Antibiotics in Combination with Antifungals to Combat Drug Resistant Candida – A Concept on Drug Repurposing

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Authors’ contributions

This work was carried out in collaboration among all authors. Author HM designed the study, performed the statistical analysis and wrote the protocol and the first draft of the manuscript. Authors RS, KVL and VB managed the analysis of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2020/v20i830274

Editor(s):

(1) Dr. Muhsin Jamal, Abdul Wali Khan University, Pakistan.

Reviewer(s):

(1) Lauana Aparecida Santos, Federal University of Alfenas, Brazil.
(2) Davi Porfírio da Silva, Federal University of Alagoas, Brazil.

Complete Peer review History: http://www.sdiarticle4.com/review-history/60895

Received 26 June 2020
Accepted 02 September 2020
Published 09 September 2020

ABSTRACT

Candida spp. are emerging pathogens of hospital-acquired infections that causes invasive candidiasis, leading to human mortality and morbidity. Evolution of resistance to different groups of antifungal drugs is a major concern nowadays. Resistance mechanism in some of the antifungal drugs are formation of biofilms, alterations in drug target and lowered intracellular drug levels caused by efflux pumps. Introduction of novel strategies are necessitous to eliminate the phenomenon of drug resistance. Drug repurposing is a promising therapeutic strategy that can improve the efficacy of antifungal therapy for invasive candidiasis. Antibiotics and antifungal drugs were combined against resistant Candida spp. and the in vitro antifungal synergy were analyzed by disk diffusion methods, checkerboard microbroth dilution method and time-kill curves. Synergistic effects were seen against drug-resistant strains, but drug-susceptible strains show indifferent effects in experimental studies. Profiting from the synergistic effects of combination therapy, alternative therapeutic approaches for drug resistance could be designed. This review will discuss different antifungal drugs and their mechanism, drug-resistance mechanisms and some of the antibiotic and antifungal combinations that provide novel insights in treating invasive fungal infections.

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Keywords: Antibiotics; antifungal drugs; Candida; checkerboard assay; combination; drug resistance; in vitro interactions.

1. INTRODUCTION

*Candida* spp. are a group of opportunistic pathogens that are responsible for a spectrum of diseases ranging from superficial to invasive infections, leading to high mortality and morbidity in a huge population worldwide [1,2]. *Candida* spp., *Cryptococcus* spp., *Pneumocystis* spp. and *Aspergillus* spp. are the main fungi, accounting for an estimated 90% of human mortality cases [3]. The main cause of invasive candidiasis is colonization of the mucous membranes and skin, modifications in natural barriers of host like soft skin infections and indwelling catheters insertion [4,5,6].

The most common form of invasive candidiasis is candidemia. During 2009-2013, incidence of candidemia declined but in 2013-2017, it was stable at about 9 cases per 100,000 population as per American Centre for Disease Control (CDC) 2018 [7]. Candidemia rates have decreased significantly in infants, but were highest among people aged 65 and older [8,9]. The cause of candidemia rate declination in infants may due to prophylaxis of fluconazole in high-risk premature babies or hand hygiene, catheter care and other factors in improved infection control practices [10]. Rates of candidemia are almost double times higher in blacks in comparison with non-blacks due to incongruity in underlying conditions, socioeconomic status and several other factors [10].

*Candida albicans* is the predominant fungus causing invasive candidiasis but majority of the resistance is seen in *Candida auris, Candida glabrata,* and *Candida parapsilosis* [11]. *Candida* is emerging resistance to antifungal drugs (both 1st line and 2nd line), such as echinocandins (micafungin, caspofungin, anidulafungin), amphotericin B and fluconazole [10]. Among *Candida* spp., an emerging multidrug-resistant species is *C. auris*, causing serious infections and spreading easily in healthcare provisions as per CDC 2018. In United States, 90% of *C. auris* were resistant to fluconazole. Among candidemia isolates tested at CDC, 7% were fluconazole resistant in which *C. glabrata* and *Candida krusei* were more common (70%) [12,13].

Innovative and newer treatment strategies must be developed to combat invasive candidiasis as antifungal resistance is a severe harm staring at public health. Formation of biofilms, overexpression of multidrug efflux pumps, abnormalities in the chromosome, the capability to escape immune defenses of host and spontaneous mutations are the aspects responsible to confer antifungal resistance [14].

2. ANTIFUNGAL DRUGS AND THEIR MECHANISM

The common antifungal drugs employed for invasive candidiasis are: (i) polyenes (nystatin, amphotericin B), (ii) azoles (voriconazole, fluconazole, posaconazole, itraconazole), (iii) echinocandins (micafungin, anidulafungin, caspofungin) and (iv) pyrimidine analogues (5-flucytosine).

2.1 Polyenes

Polyenes are heterocyclic amphipathic molecules that target ergosterol (major sterol in the fungal cell membrane) [14]. Amphotericin B and nystatin are natives to this group. They cause cell death by entering into the phospholipid bilayers and permitting tiny particles to leak out of the membrane by generating pores in the cell membrane [15].

2.2 Azoles

Azoles are synthetic heterocyclic compounds and inhibitors of fungal cytochrome P450 14α-lanosterol demethylase enzyme, encoded by CYP51 or ERG11 gene that catalyze ergosterol biosynthesis. This reduces the ergosterol content in fungal cell membrane, thus toxic intermediates of methylation get accumulated resulting in destruction of the function and division of fungal membrane leading to cell death [16]. The triazoles (fluconazole, itraconazole, voriconazole and posaconazole) act as a fungicide against Aspergillus but are fungistatic in action against Candida [14].

2.3 Echinocandins

Echinocandins are semi-synthetic, cyclic lipopeptides used as the first-line drug for candidiasis. Clinically used echinocandins are
caspofungin, micafungin, and anidulafungin. The above-mentioned drugs act as a fungicide in vitro towards Candida spp. They act by inhibition of β-(1,3) glucan synthases (an enzyme-complex in the fungal cell membrane) thus reducing the synthesis of β-(1,3) glucan [15].

2.4 Pyrimidine Analogues

Pyrimidine analogues are nucleoside analog antimetabolites that acts by disrupting pyrimidine metabolism in the fungal cell nucleus by its fungistatic activity. 5-Flucytosine works by conversion to 5-FU (5-fluorouracil) by an enzyme-cytosine deaminase, inhibiting cell division by incorporating into DNA and RNA. Combination of 5-flucytosine and other antifungal agents (e.g. amphotericin B) confer higher resistance than in monotherapy [15].

3. RESISTANCE MECHANISM OF ANTIFUNGAL DRUGS

Antifungal drug resistance is said to be when a fungus is non-susceptible to an antifungal drug and the drug’s MIC (Minimum Inhibitory Concentration) value is higher than the susceptibility breakpoint for that species during in vitro susceptibility testing. The antifungal drug resistance is divided into two main categories: (i) Clinical or intrinsic resistance and (ii) in vitro resistance (primary resistance and secondary or acquired resistance).

3.1 Polyenes

Amphotericin B (AMB) resistance results from low concentration of fungal membrane ergosterols due to mutations in the ERG3 gene (gene encoding C-5 sterol desaturase enzyme) and increased catalase activity causing reduction in susceptibility to oxidative damage [16,17]. AMB resistance is not common among C. albicans isolates [18].

3.2 Azoles

High level resistance to azoles in C. albicans is due to overexpression of cell membrane efflux pumps that lowers the concentration of drugs, overexpression or upregulation of CDR1 and CDR2 genes, point mutation in ERG11 gene that prevents drug from binding to the target by decreasing binding site affinity and modification of the Erg11 (target enzyme) [14]. FLC resistance is mainly due to upregulation of MDR1 gene. The Mr1 (multidrug resistant regulator 1) is one of the factors that mediates transcription, controls expression and upregulation of MDR1 in drug resistant Candida [19].

3.3 Echinocandins

Resistance to echinocandins in Candida spp. resulted from point mutations in the FKS1 gene, which is a subunit of β-(1,3)-glucan synthase and FKS2 gene that induces amino acid substitutions [20]. Additional mutations in both genes (FKS1 and FKS2) were identified in many Candida isolates at positions - 645 (Serine), S645P (serine to proline), S645Y (serine to tyrosine) and S645F (serine to phenylalanine) [21,22]. Hot spot mutations have chances of conferring more resistance to caspofungin compared to micafungin or anidulafungin.

3.4 Pyrimidine Analogues

5-Flucytosine resistance results from mutations in the uracil phosphoribosyl transferase (Fur1p) enzyme that converts 5-FU to 5-fluorouridine 5'-monophosphate [23].

4. AMALGAMATION OF ANTIBIOTIC AND ANTIFUNGAL DRUGS

Antibiotics are often combined with antifungal drugs, simultaneously or sequentially to confer enhanced efficacy and specificity of presently available drugs that can combat drug resistant fungi. Some of the drug combinations and their in vitro interactions that show considerable promise in treating invasive candidiasis are discussed below.

4.1 Combination of Gentamicin (GM) and Azoles

GM (a conventional aminoglycoside antibiotic) was combined with triazoles such as itraconazole (ITZ), voriconazole (VRC) and fluconazole (FLC), against resistant C. albicans [24]. Antifungal susceptibility testing was done by broth microdilution followed by checkerboard assay. The minimum inhibitory concentration (MIC) results showed reduction from 16 μg/mL to 0.25 μg/mL for ITZ, from 16 μg/mL to 0.03 μg/mL for VRC and from 512 μg/mL to 1 μg/mL for FLC. Their study proved that the GM combination with azoles indicated an in vitro strong synergism against the resistant C. albicans. GM in
combination with FLC is susceptible against biofilms of *Candida albicans*. The results revealed a synergism against planktonic cells of drug-resistant *Candida albicans* with FICI-fractional inhibitory concentration index of about 0.13 to 0.14.

4.2 Combination of Aspirin and Amphoterican B

Aspirin (also called ASA-acetylsalicylic acid) was combined with amphoterican B (a polyene macrolide, AMB) against biofilms and planktonic cells of resistant *C. albicans* and *C. parapsilosis* [25]. The *in vitro* interactions were analyzed by the checkerboard microdilution method followed by time-kill test, obtained data was analyzed using Loewe additivity-based model (FICI) and Bliss Independence-based model (ΔE). Their results suggest that strong synergism was analyzed by FICI against biofilms while indifferent synergistic impacts were found against planktonic cells of *C. albicans*. Further studies state that the biofilm formation in *C. glabrata*, *C. parapsilosis*, *C. guilliermondii* and *Candida kefyr* is also suppressed by ASA [26].

4.3 Combination of Tacrolimus (FK506) and Azoles

*In vitro* synergy between FK506, a macrolide antibiotic and azoles (ITZ, VRC, FLC) against resistant *Candida albicans* were evaluated by method of checkerboard microdilution based on antifungal growth and time-killing test by XTT reduction assay and colony counting [27,28]. Their studies showed that azole-sensitive strains had synergistic and impassive impacts, but high synergy was seen against azole-resistant strains in FICI analysis. Their studies demonstrated that the property of azoles that target biosynthesis of ergosterols can be improved *in vitro* by FK506 (calcineurin pathway inhibitor) mainly against azole-resistant *C. albicans*.

4.4 Combination of Minocycline and Fluconazole

*In vitro* interaction between minocycline (a second-generation semi-synthetic tetracycline antibiotic) and fluconazole against both FLC-sensitive and FLC-resistant *Candida albicans* were investigated using nonparametric models of FICI model and ΔE model [29]. The combination mechanisms were evaluated by assessing their effect on biofilms of *C. albicans*, uptake and efflux of FLC and intracellular calcium parity. Their study suggests that there is a strong synergism against fluconazole-resistant *C. albicans*.

4.5 Combination of Aminoglycoside K20 and Azoles

Amphiphilic aminoglycoside K20, an artificial derivative of kanamycin A antibiotic combined with azoles (FLC, ITZ, VRC, clotrimazole-CTZ, Posaconazole-POS) against resistant *C. albicans* and *Cryptococcus neoformans* were analyzed using checkerboard microbrot dilution method, disk diffusion methods and time-kill curves [30]. Their results revealed that K20 and all azoles were synergistic inhibitors against azole-resistant *C. albicans*. K20 has synergistic inhibitory activities with four (FLC, ITZ, CTZ, POS) and three (FLC, ITZ, VRC) azoles against *C. tropicalis* and *Candida lusitaniae*, respectively.

4.6 Miscellaneous Drug Combinations

Amphiphilic tobramycin analogues (C12 and C14) were combined with azoles (POS, VOR, FLC and ITC) *in vitro* against *C. albicans* to investigate their synergistic activities and they reported that the combination effects of these drugs were non-toxic to mammalian cells [31]. *In vitro* antifungal synergy between doxycycline (tetracycline antibiotic) and fluconazole were tested against resistant *C. albicans* biofilms; their results concluded that combination testing exhibited higher antifungal effects than testing of single drugs [32,33]. Both *in vitro* and *in vivo* antifungal synergy of linezolid (oxazolidinone antibiotic) and azoles (FLC, ITZ and VRC) were evaluated and they suggested that this combination might be a new therapeutic approach for *C. albicans* resistance [34]. Some of the non-antibiotic and antifungal drug combinations against resistant *Candida* spp. are licofelone and FLC [35], fluoxetine and azoles [36], cyclosporine A and FLC [37,38], ribavirin and fluconazole [39], D-Penicillamine and FLC [40], N-butylnaphthalide and FLC [41] and gypenosides and FLC [42].

5. CONCLUSION

Resistance to antifungal drugs is a serious global threat prevalent in medical arena. The drug repurposing can be a better diagnostic tool that can safeguard and help in the survival of invasive candidiasis as it can combat the antifungal drug resistance. The amalgamation of antibio and antifungal drugs can eliminate the problems of
Candida resistance in antimicrobial therapy and helps in discovery of new antifungals. Based on above discussion, GM-FLC, aminoglycoside K20-azole, linezolid-azoles and FK506-azoles seems to be some of the promising combinations. Studies on in vitro interactions alone cannot help in bringing the combination therapy into clinical use, so significant researches have to be done on in vivo interactions to develop narrow spectrum antifungals with improved activity. More investigations are necessitous in drug repurposing as it is a promising therapeutic strategy.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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