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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.

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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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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 e□ux 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 e□ux 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 Mrr1 (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 Amphotericin B

Aspirin (also called ASA-acetylsalicylic acid) was combined with amphotericin 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 (∆E). 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 ∆E 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 microbroth 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 (C_{12} and C_{14}) 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-butylphthalide 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 antibiotic 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-azoles, 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.

REFERENCES

1. Spitzer M, Robbins N, Wright GD. Combinatorial strategies for combating invasive fungal infections. Virulence. 2016; 8(2):169-185.
2. Azevedo MM, Teixeira-Santos R, Silva AP, Cruz L, Ricardo E, Pina-Vaz C et al. The effect of antibacterial and non-antibacterial compounds alone or associated with antifungals upon fungi. Frontiers in Microbiology. 2015;6(669):669.
3. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden Killers: human fungal infections. Science Translational Medicine. 2012;4(165): 165rv13.
4. Costa-De-Oliveira S, Pina-Vaz C, Mendonça D, Rodrigues AG. A first Portuguese epidemiological survey of fungaemia in a university hospital. European Journal of Clinical Microbiology & Infectious Diseases. 2008;27(5):365-374.
5. Dimopoulos G, Karabinis A, Samonis G, Falagas ME. Candidemia in immune-compromised and immunocompetent critically ill patients: A prospective comparative study. European Journal of Clinical Microbiology & Infectious Diseases. 2007;26(6):377-384.
6. Blumberg H, Jarvis W, Soucie J, Edwards J, Patterson J, Pfaller M et al. Risk factors for *Candida* bloodstream infections in surgical intensive care unit patients: The NEMIS prospective multicenter study. Clinical Infectious Diseases. 2001;33(2): 177-186.
7. Magill SS, Wilson LE, Thompson DL, Ray SM, Nadle J, Lynfie,ld R et al. Reduction in the Prevalence of Healthcare-Associated Infections in U.S. Acute Care Hospitals, 2015 vs 2011. Open Forum Infectious Diseases. 2017;4(1):S49.
8. Benedict K, Roy M, Kabbani S, Anderson EJ, Farley MM, Harb S, et al. Neonatal and Pediatric Candidemia: Results from Population-Based Active Laboratory Surveillance in Four US Locations, 2009–2015. Journal of the Pediatric Infectious Diseases Society. 2018;7(3):e78-e85.
9. Cleveland AA, Farley MM, Harrison LH, Stein B, Hollick R, Lockhart SR et al. Changes in Incidence and Antifungal Drug Resistance in Candidemia: Results from Population-Based Laboratory Surveillance in Atlanta and Baltimore, 2008-2011. Clinical Infectious Diseases. 2012;55(10): 1352-1361.
10. CDC. Centers for Disease Control and Prevention. Fungal Diseases (Invasive Candidiasis Statistics); 2018.
11. Toda M, Williams SR, Berkow EL, Farley MM, Harrison LH, Bonner L, et al. Population-Based Active Surveillance for Culture-Confirmed Candidemia — Four Sites, United States, 2012–2016. MMWR. Surveillance Summaries. 2019;68(8):1-15.
12. Lockhart SR, Iqbal N, Cleveland AA, Farley MM, Harrison LH, Bolden CB et al. Species identification and antifungal susceptibility testing of *Candida* bloodstream isolates from population-based surveillance studies in two U.S. Cities from 2008 to 2011. Journal of Clinical Microbiology. 2012;50(11):3435-3442.
13. Vallabhaneni S, Westercamp M, Cleveland A, Farley MM, Harrison L, Schaffner W, et al. Emergence of echinocandin and multi-drug resistant *Candida glabrata* Bloodstream Infection: Data from a Large Multi-site Population-Based Candidemia Surveillance Program. Open Forum Infectious Diseases. 2014;1(1):S383.
14. Costa-De-Oliveira S, Rodrigues AG. *Candida albicans* antifungal resistance and tolerance in bloodstream infections: The Triad Yeast-Host-Antifungal. Micro-organisms. 2020;8(2):154.
15. Peman J, Canton E, Espinel-Ingroff A. Antifungal drug resistance mechanisms.

Expert Review of Anti-infective Therapy. 2009;7(4):453-460.

16. Sokol-Anderson ML, Brajtburg J, Medoff G. Amphotericin B-induced oxidative damage and killing of *Candida albicans*. Journal of Infectious Diseases. 1986; 154(1):76-83.
17. Kelly S, Lamb D, Kelly D, Manning N, Loeffler J, Hebart H et al. Resistance to fluconazole and cross-resistance to amphotericin B in *Candida albicans* from AIDS patients caused by defective sterol Δ5,6-desaturation. FEBS Letters. 1997; 400(1):80-82.
18. Arendrup M. Update on antifungal resistance in *Aspergillus* and *Candida*. Clinical Microbiology and Infection. 2014; 20(6):42-48.
19. Morschhauser J, Barker KS, Liu TT, BlaB-Warmuth J, Homayouni R, Rogers PD. The transcription factor Mrr1p controls expression of the MDR1 efflux pump and mediates multidrug resistance in *Candida albicans*. PLoS Pathogens. 2007;3(11): e164.
20. Park S, Kelly R, Kahn JN, Robles J, Hsu M, Register E, et al. Specific substitutions in the echinocandin target Fks1p account for reduced susceptibility of rare laboratory and clinical *Candida* sp. isolates. Antimicrobial Agents and Chemotherapy. 2005;49(8):3264-3273.
21. Perlin DS. Resistance to echinocandin-class antifungal drugs. Drug Resistance Updates. 2007;10(3):121-130.
22. Garcia-Effron G, Park S, Perlin DS. Correlating echinocandin MIC and kinetic inhibition of fks1 mutant glucan synthases for *Candida albicans*: Implications for Interpretive Breakpoints. Antimicrobial Agents and Chemotherapy. 2008;53(1): 112-122.
23. Akins RA. An update on antifungal targets and mechanisms of resistance in *Candida albicans*. Medical Mycology. 2005;43(4): 285-318.
24. Lu M, Yu C, Cui X, Shi J, Yuan L, Sun S. Gentamicin synergises with azoles against drug-resistant *Candida albicans*. International Journal of Antimicrobial Agents. 2018;51(1):107-114.
25. Zhou Y, Wang G, Li Y, Liu Y, Song Y, Zheng W et al. *In vitro* Interactions between aspirin and amphotericin B against planktonic cells and biofilm cells of *Candida albicans* and *C. parapsilosis*. Antimicrobial Agents and Chemotherapy. 2012;56(6):3250-3260.
26. Stepanovic S, Vukovic D, Jesic M, Ranin L. Influence of acetylsalicylic acid (aspirin) on biofilm production by *Candida* Species. Journal of Chemotherapy. 2004;16(2):134-138.
27. Sun S, Li Y, Guo Q, Shi C, Yu J, Ma L. *In vitro* interactions between Tacrolimus and Azoles against *Candida albicans* determined by different methods. Antimicrobial Agents and Chemotherapy. 2007;52(2):409-417.
28. Maesaki S, Marichal P, Hossain MA, Sanglard D, Bossche HV, Kohno S. Synergic effects of tacrolimus and azole antifungal agents against azole-resistant *Candida albicans* strains. Journal of Antimicrobial Chemotherapy. 1998;42(6): 747-753.
29. Shi W, Chen Z, Chen X, Cao L, Liu P, Sun S. The combination of minocycline and fluconazole causes synergistic growth inhibition against *Candida albicans*: An *in vitro* interaction of antifungal and antibacterial agents. FEMS Yeast Research. 2010;10(7):885-893.
30. Shrestha SK, Grilley M, Anderson T, Dhiman C, Oblad J, Chang CT et al. In vitro antifungal synergy between amphiphilic aminoglycoside K20 and azoles against *Candida* species and *Cryptococcus neoformans*. Medical Mycology. 2015;53(8):837-844.
31. Shrestha SK, Fosso MY, Garneau-Tsodikova S. A combination approach to treating fungal infections. Scientific Reports. 2015;5(1):17070.
32. Gao Y, Zhang C, Lu C, Liu P, Li Y, Li H et al. Synergistic effect of doxycycline and fluconazole against *Candida albicans* biofilms and the impact of calcium channel blockers. FEMS Yeast Research. 2013; 13(5):453–462
33. Gao Y, Li H, Liu S, Zhang X, Sun S. Synergistic effect of fluconazole and doxycycline against *Candida albicans* biofilms resulting from calcium fluctuation and downregulation of fluconazole-inducible efflux pump gene overexpression. Journal of Medical Microbiology. 2014;63(7):956-961.
34. Lu M, Yang X, Yu C, Gong Y, Yuan L, Hao L et al. Linezolid in Combination with azoles induced synergistic effects against *Candida albicans* and protected *Galleria mellonella* against experimental

Candidiasis. Frontiers in Microbiology. 2019;9:3142.
35. Liu X, Li T, Wang D, Yang Y, Sun W, Liu J et al. Synergistic antifungal effect of fluconazole combined with licofelone against resistant *Candida albicans.* Frontiers in Microbiology. 2017;8:2101.
36. Gu W, Guo D, Zhang L, Xu D, Sun S. The synergistic effect of azoles and fluoxetine against resistant *Candida albicans* strains is attributed to attenuating fungal virulence. Antimicrobial Agents and Chemotherapy. 2016;60(10):6179-6188.
37. Shinde RB, Chauhan NM, Raut JS, Karuppayil SM. Sensitization of *Candida albicans* biofilms to various antifungal drugs by cyclosporine A. Annals of Clinical Microbiology and Antimicrobials. 2012; 11(1):27.
38. Jia W, Zhang H, Li C, Li G, Liu X, Wei J. The calcineurin inhibitor cyclosporine A synergistically enhances the susceptibility of *Candida albicans* biofilms to fluconazole by multiple mechanisms. BMC Microbiology. 2016;16(1):113.
39. Zhang M, Yan H, Lu M, Wang D, Sun S. Antifungal activity of ribavirin used alone or in combination with fluconazole against *Candida albicans* is mediated by reduced virulence. International Journal of Antimicrobial Agents. 2020;55(1):105804.
40. Li Y, Jiao P, Li Y, Gong Y, Chen X, Sun S. The Synergistic antifungal effect and potential mechanism of d-penicillamine combined with fluconazole against *Candida albicans.* Frontiers in Microbiology. 2019;10:2853.
41. Gong Y, Liu W, Huang X, Hao L, Li Y, Sun S. Antifungal activity and potential mechanism of n-butylphthalide alone and in combination with fluconazole against *Candida albicans*. Frontiers in Microbiology. 2019;10:1461.
42. Liu Y, Ren H, Wang D, Zhang M, Sun S, Zhao Y. The synergistic antifungal effects of gypenosides combined with fluconazole against resistant *Candida albicans* via inhibiting the drug efflux and biofilm formation. Biomedicine & Pharmacotherapy. 2020;130:110580.

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