungal opportunistic infections such as oropharyngeal candidiasis, cryptococcosis, histoplasmosis or penicilliosis are now an important cause of morbidity and mortality for patients with AIDS. Advances in surgical techniques and in immunosuppressive regimens have accounted for a decline in the incidence of invasive candidiasis in organ transplant recipients at high risk for *Candida* infections, but which has also resulted in a rise in the frequency of non albicans *Candida* species as pathogens. The increasing use of more aggressive immunosuppressive drugs in hematopoietic stem cell transplant recipients has resulted in an increase in the incidence of invasive filamentous mycoses (such as aspergillosis, fusariosis or mucorales) among these patients[4,5,7,9-12,14-16,20]. Recent changes in the epidemiology of invasive fungal infections are having important implications in the management of these infections. An early diagnosis of invasive mycoses is very important in order to treat patients at a stage of the disease when the fungal cell concentrations and body tissues damage burdens are low. However, diagnosis is difficult and in many cases is not possible to obtain a reliable evidence of invasive mycosis[21-23].

Against this problem, antifungal combination therapy is one of the available management strategies to provide the clinicians with effective tools. Also new potent generation of new triazole derivative molecules; liposomal and other formulations for delivering amphotericin B (AMB) or azole drugs; and immunomodulators are available and also in the pipeline. Nevertheless, some safety, toxicological, pharmacokinetic aspects or spectrum profile are not perfect for every drug. Moreover, the industry should develop newer families of antifungal drugs, while combination therapy tends to maximize the potency of known drugs and also of other substances combined with antifungal drugs, without any antagonist mode of action, in order to reduce clinical failure. The rationale for combination therapy is to maximize antifungal effects by attacking different fungal targets at the same time with additive or synergistic effects, but some combinations offer good results when using the same target. However, clinical studies are required to provide a correlation with in vitro studies. This review has tried to summarize the accumulated experience on the combination of current antifungal agents used in the treatment of invasive fungal infections.

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COMBINATION THERAPY

Some methods have been developed to study the interaction of the combined antifungal agents in vitro. The lack of standardization of these methods is the main problem, although most of them are CLSI and EUCAST guidelines-based[24,25]. Also commercialized methods (Etest and diffusion methods) are performed in some laboratories to get a description of the in vitro antifungal activity of combined drugs[20,26-28]. Unfortunately, changes must be made in the standardized methodologies to update the procedure in order to study two or more drugs providing data of in vitro combined susceptibility. Within this context, different antifungal activity results have been reported depending on the study method used (E-test, time-kill and checkerboard methods against *Candida*) for the same combination of antifungal agents, such as fluconazole (FNZ) / voriconazole (VRZ) plus terbinafine (TRB) (table 1 and 2). Nevertheless, in vitro interaction of anidulafungin (AND) with VRZ tested by checkerboard and E-test have resulted coincident within variations of +/-3 $\log_2$ dilutions in studies performed against 30 *Aspergillus* clinical isolates, obtaining indifferent interactions by both methods.

On the other hand, these published data are frequently obtained with a reduced number of clinical isolates due to the complexity of their performance. At this point, interaction is usually not only determined by the antifungal combination but also by the choice of the endpoint[29]. In this way, usefulness of these tests is related to some experimental variables and also to the antifungal combined, fungi, isolates and method. Complementary data were obtained by graphical isobolograms and statistical response surface methods useful for the study of three drugs combinations in the same way that it happens with time-kill curves and flow-cytometry[27,30-32]. As an advantage, flow-cytometry method provides results of combination in 2h time determining the total amount of viable cells by means of metabolic markers[31]. However, the scientific basis for this approach requires further evidence from prospective clinical trials of antifungal combinations on the basis of proved antifungal efficacy not successfully performed or relatively done[33-35].

Published references illustrate the possibility that real advantages of this strategy could be seen for particular combinations and only in particular mycoses and/or, certain clinical isolates, particular types of patients on the basis of desirable antifungal standardized susceptibility tests, animal models and clinical reports (tables 1 and 2)[31,36-41].

Another methodological problem that affects the way to interpret the value of combination is the MIC determination. This especially affects those drugs with different endpoint determination because some fractional inhibitory concentrations indexes (FICI) calculation could be done ($FICI_0$, $FICI_1$, $FICI_2$) or when filamentous fungi are tested. Additionally, the same drug combination against the same isolate can be interpreted as synergy, indifference or antagonism[29,31,42]. Due to this problem, "Response Surface Model" was proposed by Te Dorsthorst to determine the interaction index alpha ($IC\alpha$) as a consistent alternative to FICI values to evaluate interactions by using spectrophotometric determinations of growth in wells with a colorimetric indicator in the culture media[29]. Another proposal to solve the lack of reproducibility of results obtaining FICI values was the Monte Carlo simulation analysis, performed also with spectrophotometric determinations of endpoints with a colorimetric indicator in the culture media[42].

Objectively, combination therapy tends to reduce clinical failure when resistant strains have been recovered from patients, although interactions and cross-resistance may result in some drug associations. Interaction between antifungal drugs depends on the selected method, on antifungal combination, the sequence of administration, and genera, species and strain of the pathogen. Synergy has been established among conventional antifungal agents and also among investigational molecules under development[41]. Alternatively, the combination with antifungal drugs and other molecules without effect on fungi, such as FNZ and cyclosporine, results in a fungicidal effect against yeasts, indeed against FNZ-susceptible strains of *C. albicans*[43-45]. This combination also results effective against animal models of endocarditis due to *C. albicans*[43]. Furthermore, as a result of the use of combination among azole antifungal drugs such as FNZ, caspofungin (CAS), pneumocandin or TRB with calcineurin inhibitors (cyclosporin and tacrolimus), an overall enhanced susceptibility has been described in intrinsically resistant species of *Candida*, such as *Candida krusei*, and also for some clinical isolates of *Cryptococcus neoformans*[46-51]. These combinations can be extended to a synergic fungicidal action obtained with ergosterol biosynthesis inhibitors (TRB, fenpropimorph and FNZ) against *C. albicans*, *C. glabrata* and *C. krusei*, even in some cases this action is calcineurin dependent[46-49]. This synergy is species-dependent[49]. Other alternative combinations are those including antifungal plus antibacterial drugs (tetracyclines and quinolones) or other non-antifungal agents (amiodarone, eugenol, galdanamycin, etc.)[51-53].

Synergy between antifungal drugs is considered as a positive interaction when two or more drugs can develop a cumulative effect, while antagonism would be related to a negative interaction[33,37,54-56]. Under in vitro considerations, FICI obtained by checkerboard dilutions, express the lowest concentration of two or three drugs that inhibit growth[29,31,33,37,56,57]. Other models, such as response surface modelling, are available to avoid problems of combined agents with different MIC endpoints or even as tools for synergy screening of new antifungal agents[29,42,57,58]. Synergy is defined when a FICI ≤0.5 is obtained and antagonism for FICI >4; the range of no interaction is for FICI values between >0.5 and 4[37,58]. In time-kill studies, synergy is defined when combination achieves an increase rate in killing cells of ≥2 $\log_{10}$ (CFU/ml) at 24h; < 2 but > 1 $\log_{10}$ increase is additive; a decrease from the least active antifungal <2 $\log_{10}$ CFU/ml is indifference; and a reduction in killing of >2 $\log_{10}$ is considered as antagonism[27,59]. The current review focuses on some of the most frequent of these drug combinations.

Amphotericin B plus 5-fluorocytosine

The traditional combinations of AMB plus 5-fluorocytosine

(5FC) or AMB plus rifampicin with higher efficacy, have been replaced by newer combinations. Some controversial opinions affect the combination of AMB plus 5FC because no data about either interaction in vitro or synergy against *C. albicans*, *C. neoformans* and *A. fumigatus*, and also synergy against *Aspergillus* have been published[31,37,56,60,61]. This combined therapy is the treatment of choice used against cryptococcal meningitis but not in other infections with the exception of some cerebral sinusitis, and arteritis by *Aspergillus* spp.[21,37,60,61]. Balance of produced adverse effects by monotherapy and improved combination seems not to be positive[37]. AMB plus 5FC interacted in synergy, indifference or antagonism against some isolates of *A. fumigatus* and *A. flavus* depending on the way that MICs were obtained for FIC calculation (MIC_0, MIC_1, MIC_2). When Greco Model for R2s calculation was used, synergy was detected in 61.9% of isolates[31].

Amphotericin B plus azole drugs

Combinations of AMB and azole antifungal drugs have shown therapeutic efficacy but there are some controversial opinions. In vitro antagonism has been reported between AMB and some azole drugs such as FNZ, but little evidence of clinical synergism, antagonism or no interaction has been found in animal models of invasive aspergillosis or in the clinical setting[37,56,61-65]. However, this combination was more active than monotherapy against *C. neoformans* without any apparent antagonism[66]. Antagonism could be based on two antifungal drugs with the same target but, opposite this fact, AMB and FNZ do not act in or with the same reaction of the ergosterol biosynthesis route[63]. Sequential administration with FNZ and itraconazole (ITZ) can reverse the in vitro antagonism AMB-ITZ altering the experimental conditions of culture media in which this interaction is studied[40,56,67]. Nevertheless, interaction between AMB and FNZ is dependent on the drugs concentrations, requiring 2-4 mg/L of FNZ to achieve ergosterol damage followed by an antagonism with AMB due to the reduction of targets or sites of action[62]. Effectiveness of the combination of AMB plus FNZ was studied in animal models for invasive candidiasis and aspergillosis resulting in better survival rates than monotherapy[37,40,63]. This association is successfully used in the management of candidal endocarditis and systemic trichosporonosis in bone marrow transplantation recipients[37].

Mechanism of action of this synergic combination has been related to the phospholipid content in the fungal cell and to the AMB mode of action, as we as to the saturation process of fatty acid chains and also to per oxidative process regardless of the ergosterol inhibition performed by FNZ[63,68]. A reduction of the intake of azoles has been observed with the simultaneous administration of AMB or even a competition between azoles and AMB for the same targets at different sites in the sequential administration[37,40,56,61,68]. This could be related to the described differences associated with the order of the sequential administration of antifungal drugs in patients[35]. Besides, it is the origin of the mechanism of antagonism observed with new triazole derivatives because antagonism is described when azoles are administered before AMB, even reducing the in vitro susceptibility to AMB in *C. albicans* or inducing a transient resistance directly related to the time of preincubation with FNZ or also the same fungistatic FNZ effect[31,40,56,64,69]. Changing the sequence and with a previous AMB administration before the AMB plus FNZ, Louie et al obtained a rapidly sterilization of kidneys and cardiac vegetations in animal models of pyelonephritis and endocarditis compared to the simple combination of AMB plus FNZ[40]. Conversely, Barchiesi et al concluded that pre-exposure to FNZ abolished the fungicidal activity of the polyene in a systemic cryptococcosis model in mice[68]. Nevertheless, administration of azoles after AMB has a synergistic effect in contrast with the effect observed when hydrophilic azoles and AMB are simultaneously administered, because of a reduction of the ergosterol in the cell membrane[37,40]. Against this model of theoretical action predictions, combination results between antifungal class drugs are dependent on the method used for the evaluation, the pathogen, and the choice of combination or sequential combination of antifungal drugs[37,38,67-71]. Therapeutic value of these combinations is limited and an efficacy improvement in *Candida* infections has not been proved when compared to monotherapy schedules, without reducing adverse effects[37]. The pre-exposure to ITZ induces a reduction of the efficacy of conventional or lipid formulations of AMB in murine models of acute invasive pulmonary aspergillosis, endocarditis and pyelonephritis by *C. albicans*[40,67]. This effect was not detected when the administration was sequentially started with AMB and ended with ITZ[67]. The impact of the order of initiating was also observed with FNZ and AMB. Previous exposure to FNZ reduced the susceptibility to AMB in *C. albicans* in a rabbit model of endocarditis and pyelonephritis but not in murine model of cryptococcosis[66,67]. This effect was not detected when the administration was sequentially started with AMB and ended with ITZ[66]. Pre-exposure to FNZ can induce resistance to AMB in *C. albicans* in a period of 8-24h but also interactions with some other drugs, such as prednisolone, methylprednisolone, midazolam, warfarin, cyclosporine, nifedipine, phenytoin and/or omeprazole[40,44,56,72,73]. This resistance was more persistent when the combinations of AMB plus FNZ was the inductor[40]. Triazole derivatives, such as VRZ, ITZ and FNZ are being tested in combination with AMB, CAS or TRB[21,57,60,68,74,75]. AMB plus VRZ offered better in vivo results against FNZ-susceptible *C. dubliniensis* isolates in comparison with that from AMB plus ITZ (60% and 16,66% respectively of synergy)[74]. Combination of AMB plus ITZ showed good results in the management of sinonasal infection by *S. brevicaulis*, abdominal mucormycosis and some aspergillosis while others failed[37]. At any case, a significant reduction of MIC was obtained against *C. glabrata* with AMB plus VRZ with synergy as it was demonstrated by time kill-curves[26].

Sandoval-Denis et al.[76] observed that combination of AMB at suboptimal dose (0.3 mg/kg) with VZN shown efficacy in prolonging survival and reducing tissue burden in a murine model of disseminated aspergillosis caused by an isolate of *A. fumigatus* with poor in vivo response to this azole. The efficacy of the combined treatment was higher than the obtained with

amphotericin B alone at 0.8 mg/kg[76].

Although combination of AMB and ravuconazole (RVZ) has synergic interaction against clinical isolates of *Fusarium*, interaction of liposomal AMB with RVZ or VRZ has been proved antagonistic or indifferent, respectively, against invasive aspergillosis in neutropenic rabbits. This conclusion may affect all new triazole derivatives and polyene antifungal drugs in this fungal infection although the opposite effect is produced by the combination of AMB and FNZ up to levels of >85% in the case of AMB plus VRZ[69,77,78]. Liposomal AMB or nystatin showed synergic or additive effects when combined with ITZ, 5FC, CAS, rifampicin or cyclosporine and was successfully used in the management of renal infections in a child, and mucormycosis[37]. AMB plus PSZ was effective in a murine model of disseminated infection by *Rhizopus oryzae*[79].

The standard therapies for histoplasmosis and the rest of endemic mycoses include ITZ and AMB. The role of ITZ in histoplasmosis is limited by drug interactions and variable drug levels, and it has been reported that the echinocandin molecules are not effective in murine histoplasmosis[80]. Against *A. fumigatus* and *A. flavus* isolates, interaction of AMB plus ITZ is also influenced by the way FICI is determined, but when Greco model was applied, antagonism was detected in 33.3%, indifference in 57.2% and synergy in 9.5%[31].

5-fluorocytosine plus azoles

Effects of 5FC combined with FNZ, VRZ or PSZ have been studied and reported as synergic against *C. neoformans* and *C. glabrata,* in comparison with the antagonistic effect observed against most *C. albicans* or *Aspergillus* clinical isolates[31,32,37,38,67,81]. Mode of action of this fungistatic combination could be related to the fact that azoles inhibit the synthesis of ergosterol producing a fungal membrane more permeable to 5FC. This 5FC plus FNZ combination against *C. neoformans* caused a significant reduction of MIC values for both drugs, with a 62% of synergy without antagonism[81]. A related problem could be the isolation of FNZ-resistant *C. neoformans*[56].

These results and others contrasted clearly with those obtained with the combination of 5FC and FNZ in the management of cryptococcosis in a HIV-infected patient as alternative treatment, in terms of effectiveness and safety, but not from those of George *et al.*, in experimental invasive aspergillosis and other combinations with newer triazole derivatives[37,38,71]. Also FNZ plus 5FC have a synergic effect when combined with some antifungal peptides under development and can be used in oral administration being suitable for those patients with renal failure[37,82]. Combination of 5FC plus ITZ is also suitable against esophagitis infection produced by FNZ-resistant *Candida* isolates[37]. 5FC also antagonizes with TRB against *Aspergillus*[38]. Combination of PSZ and 5FC is able to produce a significant reduction of the CMI for *C. neoformans* in comparison to CMI values of PSZ and 5FC offering a 33% of synergic effect between both drugs and a 67% of additive effect[26,67]. This interaction was correlated with a higher reduction of fungal burden, measured as UFC/ml, in brain and also from invaded tissues in a murine model of cryptococcosis[26,68].

Echinocandins

The alteration in the cell wall architecture induced by echinocandins seems to enhance the action of a second or a third antifungal drug resulting in a simultaneous disruption of the fungal cell wall and cell membrane. In this way, cell stability is reduced causing fungal cells death[32,33,60,61,70,83-85]. Mode of action of echinocandins consists in inhibiting 1,3-β-D-glucan synthase[84]. Echinocandins enhance the access of polyenes to ergosterol in the fungal cell membrane. A positive interaction between CAS and AMB has been described by different methods and confirmed by time-kill studies against *C. parapsilosis* by Barchiesi et al., correlating their findings with an in vivo murine infection model[30]. Also this enhancing effect has been demonstrated between micafungin (MCF) and AMB against different species of *Scedosporium*/*Pseudallescheria*, with 82.4% for *Scedosporium prolificans* and 31.6% for *Scedosporium apiospermum* of synergy effect reducing the minimal effective concentrations (MEC) of individual antifungal drugs in all the isolates[85,86]. MCF combined with AMB produced a synergic interaction against clinical isolates of *Trichosporon ashaii* that were indifferent to combination of TRB plus FNZ[28]. At any case, MICs were reduced when combined drugs were applied. These results correlated with in vivo data in a murine model, in which lower UFC/ml were obtained after combined therapy as well as an absence of fungal elements in pathological study compared with animals with monotherapy[87]. Interaction of MCF and AMB was more effective than that observed with MCF against 37 *Cryptococcus* isolates. FNZ, ITZ, VRZ or RVZ was species dependent while MFC was inactive against all[88].

A synergistic effect of CAS combined with AMB and PSZ or VRZ has been shown not only against *C. glabrata* but also against *C. glabrata* resistant to FNZ and also with a moderate susceptibility to CAS. This combination CAS plus azole drug, including ITZ, has shown active against *A. fumigatus*, other species of *Aspergillus, Fusarium* and *S. brevicaulis*[21,37,38,61,68,89,90]. Oliveira et al. performed one of the studies containing the highest number of clinical isolates (n=119), describing 21% of synergy between CAS and PSZ against *C. glabrata* and 82% of indifference[74].

The synergic and even the additive effect of CAS with VRZ and AMB was also demonstrated against those yeast-like species resistant to echinocandins (*C. neoformans*), *Aspergillus* and other opportunistic moulds[37]. A synergic effect in 87.5% and additive in the 12.5% of *Aspergillus* isolates (*A. flavus*, *A. terreus*, *Aspergillus niger* and *A. fumigatus*) was observed, in the same way that it was obtained with AND[38,90]. Nevertheless, combination of AND plus AMB resulted in indifference or antagonism against *Aspergillus* in a greatest percentage than synergy[90]. This synergy was observed with MICs of all *Aspergillus* isolates against combination TRB plus VRZ were lower than TRB and VRZ alone MICs[70,91]. This has been confirmed in the case of CAS and ITZ or VRZ in the treatment of invasive

pulmonary aspergillosis or CAS and conventional or liposomal AMB in the management of invasive aspergillosis or disseminated aspergillosis, hyalohyphomycosis (*Paecilomyces lilacinus*) and cerebral phaeohyphomycoses (*Cladiophialophora bantiana*)[91]. Yet, FNZ combined with CAS or AND seems to be useful although few results are obtained in murine models of candidiasis but combined CAS and VRZ do not produce better results than monotherapy in animal models of invasive aspergillosis[37,61]. CAS plus RVZ was the most active combination against *S. prolificans*[86]. Combination of CAS and 5FC showed a synergy in more than 60% of *Aspergillus* isolates[38]. The combination of MCF with conventional AMB resulted in a moderate activity. A synergic activity was demonstrated with MCF in combination with AMB against isolates of *Scedosporium* and *Cryptococcus* in vitro and against *C. glabrata* in animal models of disseminated candidiasis, contrary to pulmonary aspergillosis animal model[77]. Good results were obtained with MCF and RVZ against pulmonary aspergillosis infections in rabbits and an animal model of disseminated infection by *Trichosporon asahii*, in which the efficacy of the combination was measured in an increased survival rate and a reduction of the kidney fungal burden over those obtained in the same experimental model with MCF and FNZ[28]. Also data about the lack of antagonism between FNZ and MCF was reported in *C. albicans* and *C. tropicalis*[92]. This combination used in a murine model of disseminated blastoschizomycosis reducing the tissue burden and achieving a 100% of survival rate of animals in comparison with monotherapy[93]. Variations in the interaction of MFC and ITZ was dependent of specie[94]. In this way, up to 50% of synergic interactions were obtained in *A. fumigatus*, *A. flavus*, *A. terreus*, *Fonsecaea* spp. and *Sporothrix schenckii*[94]. Combined treatment with AND and VRZ prolonged the survival in a murine model of disseminated infection by *A. flavus* and reduced the fungal load in comparison with AND alone, and only in a few cases, it improved the results of the VRC monotherapy, although other studies demonstrated the indifference of this combination in the 97% of isolates in some studies[95-97]. The combination of the two drugs and VRC alone reduced the galactomannan levels in serum in comparison with the control group[98]. However, antagonism between AND and azoles has been observed in one isolate of *A. niger*[88].

Terbinafine and other drugs

The combination of TRB with, broad-spectrum triazoles (ITC, RVC, VRZ or albaconazole) resulted in synergy against, *A. fumigatus* and *S. prolificans,* some dimorphic moulds (*Sporothrix schenkii*) and the opportunistic moulds *S. brevicaulis*, *Fusarium*, *Paecilomyces*, or dematiaceous fungi and yeast, such as *C. glabrata*[26,34,37,64,78,89,91,99,100,101]. Concomitant reductions of TRB CMIs were 2-32-fold dilutions for TRB and ITZ[99]. TRB is an ergosterol biosynthesis inhibitor that acts at different targets from azole derivatives (lanosterol 14α-demethylase and squalene epoxidase and lanosterol 14α-demethylase, respectively) and accumulate in skin and adipose tissues[35,37]. Other triazoles, such as FNZ, ITZ or PSZ, can act in a synergic way when combined with TRB, providing good results against *Candida* isolates triazole-resistant *S. prolificans*, *S. brevicaulis* and also *Aspergillus* even when ITS-resistant isolates were tested[27,34,37,89,99]. TRB plus FNZ offered a reduction of MIC ≤2 mg/L and ≤32 mg/L in the 79% and 50% of isolates, respectively for TRB and FNZ of *Candida* isolates with 37.4% of synergy and 62.5% or additive effect[34]. Other studies remark the reduction of the MIC values dilutions for TRB and 4-16 fold dilutions for FNZ when combined against *Aspergillus* isolates[99]. In the same study, the combination of TRB and ITZ also produced a reduction of TRB MIC values ≤2 mg/L in 58% and ITZ ≤0.5 mg/L in 21%[99]. Synergy was found in 58% while combination of TRB plus VRZ produced the highest degree of TRB MICs reduction and the lowest synergic percentage (25%); TRB plus PSZ produced a reduction of TRB MIC values in 62% of isolates to ≤2 mg/L[34]. Even better results of synergy (100%) were found with the combination TRB plus VRZ against dematiaceous fungi, although a higher synergy was obtained with TRB plus AMB (96.5%) and TRB plus ITZ (75.9%)91. Of special interest is the reduction of MIC values for AMB (96.5% against *Fonsecaea pedrosoi*, *Curvularia clavata, Curvularia senegalensis, Curvularia geniculata, Exophiala jeanselmei, Alternaria alternata, Bipolaris* and *Cladophialophora bantiana* when combined with TRB from mean MIC of 4 to 0.125 mg/L[91].

Also, combination of TRB and ITZ provided lower MIC values for mould and also yeast phase of *Sporothrix schenkii* compared with TRB or ITZ alone[101]. The interaction of this combination depended of the phase (mycelial and yeast-like form) obtaining FICI 3 (indifferent) and 5 (additive) with mycelial form to FICI 2 (synergy) and 3 additive[101].

Combination of TRB and AMB had uncertain results against several fungal pathogens, being this combination antagonistic or synergy against some isolates of *A. niger* and *A. fumigatus*[29,37,38,99]. TRB was evaluated with AMB to assess antagonism or synergy in a rabbit animal model of invasive aspergillosis and even TRB had little activity, no antagonism could be demonstrated against AMB in this animal model[39]. Disseminated phaeohyphomycosis is primarily seen in immunocompromised patients, with *S. prolificans* accounting for nearly half of the cases. In several patients without immunodeficiency, prior cardiac surgery was a possible risk factor for endocarditis. Overall mortality was above 70%, and was not significantly different with or without immunodeficiency. Many isolates of dematiaceous fungi are resistant to AMB. ITZ and VRZ have the most consistent and potent activity. PSZ and RVZ also have generally broad spectrum of activity against dematiaceous fungi. There are promising data that the combination of TRB with ITZ or VRZ is synergistic against *S. prolificans*, which is typically resistant to most antifungal agents during therapy[37]. Successful data are available about the use of TRB with VRZ to treat infections due to *S. prolificans*[37]. Combination of TRB plus ITZ evidenced antagonism against FNZ-susceptible *C. dubliniensis* contrary to observations with FNZ-resistant isolates (66.7% of antagonism and 30% of synergy, respectively)[75].

TRB and CAS were tested against *Pythium insidiosum,* obtaining better results of synergy (41.2%, the same than TRB

and FNZ combination) in comparison to those obtained with the combination of TRB and ketoconazole (29.4%) or miconazole (11.8%) and also VRZ and ITZ (17%)[102,103]. Even the combination of TRB with azoles for the treatment of invasive aspergillosis seems promising, being TRB with AMB or with 5FC combinations less effective[99].

Triple combination of antifungal drugs

The combinations of three or more antifungal drugs has also been studied and used in the clinical setting on the basis of the obtained results with the synergic effect of the combination of CAS plus 5FC plus AMB against *Aspergillus*[38]. In that case, synergic effect was also detected in double combinations. Other triple associations, such as CAS and 5FC and VRZ, have shown paradoxical antagonistic and synergy effects against *Aspergillus* based on the absence of a completely synergic effect in dual combinations[38,59]. Associations between VRZ plus AMB plus CAS against *A. fumigatus*, *A flavus* and *A. terreus*, led to the conclusion that in vitro antifungal concentration creates the dynamic and potential success of combination[58,104]. In this way, synergy was observed at low concentrations of VRZ (<0.03 mg/L), AMB (<0.17 mg/L) and intermediate of CAS (0.95-14.9 mg/L), while increased concentrations of antifungal drugs produced antagonism[58]. Presence of a third antifungal affects the dual association of two others: this has been observed with high concentrations of AMB in relation to VRZ plus CAS that reduces its synergy. Similar results have been observed with VRZ in relation to CAS plus AMB combination[58].

Antifungal drugs plus non antifungal drugs

Combination of antifungal drugs with non antimicrobial agents is prolific and data are available with calcineurin inhibitors (cyclosporine A and tacrolimus), proton pump inhibitors, antiarrythmic agents; cholesterol-lowering agents, immunomodulators, antineoplastic drugs, antiparasitic agents, microbial metabolites, human recombinant antibodies[36,37,43-46,49,105-121]. Calcineurin inhibition results lethal for *C. albicans* yeast cells exposed to FNZ[46]. The proposed mechanism consists on the inhibition of ergosterol biosynthesis by FNZ promoting calcineurin inhibitor entrance or avoiding calcineurin production. Even calcineurin is not essential for yeast cells of *C. albicans*, it mediates in cell survival during the FNZ action[46]. These phenomena can be achieved by other azole drugs such as VRZ and PSZ46. Reconstitution of antifungal defence by either exogenous administration of enhancing cytokines or transfusion of allogenic phagocytes treated with enhancing cytokines appears to be a promising combination in addition to antifungal chemotherapy for these difficult-to-treat infections[111,119,120]. The combination of FNZ plus cyclosporine resulted in a fungicidal synergism against *C. albicans* with an unclear mode of action that was not dependent neither on multidrug efflux transporters encoded by *CDR1*, *CDR2*, *CaMDR1* nor on *FLU1* genes[44]. This effect may be related to a higher susceptibility to FNZ due to efflux pump deletion or alteration by cyclosporine, resulting in a fungicidal action of FNZ because cyclosporine alone is not able to inhibit the fungal growth[111]. Haemopoietic growth factors, such as granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF), and Th1 cytokines including interferon-αor granulocyte infusions have a general enhancing activity on the antifungal function of phagocytes and the efficacy of antifungal agents[50,108,111]. These cells exert their antifungal activity by damaging the fungal cell wall and membrane, the target of action of both antifungal metabolites of phagocytes as well as of polyenes, triazoles and echinocandins. Cytokines may collaborate with antifungal drugs in producing larger antifungal effects when they are combined. Some of these combinations with recombinant interferon-gamma have been tested against cryptococcal meningitis, invasive aspergillosis and candidaemia[111]. Antifungal drugs such as polyenes and azoles, which alter the fungal membranes, may render fungi more susceptible to cytokines. Moreover, some antifungal drugs, like AMB and VRZ, may have direct immunomodulatory properties on phagocytes enhancing the conidiocidal and antihyphal activity of pulmonary alveolar macrophages and polymorphonuclear leukocytes against *A. fumigatus* and also the use of itraconazole as a corticosteroid sparing[35]. Triazoles and polymorphonuclear leukocytes synergize to increase *S. prolificans* and *S. apiospermum* damages[122]. Transfusion of cytokine-elicited polymorphonuclear leukocytes can assist recovery from antifungal chemotherapy refractory filamentous fungal infections[123]. Some studies evaluated the safety and efficacy of transfusions of G-CSF-elicited polymorphonuclear leukocytes in patients with invasive fungal infections that were refractory to therapy with AMB alone and showed a favourable outcome in some patients with aspergillosis[123]. A randomized trial to determine the effect of interferon-αplus VRZ in patients with invasive aspergillosis and other filamentous fungal infections is being initiated, suggesting that cytokines can increase the antifungal effect of azole new derivatives against *Candida* spp. and also against those FNZ-resistant and mucormycoses[96]. Mycograb®, a human recombinant antibody to heat shock protein 90 (HSP90) of *Candida* spp, shows an intrinsic antifungal activity and has a synergic effect when combined with AMB or their lipidic or liposomal formulations and caspofungin, both in vitro and in vivo and in humans to treat the invasive candidiasis[36,96,113-116,118,121,124]. Mycograb® has currently been evaluated in a multinational trial in patients suffering invasive candidiasis (*C. albicans, C. krusei* and *C. glabrata*) receiving AMB, with FNZ and CAS for the management of infections produced by *C. neoformans*[36,106,109,114-117,121,124]. However, this study has been stopped without knowing the exact causes. This combination between a monoclonal antibody against HSP[90] and antifungal drug apparently improves the possible toxic effects of drugs and also the inherent risk of resistance to some therapies[36].

Another proposed approach is the combination of antifungal drugs with antibacterial agents[51]. Available data are provided with those antibacterial based on a mode of action related to targets present in both cell models and a collaborative effect. Rifampicin or rifabutin act in the RNA polymerase by inhibiting the transcriptional process. Anti-

fungal molecules with a mode of action at the cell membrane site (AMB or nystatin) could enhance the entering of active concentrations of rifabutin or rifampicin to act at RNA- polymerase. This is possible in *Candida*, *Aspergillus Fusarium*, and *C. neoformans*[37]. Combination of rifampicin and azoles has been dismissed due to the reduction of azole concentration produced by the antibacterial agent[37]. This combination only offers better results than monotherapy in animal model of yeast keratitis opposed to that in pulmonary aspergillosis or fusariosis[37]. Other promising data are obtained with the combination of fluorquinolones (ciprofloxacin, levofloxacin, or ofloxacin) and AMB or the echinocandins[37]. In these cases, different results have been obtained depending on the combination and the animal model infection studied[37]. Synergy between MCF and nikkomycin Z and between MFC and AMB or ITZ has also been described against *A. fumigatus*[57]. In this case, synergy was observed between nikkomycin Z and MFC without AMB as a binary combination depending on the concentration used, with synergic results in experimental histoplasmosis and murine aspergillosis[37,57]. Ternary combination of these drugs has a synergic or antagonistic effect dependent on drug concentrations[57].

Combination of antifungal drugs studied in animal models has provided reliable data to predict their usefulness in the management of human fungal infections, although controversy is present due to the lack of large scale studies and unpredictable antifungal doses[37]. Complexity about the great diversity of animal models to study the combinative antifungal therapy is one of the difficulties to predict or establish the pharmacokinetics and host inflammatory in vivo to correlate the clinical settings[57]. Complexity about in vitro testing is related to the problem of the differences and changes synergism-antagonism observed in some fungal pathogens related to minimal inhibitory concentrations[57].

CONCLUSIONS

Superficial mycoses are very frequent and relatively easy to treat with the available large range of topic and systemic antifungal drugs. However, invasive fungal infections although uncommon are important causes of morbidity and mortality in immunocompromised patients because the high difficulty for a prompt accurate diagnosis and are usually managed with empirical treatment. Treating these infections at an early stage is often essential for a favourable outcome but toxicity and antifungal resistance limit their use.

Antifungal resistance is a real problem in filamentous fungi invasive mycoses, and the appropriate surveillance and research in new antifungal targets and agents is necessary. Hyalohyphomycoses caused by *Fusarium*, *Scedosporium* or *Acremonium*, and mucormycoses have a poor prognosis, consequence of the combination of severe immunosuppression, severity of the underlying diseases and the poor susceptibility of these fungi to current antifungal drugs. Combination therapy could maximize antifungal therapeutic efforts in all those mycoses recalcitrant to current therapy by attacking different fungal targets at the same time. However, real advantages will be probably reached only for particular combinations and in a limited number of mycoses and/or specific patients. Combination tends to reduce clinical failure when resistant strains could be recovered from patients, although drug interactions and cross-resistance may result. Synergy has been established between conventional antifungal agents and also between investigational molecules. Combinations of echinocandins and azoles or AMB with these echinocandins and some azoles. In animal models, combinations between echinocandins and azoles or AMB and the broad-spectrum triazole, VRC or PSC, or echinocandins, CAS, AND and MCF, have been promising for the treatment of invasive candidiasis, aspergillosis and cryptococcosis, and for the therapy of some mycoses caused by recalcitrant filamentous fungi to monotherapy with the same drugs. However, most of the combined antifungal treatments described in humans continue being anecdotal or not supported in blind and controlled clinical studies.

CONFLICT OF INTEREST

In the past 5 years, GQA has received grant support from Astellas Pharma, Gilead Sciences, Pfizer, Schering Plough and Merck Sharp and Dohme. He has been an advisor/consultant to Merck Sharp and Dohme, and has been paid for talks on behalf of Astellas Pharma, Esteve Hospital, Gilead Sciences, Merck Sharp and Dohme, Pfizer, and Schering Plough.

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Table 1 **Interaction mode of double and triple antifungal drug combinations against pathogenic fungi.**

Combination	Fungi	Method	Interactions (%, * number of isolates)				Reference
			Synergic	Aditive	Indifference	Antagonism	
VRZ+FC	*Candida glabrata* (n=20)	Checkerboard	5	-	95	-	83
VRZ+AMB			10	-	90	-	
VRZ+TRB			75	-	25	-	
FNZ+FC	*Cryptococcus neoformans* (n=31)	Checkerboard	8	-	23	-	81
AMB+ITZ	*Paecilomyces variotii* (n=4) *Paecilomyces lilacinus* (n=3)	Checkerboard	-	-	7*	-	100
AMB+VRZ			1*	-	4*	-	
AMB+ABC			1*	-	6*	-	
AMB+TRB			1*	-	4*	-	
AMB+RVZ			-	-	7*	-	
TRB-ITZ			3*	-	4*	-	
TRB-VRZ			6*	-	1*	-	
TRB-ABZ			2*	-	5*	-	
TRB-RVZ			3*	-	3*	-	
TRB-MCF			2*	-	5*	-	
MCF-ITZ			1*	-	6*	-	
MFC-VRZ			2*	-	5*	-	
MCF-ABZ			-	-	7*	-	
MFC-RVZ			-	-	7*	-	
MFC-AMB			-	-	7*		
TRB-ITZ			4*	-	3*	-	
TRB-VRZ			6*	-	1*	-	
TRB-ABZ			2*	-	5*	-	
TRB-RVZ			3*	-	3*	-	
TRB-MFC			2*	-	5*	-	
AMB+FNZ	*Cryptococcus gattii* (n=14)	Checkerboard	-	-	100	-	62
AMB+FNZ	*Cryptococcus neoformans*		-	-	100	-	66
AMB+ITZ			-	-	100	-	
AMB+PSZ			30	-			

Table 1 **Interaction mode of double and triple antifungal drug combinations against pathogenic fungi (cont.)**

AMB+MCF	*Candida krusei* (n=35)	Checkerboard (CMI-0/CMI-1)	26/37	-	74/63	-	87
	Candida albicans (n=35)		8.5/71	-	91.5/29	-	
	Candida parapsilosis (n=15)		40/60	-	60/40	-	
	Candida tropicalis (n=15)		47/53	-	53/47	-	
	Candida dubliniensis (n=20)		35/50	-	65/50	-	
	Candida glabrata (n=15)		-/53	-	100/47	-	
	Candida lusitaniae (n=10)		-/20	-	100/80		
AMB+MCF	*Scedosporium apiospermum* (n=19)	Checkerboard	31.6	-	68.4	-	85
	Scedosporium prolificans (n=17)		82.4	-	17.6	-	
AMB+AND	*Candida albicans* (n=14)	Checkerboard/flow-citometry	6*/7*	-	5*/5*	3/2	32
	C. glabrata (n=8)		3*/2*	-	5*/6*	-	
	C. guilliermondii (n=1)		-	-	1*/1*	-	
	C. krusei (n=2)		1*/-	-	1*/2*	-/-	
	C. lusitaniae (n=1)		1*/1*	-	-/-	-/-	
	C. parapsilosis (n=9)		6*/6*	-	3*/3*	-/-	
	C. tropicalis (n=4)		2*/3*	-	2*/1*	-/-	
AND+ITZ	*A. flavus* (n=8)		8*	-	-	-	90
	A. fumigatus (n=8)		8*	-	-	-	
	A. niger (n=5)		-	-	4*	1*	
	A. terreus (n=5)		2*	-	3*	-	
	F. oxysporum (n=2)		-	-	2*	-	
	F. solani (n=5)		-	-	5*	-	
AND-VRZ	*A. flavus* (n=8)		7*	-	1*	-	
	A. fumigatus (n=8)		8*	-	-	-	
	A. niger (n=5)		-	-	5*	-	
	A. terreus (n=5)		3*	-	2*	-	
	F. oxysporum (n=2)		-	-	2*	-	
	F. solani (n=5)		-	-	5*	-	
AND-AMB	*A. flavus* (n=8)		-	-	5*	3*	
	A. fumigatus (n=8)		5*	-	3*	-	
	A. niger (n=5)		-	-	5*	-	
	A. terreus (n=5)		-	-	5*	2*	
	F. oxysporum (n=2)		-	-	2*	-	
	F. solani (n=5)		-	-	5*	-	

Table 1	Interaction mode of double and triple antifungal drug combinations against pathogenic fungi (cont.)						
FNZ+MCF	*C. albicans* (n=15)	Checkerboard (CMI-2)	33	-	67	-	92
	C. dubliniensis (n=20)		-	-	100	-	
	C. glabrata (n=15)		7	-	93	-	
	C. krusei (n=20)		-	-	100	-	
	C. lusitaniae (n=10)		-	-	100	-	
	C. parapsilosis (n=10)		-	-	100	-	
	C. tropicalis (n=15)		26	-	74	-	
ITZ+MCF	*C. parapsilosis* (n=25)	Checkerboard (CMI-2)	12	-	88	-	113
	C. albicans (n=20)		50	-	50	-	
	C. dubliniensis (n=20)		15	-	85	-	
	C. krusei (n=20)		15	-	85	-	
	C. tropicalis (n=10)		20	-	80	-	
	C. lusitaniae (n=10)		20	-	80	-	
AND+FNZ	*Candida albicans* (n=16)	Checkerboard/flow-citometry	10*/10*	-	6*76*	-/-	32
	C. glabrata (n=9)		2*/4*	-	7*/5*	-/-	
	C. krusei (n=1)		1*/1*	-	-/-	-/-	
	C. parapsilosis (n=7)		4*/6*	-	2*/-	1*/1*	
	C. tropicalis (n=3)		2*/2*	-	1*/1*	-/-	
AMB-MCF	*Cryptococcus neoformans* (n=10)	Checkerboard	7*	-	3*	-	88
	Cryptococcus gattii (n=10)		8*	-	2*	-	
	*Cryptococcus albidus (*n=10)		5*	-	5*	-	
	Cryptococcus laurentii (n=7)		6*	-	1*	-	
FNZ-MCF	*Crypotococcus neoformans* (n=10)	Checkerboard	3*	-	7*	-	88
	Crypotococcus gatii (n=10)		2*	-	8*	-	
	*Crypotococcus albidus (*n=10)		4*	-	6*	-	
	Crypotococcus laurentii (n=7)		3*	-	4*	-	
ITZ-MCF	*Crypotococcus albidus (*n=10)	Checkerboard	3*	-	7*	-	88
	Crypotococcus laurentii (n=7)		3*	-	4*	-	
	*Crypotococcus albidus (*n=10)	Checkerboard	5*	-	5*	-	
	Crypotococcus laurentii (n=7)		3*	-	4*	-	
PSZ-CAS	*C. glabrata* (n=119)	Checkerboard	18	-	82	-	74

Table 1 **Interaction mode of double and triple antifungal drug combinations against pathogenic fungi (cont.)**

TRB-FNZ	*C. glabrata* (n=14)	Checkerboard	33.3	66.6	-	-	34
TRB-ITZ			37.5	62.5	-	-	
TRB-VRZ			58	42	-	-	
TRB-PSZ			25	-	75	-	
TRB-ITZ	*Pythium insidiosum* (n=30)	Checkerboard	5	-	25	-	102
TRB-VRZ			5	-	25	-	
TRB-FNZ	*C. albicans* (n=5)	Checkerboard	80	-	10	-	27
	C. glabrata (n=5)		80	-	20	-	
	C. tropicalis (n=5)		80	-	20	-	
	C. krusei (n=5)		-	-	100	-	
TRB-VRZ	*C. albicans* (n=5)	Checkerboard	80	-	20	-	27
	C. glabrata (n=5)		80	-	20	-	
	C. tropicalis (n=5)		80	-	20	-	
	C. krusei (n=5)		20	-	80	-	
TRB-ITZ	*S. schenkii* (n=8) yeast-like phase ITZ-resistant	Checkerboard	-	50	50	-	101
	S. schenkii (n=8) miceliar phase ITZ-resistant		25	50	50	-	
	S. schenkii (n=8) yeast-like phase ITZ-susceptible		-	25	75	-	
	S. schenkii (n=8) miceliar phase ITZ-susceptible		25	-	75	-	
CAS-FC	*Aspergillus fumigatus* + *A. terreus* (n=16)	Checkerboard	62	38	-	-	38
VRZ-FC			-	7	-	93	
CAS-AMB			-	100	-	-	
CAS-VRZ			-	100			
CAS-AMB-FC			100	-	-	-	
CAS-VRZ-FC			67	33	-	50	

AMB: amphotericin B; FC: flucytosine; FNZ: fluconazole; ITZ: itraconazole: MFC: micafungin; PSZ: posaconazole; VRZ: voriconazole; RVZ, ravuconazole; ABZ: albaconazole; CAS: caspofungin; TRB: terbinafine; AND: anidulafungin

Table 2 **Variation of MIC values of single antifungal drugs and combined (mg/L)**

Combination	Pathogen	gMIC single drugs	gMIC combination	Method	Reference
FC+FNZ	*Cryptococcus neoformans* (n=31)	3.4 / 3.2	0.44 / 1.02	Checkerboard	
FC+VRZ	*Candida albicans* (n=20)	0.03 / 0.2	0.02 / 0.02	Checkerboard	12
TRB+VRZ		9.8 / 0.2	1.2 / 0.05		
AMB+VRZ		1.5 / 0.2	0.39 / 0.08		
AMB+FNZ	*Cryptococcus neoformans*	0.69 / 4.19	0.066 / 1.65	Checkerboard	66
AMB+ITZ		0.69 / 0.41	0.1 / 0.17		
AMB+PSZ		0.69 / 0.45	0.15 / 0.11		
AMB+MCF	*Candida kusei* (n=35)	2.11 / 0.96	0.81 / 0.15	Checkerboard (MIC-0)	87
	C. albicans (n=35)	0.73 / 0.31	0.36 / 0.08		
	C. parapsilosis (n=15)	2 / 14.94	0.67 / 1.15		
	C. tropicalis (n=15)	1.66 / 1.44	0.49 / 0.12		
	C. dubliniensis (n=20)	0.41 / 1.46	0.14 / 0.29		
	C. glabrata (n=15)	0.75 / 0.27	0.31 / 0.11		
	C. lusitaniae (n=10)	0.44 / 1.18	0.24 / 0.60		
AMB+MCF	*Candida kusei* (n=35)	1.25 / 0.65	0.49 / 0.10	Checkerboard (MIC-2)	87
	C. albicans (n=35)	0.7 / 0.23	0.16 / 0.04		
	C. parapsilosis (n=15)	0.74 / 9.28	0.22 / 0.16		
	C. tropicalis (n=15)	0.87 / 0.47	0.17 / 0.06		
	C. dubliniensis (n=20)	0.14 / 0.32	0.08 / 0.07		
	C. glabrata (n=15)	0.69 / 0.24	0.23 / 0.06		
	C. lusitaniae (n=10)	0.35 / 0.86	0.19 / 0.31		
AMB+MCF	*S. apiospermum* (n=19)	4,62 / 5.76	1.15 / 1.43	Checkerboard	85
	S. prolificans (n=17)	11.54 / >32	2.66 / 6.23		
FNZ+MCF	*C. albicans* (n=15)	0.87 / 0.25	0.39 / 0.07	Checkerboard	92
	C. dubliniensis (n=20)	0.27 / 0.38	0.26 / 0.36		
	C. glabrata (n=15)	6.65 / 0.13	2.2 / 0.05		
	C. krusei (n=20)	47.8 / 1.55	8.3 / 0.66		
	C. lusitaniae (n=10)	0.61 / 0.92	0.37 / 0.17		
	C. parapsilosis (n=10)	0.61 / 12.13	0.53 / 6		
	C. tropicalis (n=15)	1.57 / 0.16	0.57 / 0.11		

Table 2 **Variation of MIC values of single antifungal drugs and combined (mg/L) (cont.)**

ITZ+MCF	*C. parapsilosis* (n=25)	0.16 / 6.42	0.1 / 0.36	Checkerboard	113
	C. albicans (n=20)	0.8 / 0.31	0.11 / 0.1		
	C. dubliniensis (n=20)	0.41 / 1.18	0.26 / 0.47		
	C. krusei (n=20)	0.11 / 0.25	0.01 / 0.05		
	C. tropicalis (n=10)	0.36 / 0.28	0.29 / 0.16		
	C. lusitaniae (n=10)	0.15 / 0.94	0.06 / 0.31		
TRB+CAS	*Pythium insidiosum* (n=17)	14.7 / 19.6	0.42 / 5.77		103
TRB+MCZ		14.7 / 13.6	0.81 / 5.77		
TRB+KTZ		14.7 / 23.1	10.2 / 0.66		
TRB+FBZ		14.7 / 59	1.44 / 3.54		
TRB+ITZ	*Pythium insidiosum* (n=30)	4 / >16	0.56 / 3.17	Checkerboard	102
TRB+VRZ		4 / >16	0.61 / 3.48		
TRB+FNZ	*Candida glabrata* (n=24)	>8 / ≥64	1.059 / 31.09	Checkerboard	34
TRB+ITZ		>8 / 1.88	0.97 / 0.68		
TRB+VRZ		>8 / 3.77	0.98 / 0.97		
TRB+PSZ		>8 / 1.78	1.56 / 0.68		
TRB+FNZ	*C. albicans* (n=5)	9.19 / 18.38	0.33 / 0.06		27
	C. glabrata (n=5)	>16 / 24.25	1.51 / 3.48		
	C. tropicalis (n=5)	27.8 / 48.5	0.5 / 1.32		
	C. krusei (n=5)	>16 / 74.51	0.57 / 64		
TRB+VRZ	*C. albicans* (n=5)	10.55 / 1.54	0.43 / 0.003		
	C. glabrata (n=5)	>16 / 2.64	1.15 / 0.57		
	C. tropicalis (n=5)	>16 / 0.56	3.03 / 0.002		
	C. krusei (n=5)	>16 / 0.57	0.25 / 0.33		
TRB+ITZ	*Sporothrix sheckii* (n=8) fase miceliar Resistente a ITZ	0.21 / 4.76	0.24 / 0.15		101
	Sporothrix sheckii (n=8) fase levadura Resistente a ITZ	0.71 / 1.68	0.6 / 0.1		
	Sporothrix sheckii (n=8) fase miceliar Sensible a ITZ	0.18 / 2.38	0.25 / 0.5		
	Sporothrix sheckii (n=8) fase levadura Sensible a ITZ	0.84 / 0.59	0.21 / 0.21		

Table 2 Variation of MIC values of single antifungal drugs and combined (mg/L) (cont.)

TRB+VRZ	*Fonseca pedrosoi* (n=8)	0.25-4.1 / 0.06-0.12	<0.002 / <0.008	70
	Curvularia Clavata (n=1)	2.05 / 0.512	0.008 / 0.016	
	C. senegalensis (n=1)	4.1 / 0.256	0.008 / 0.016	
	C. geniculata (n=1)	4.1 / 0.512	0.004 / 0.004	
	C. lunata (n=4)	0.008-2.05 / 0.06-0.25	<0.008 / 0.016	
	Exophiala jeanselmei (n=6)	1.02 / 0.06-0.51	<0.002 / <0.008	
	Alternaria alternata (n=5)	0.016-4.1 / 0.25-1.02	<0.004 / 0.128	
	Bipolaris spp. (n=2)	1.02 / 0.256	0.008 / <0.016	
	Cladiophialophora bantiana (n=1)	8.2 / 2.04	0.008 / 0.016	
TRB+ITZ	*Fonseca pedrosoi* (n=8)	0.25-4.1 / 0.25-0.5	<0.004 / >0.062	
	Curvularia Clavata (n=1)	2.05 / 0.25	0.008 / 0.062	
	C. senegalensis (n=1)	4.1 / 0.5	0.008 / 0.062	
	C. geniculata (n=1)	4.1 / 0.125	0.004 / 0.062	
	C. lunata (n=4)	0.008-2.05 / 0.25-8	<0.008 / <0.25	
	Exophiala jeanselmei (n=6)	1.02 / 0.25-8	<1 / 0.062	
	Alternaria alternata (n=5)	0.016-4.1 / 0.125-1	<0.004 / <0.25	
	Bipolaris spp. (n=2)	1.02 / 2	0.008 / 0.062	
	Cladiophialophora bantiana (n=1)	8.2 / 0.25	0.008 / 0.062	

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