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ORIGINAL RESEARCH

Evaluation of the Synergistic Activity of Anidulafungin with Isavuconazole, Voriconazole, Posaconazole, and Amphotericin B in *Candida auris* (*Candidozyma auris*) Isolates

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Introduction: *Candida auris* poses a global public health threat due to its multidrug resistance and association with severe invasive infections. With limited treatment options, combination therapies are being explored to enhance antifungal efficacy. This study aimed to evaluate the in vitro synergistic activity of anidulafungin combined with isavuconazole, voriconazole, posaconazole, and amphotericin B against clinical *C. auris* isolates.

Methods: Fifty clinical *C. auris* isolates were identified via MALDI-TOF MS. Antifungal susceptibility testing was performed using the EUCAST broth microdilution method to determine Minimum Inhibitory Concentrations (MICs). Subsequently, the in vitro interactions of anidulafungin with four other antifungals were assessed using a checkerboard assay. Drug interactions were classified by calculating the Fractional Inhibitory Concentration Index (FICI).

Results: High resistance rates were observed for fluconazole (72%) and amphotericin B (48%), whereas no isolates were resistant to echinocandins. In combination tests, interactions were predominantly indifferent (56–84%), followed by partial synergy (12–40%). A single instance of true synergy (FICI = 0.49) was noted with the anidulafungin-posaconazole combination in one isolate. Synergy rates (synergy + partial synergy) varied significantly across combinations (Fisher's exact test, $p = 0.0044$), with anidulafungin-azole combinations showing the highest rates (36–40%) compared to anidulafungin-amphotericin B (12%). No antagonism (FICI ≥ 4) was detected in any combination.

Discussion: The predominance of indifferent interactions, with some partial synergy, suggests that combination therapy may not consistently offer a strong synergistic benefit for these isolates. This exploratory study characterizes the in vitro interaction landscape in our isolate collection, providing foundational data to guide future research and inform clinical decisions where treatment options are scarce.

Keywords: *candida auris*, *candidozyma auris*, antifungal combination test, anidulafungin, amphotericin B, azole antifungals

Introduction

Candida auris (*Candidozyma auris*) has become a global public health problem due to its ability to survive on inanimate surfaces, resistance multiple classes of antifungal drugs, and cause healthcare associated infections. It has been listed among the high-priority fungal pathogens in guidelines published by the World Health Organization (WHO).[1–4]

C. auris, unlike other *Candida* species, is the first yeast species that can rapidly develop multidrug resistance during treatment, maintain this resistance across many clonal generations, transmit it to subsequent generations and spread through healthcare services.[5] After being first identified in Japan in 2009, *C. auris* has been reported worldwide, including Africa, North and South America, other parts of Asia, Australia, Europe, the Middle East, particularly in the last decade.[5,6]

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Various studies have reported that approximately 90% of *C. auris* isolates are resistant to fluconazole, approximately 30% to amphotericin B, and less than 2% to echinocandins.[6,7] Antifungal management and infection control measures play a key role in the treatment and prevention of the spread of multidrugresistant strains, especially *C. auris*.[8] Three classes of antifungals are currently used in the treatment of fungal infections: azoles, echinocandins and polyenes.[9] While transmission of resistant isolates between patients is a widely accepted phenomenon, extensive antifungal use is also a risk factor for azole and echinocandin resistance.[8]

Studies have found that approximately 18 to 41% of *C. auris* isolates are resistant to two different antifungal drug groups, and 4% are resistant to all three antifungal drug groups. Resistance to at least two different antifungal drug groups is considered to indicate a multidrug-resistant (MDR) phenotype. The presence of MDR in *C. auris* severely limits treatment options.[10–12] The use of monotherapy in patients infected with *C. auris* may lead to treatment failure due to several factors, including intrinsic or acquired resistance mechanisms and the induction of tolerance, as well as inadequate penetration into specific body sites and devices prone to biofilm formation. In such cases, combination treatments containing two or more antifungal drugs may be preferred against certain fungal infections.[8,13]

Echinocandins are semi-synthetic molecules that function by inhibiting β-(1,3)-D-glucan synthase, an enzyme essential for the biosynthesis of β-(1,3)-D-glucan, a key structural component of the fungal cell wall. In contrast, polyenes such as amphotericin B are structurally complex antifungals that exert their effect by increasing the permeability of the cell membrane, which leads to the formation of transmembrane channels that disrupt ion balance and ultimately cause cell death. Furthermore, recent studies have introduced the "sterol sponge" theory, which proposes an alternative mechanism. According to this model, amphotericin B forms extramembranous aggregates that effectively pull ergosterol out of the lipid bilayer, leading to cell death through sterol depletion.[14,15]

Azole antifungals, which are commonly used for treating *Candida* species, are primarily fungistatic agents. They work by disrupting the synthesis of ergosterol, a sterol unique to fungi. This disruption ultimately compromises the stability of the cell membrane and leads to the accumulation of toxic intermediate products.[14]

The low bioavailability of echinocandins and the fungistatic nature of azoles have spurred research into new therapeutic strategies. Consequently, the use of combination therapies has become an increasingly preferred approach for treating infections caused by *Candida* species. Studies have revealed synergistic interactions between echinocandins and antifungal drugs with different mechanisms of action, such as azoles and polyenes. Researchers have reported significant in vitro synergistic activity against various *Candida* species, most notably including multidrug-resistant *C. auris*. The results from these studies suggest that targeting multiple cellular components or distinct pathways can enhance therapeutic efficacy. Supporting these findings with clinical research will be crucial for establishing the safety and reliability of using antifungal drug combinations in practice.[8,14,16]

The checkerboard method, one of the most widely used methods in synergy studies, is a test used to compare the effects of two different groups of antimicrobial drugs used alone with the effects of their use in combination.[17] However, studies on the use of antifungals in combination face some challenges. Discrepancies in findings across drug interaction studies may be attributed to differences in fungal strains, testing methodologies and interpretation of fractional inhibitory concentration indices (FICIs).[18]

In this study, we aimed to investigate the in vitro synergistic interactions of isavuconazole, posaconazole, voriconazole, and amphotericin B, both alone and in combination with anidulafungin, against *C. auris* isolates obtained from various clinical specimens using the checkerboard method.

Materials and Methods

Fungal Isolates

The study material consisted of 50 collection isolates isolated from various clinical samples and sent to the ISLAB-2 Central Mycology Laboratory from hospitals affiliated to the 2nd Service Region of the Istanbul Provincial Health Directorate between 1 November 2023 and 30 August 2024, for identification and antifungal susceptibility testing, and identified as *C. auris* by matrix assisted laser desorption ionization time of flight mass spectrometry [(MALDI-TOF MS) in vitro diagnostic (IVD) (VITEK-MS V3.2, bioMérieux, Marcyl'Etoile, France). (with a confidence ≥ 99.9%)]. Isolates

were stored at −80°C until testing, revived by subculturing onto Sabouraud dextrose agar (SDA) (RTA Labs, Turkey) twice before testing and incubated at 36 ± 1°C for 18–24 hours.

Antifungal Susceptibility Testing

Antifungal susceptibility tests were performed using the broth microdilution method per the European Committee on Antimicrobial Susceptibility Testing (EUCAST) Definitive Document E.Def 7.4 guidelines.[19] Antifungal agents were obtained from the manufacturer (Sigma–Aldrich Chemical Co., St. Louis, MO, USA).

The study was conducted in two stages. In the first stage, antifungal resistance rates of *C. auris* isolates were determined. In the second stage, the synergistic activities of the most commonly used echinocandins in combination with the azole group drugs for which low MIC values were detected in the first stage were evaluated. Anidulafungin was chosen over micafungin in the combination study because the MIC values of both drugs were similar in the preliminary study and because it is the most commonly used drug by clinicians in the treatment of *C. auris* infections in our service area.

In the first phase of the study, we studied the susceptibilities of the isolates to isavuconazole, anidulafungin, micafungin, voriconazole, posaconazole, fluconazole, and amphotericin B. To this end, we tested the following antifungal drug concentration ranges: 0.008 to 4 mg/L for isavuconazole, 0.008 to 4 mg/L for anidulafungin and micafungin, 0.015 to 8 mg/L for voriconazole and posaconazole, 0.03 to 16 mg/L for amphotericin B, and 0.12 to 64 mg/L for fluconazole. We prepared antifungal dilutions with RPMI medium supplemented with 2% glucose and buffered at pH 7 using 3-(N-morpholino) propane sulfonic acid (MOPS). We performed all dilutions on sterile 96-well flat bottom micro plates. Growth was assessed visually after 24 hours of incubation at 37°C and spectrophotometrically at 450 nm using a Multiskan Go (ThermoFisher Scientific, Waltham, MA, USA) microplate reader. We defined the minimum inhibitory concentration (MIC) as the lowest concentration that caused a 90% reduction in growth for amphotericin B and a 50% reduction in growth for the other drugs compared to a drug-free growth control.[12,20] We used the provisional breakpoints for anidulafungin (≥4 mg/L), micafungin (≥4 mg/L), amphotericin B (≥2 mg/L), and fluconazole (≥32 mg/L) recommended by the Centers for Disease Control and Prevention (CDC) to define antifungal resistance in *C. auris*, during the study,[7] and *Candida parapsilosis* American Type Culture Collection (ATCC) 22019 and *Candida krusei* ATCC 6258 as quality control strains.

Susceptibility Testing of Antifungal Combinations

In the second phase of the study, we studied combinations of anidulafungin, amphotericin B, voriconazole, posaconazole, and isavuconazole using a modified broth microdilution checkerboard method based on EUCAST guidelines.[19,21] To this end, we adjusted antifungal drug dilutions according to the predetermined MIC results for each drug by ±3 dilutions, resulting in final concentrations of 0.03 to 2 mg/L for anidulafungin, 0.015 to 8 mg/L for amphotericin B, 0.008 to 4 mg/L for voriconazole and posaconazole, and 0.004 to 2 mg/L for isavuconazole.[22] For two dimensional microplate preparation, we added 50 µL of each concentration of amphotericin B, voriconazole, posaconazole, and isavuconazole to wells 1 to 10 of each vertical column, followed by 50 µL of each concentration of anidulafungin to wells A to G of each row. Column 11 contained only anidulafungin, row H contained only other antifungals, ie, voriconazole, posaconazole, isavuconazole, and amphotericin B, and column 12 contained a growth control without antifungals, while H11 was designated as the sterility control without antifungals. Microplates were kept at −20 °C until the day of testing.[21] *C. auris* isolates were adjusted to 0.5 McFarland standard after 24 hours of incubation at 37 °C in SDA, diluted 1/10 in distilled water, and distributed as 100 µL. Microplates were evaluated visually after 24 hours of incubation at 37 °C and spectrophotometrically at 450 nm using a MultiskanGo (Ther-moFisherScientific, Waltham, MA, USA) microplate reader. All experiments were repeated twice.[23]

Analysis and Interpretation of Experimental Results

We interpreted the efficacy of drug combinations based on FICIs, which we calculated using the following formula:[23,24]

$$\mathrm{FICI} = \mathrm{FICA} + \mathrm{FICB} = (\mathrm{MICcombination}/\mathrm{MICA}) + (\mathrm{MICcombination}/\mathrm{MICB}),$$

where fractional inhibitory concentration (FICA) was calculated by dividing the MIC of the drug combination by the MIC of drug A alone, and FICB was calculated by dividing the MIC of the drug combination by the MIC of drug B alone.

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FICIs ≤ 0.5 indicated a synergistic interaction, 0.5 < FICIs < 1 indicated partial syner-gy, FICIs = 1 indicated an additive interaction, 1 < FICIs < 4 indicated an indifferent inter-action, and FICIs ≥ 4 indicated an antagonistic interaction.[12,24]

Statistical Analysis

We used R-project 4.4.2 (R: A Language and Environment for Statistical Computing, R Core Team, R Foundation for Statistical Computing, Vienna, Austria, 2024, retrieved from https://www.R-project.org) software package for the statistical analyses of the collected data, primarily the FICI values derived from the MIC values of antifungal drugs used alone and in combination against each isolate. We optimized the statistical analysis workflow by choosing various packages available in R project 4.4.2. To this end, we used the "dplyr" package for processing and manipulation of data, particularly the "mutate()" and "case_when()" functions to classify FICI values and convert them into categorical variables, and the "ggplot2" package for revealing and visualizing the differences in efficacy between drug combinations. We preferred slope charts to visualize changes in MIC values and presented the transitions between the MIC values of antifungals alone and in combination for each isolate with line plots. Additionally, we used the "viridis" package to improve color coding in graphs and the "flextable" package to create American Psychological Association (APA) compliant tables. We designed the tables to present comparative MIC values for standalone and combined use of antifungals, FICI groups, and differences between drug combinations.

A post-hoc power analysis demonstrated that our sample size (n=50) provides >99% power to detect the observed synergistic effects (Cohen's h = 0.73, α=0.05), substantially exceeding the 80% threshold.

Categorical data were summarized as frequencies and percentages. Synergistic interactions were defined as the combination of synergy (SYN) and partial synergy (PS) based on FICI criteria. Fisher's exact test was used to compare synergy rates across the four anidulafungin-based combinations. Pairwise comparisons between combinations were performed using Fisher's exact test with Bonferroni correction for multiple comparisons. Statistical significance was set at $p < 0.05$.

Results

Demographic and clinical characteristics of patients are shown in Table 1, and MIC range and summary statistics of seven different antifungal agents against *C. auris* isolates is shown in Table 2. Accordingly, fluconazole stood out with its high resistance rate (72%) and highest MIC values (MIC_{90}=128.0 mg/L), amphotericin B also showed significant resistance (48%), anidulafungin and micafungin, which are antifungals from the echinocandin group, were found to be effective with the lowest resistance rates (0%) and low MIC values (MIC_{50}=0.06 mg/L), whereas posaconazole and isavuconazole, which are antifungals from the azole group, were found to have the lowest MIC values (MIC_{50}=0.03 mg/L).

Comprehensive evaluation of the efficacy of anidulafungin based combinations based on FICI values and MIC levels revealed various interaction patterns in 50 *C. auris* isolates. Analysis of the anidulafungin-amphotericin B combination

Table 1 Demographic and Clinical Characteristics of Patients with *Candida* (*Candidozyma*) *auris* Isolates (n=50)

Characteristic	Category/Value	n	%
Gender	Male	28	56
	Female	22	44
Age	Mean ± Standard Deviation	71.2 ± 16.5	–
	Median (Range)	75 (16–95)	–
Unit	Intensive care unit (ICU)	39	78
	Non-ICU Ward	11	22
Sample	Blood	35	70
	Urine	12	24
	Skin	2	4
	Tracheal Aspirate	1	2

Table 2 Minimum Inhibitory Concentration (MIC) Range and Summary Statistics of Antifungal Agents Against *Candida (Candidozyma) auris* Isolates (mg/L)

Antifungal	MIC Range	MIC_{50}	MIC_{90}	GM	R (%)
AMB	0.25–2.0	1.0	2.0	1.101	48.0
FLZ	16-128	48.0	128.0	41.64	72.0
VOR	0.12–1.0	0.5	1.0	0.378	–
POS	0.008–0.25	0.03	0.06	0.029	–
MCF	0.03–2.0	0.06	0.13	0.079	0.0
AND	0.03–2.0	0.06	0.12	0.078	0.0
ISA	0.004–0.5	0.03	0.25	0.037	–

Abbreviations: AMB, amphotericin B; FLZ, fluconazole; VOR, voriconazole; POS, posaconazole; AND, anidulafungin; MCF, micafungin; ISA, isavuconazole; GM, geometric mean; R, resistance.

(Table S1) revealed in-different interaction in 42 (84%) isolates with FICIs ranging from 1.03 to 2.00, partial synergy in 6 (12%) isolates with FICIs ranging from 0.62 to 0.75, and additive interaction in 2 (4%) isolates with FICIs of 1.00. Analysis of the anidulafungin-voriconazole combination (Table S2) revealed indifferent interaction in 30 (60%) isolates with FICIs ranging from 1.03 to 2.58 and partial synergy in 20 (40%) isolates with FICIs ranging from 0.51 to 0.98, with significant decreases in MIC values. Analysis of the anidulafungin-posaconazole combination (Table S3) revealed indifferent interaction in 28 (56%) isolates with FICIs ranging from 1.03 to 2.37, partial synergy in 17 (34%) isolates with FICIs ranging from 0.506 to 0.98, additive interaction in 8% (4 out of 50 isolates) with FICIs of 1.00, and synergistic interaction in 1 (%2) isolate with an FICI of 0.49, which was the only isolate where synergistic interaction was detected. Analysis of the anidulafungin-isavuconazole combination (Table S4) revealed indifferent interaction in 28 (56%) isolates with FICIs ranging from 1.03 to 2.00, partial synergy in 19 (38%) isolates with FICIs ranging from 0.51 to 0.98, and additive interaction in 3 (6%) isolates with FICIs of 1.00. Overall, indifferent interaction was observed in most (56–84%) isolates, followed by partial synergy (12–40%) and additive interaction (4–8%). No antagonistic interaction (FICI $\geq$ 4) was detected in any isolate. Significant decreases in the MIC values of both anidulafungin and the antifungal drugs with which it was combined, especially in isolates featuring partial synergy, support the potential efficacy of the combination therapy. The combination with the highest rate of indifferent interaction was anidulafungin-amphotericin B (84%), followed by anidulafungin-posaconazole and anidulafungin-isavuconazole combinations, both with 56% indifferent interaction rate. The impact of combining antifungals on their MIC values is presented in Figures 1 and 2. The changes in other antifungals' (amphotericin B, voriconazole, posaconazole, isavuconazole) MIC values after they were combined with anidulafungin are shown in Figure 1, and the changes in anidulafungin's MIC values (0.03–2.0 mg/L) after it was combined with other antifungals are shown in Figure 2.

Synergy rates (Synergy + Partial Synergy) varied significantly across the four anidulafungin-based combinations (Fisher's exact test, $p = 0.0044$) (Figure 3). The highest synergy rates were observed with AND+VOR (40%, 20/50 isolates) and AND+ISA (38%, 19/50 isolates), followed by AND+POS (36%, 18/50 isolates). AND+AMB demonstrated significantly lower synergy (12%, 6/50 isolates) compared to AND+ISA ($p = 0.030$) and AND+VOR ($p = 0.016$) after Bonferroni correction for multiple comparisons. (Table 3).

Discussion

Among the antifungal combinations we tested, anidulafungin and azole combinations had the lowest FICIs, with partial synergy detected in 34–40% of isolates and a significant decrease in MIC values. In contrast, the rate of isolates with indifferent interaction was the highest (84%) in anidulafungin-amphotericin B combination. Overall, indifferent interaction was observed in most (56–84%) isolates, followed by partial synergy (12–40%) and additive interaction (4–8%). Only the anidulafungin-posaconazole combination showed a synergistic interaction in a single (0.49) isolate, and no antagonistic interaction (FICI $\geq$ 4) was detected in any combination, indicating that antifungal drugs generally exhibit

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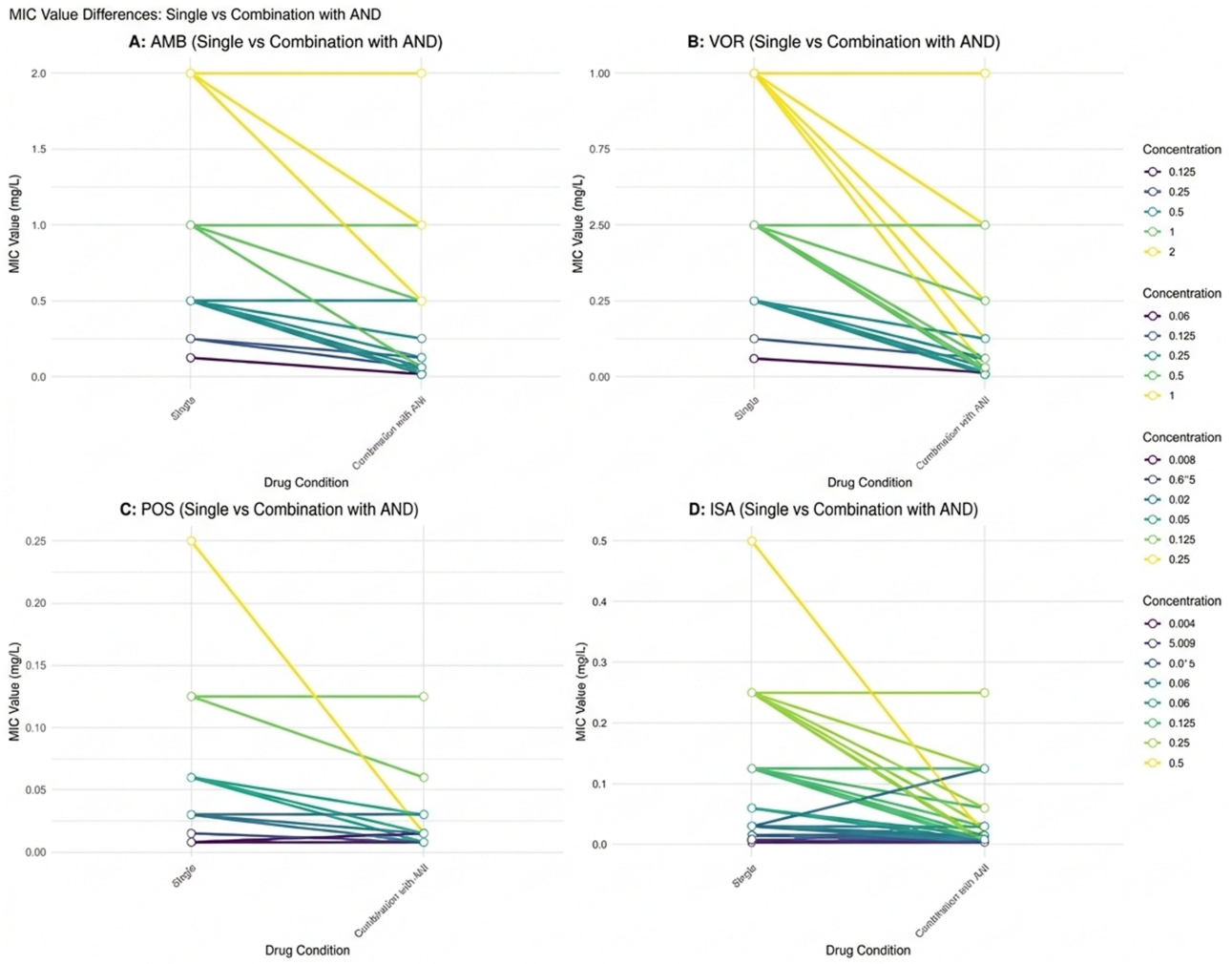

Figure 1 Comparison of MIC values of antifungal agents alone and in combination with anidulafungin (AND). (**A**): AMB (Alone vs in combination with AND); (**B**): VOR (Alone vs in combination with AND); (**C**): POS (Alone vs in combination with AND); (**D**): ISA (Alone vs in combination with AND). The graphs demonstrate the changes in MIC values of antifungals at different baseline concentrations following their combination with anidulafungin.
Abbreviations: AND, Anidulafungin; AMB, Amphotericin B; VOR, Voriconazole; POS, Posaconazole; ISA, Isavuconazole.

indifferent interactions when used in combination. Then again, the decreases observed in MIC values in combinations showing partial synergy are promising for clinical applications.

C. auris causes infections that are difficult to treat due to its multidrug resistance (MDR). In parallel with our finding that anidulafungin based combinations are effective against *C. auris*, combinations of echinocandins such as anidulafungin have been reported in the literature as an effective treatment option against *C. auris*. John et al[12] reported that anidulafungin and its combination with fosmanogepix or flucytosine showed higher synergistic activity against both resistant and susceptible *C. auris* isolates. Some studies have reported that the combinations of echinocandins and azoles, especially voriconazole, posaconazole, and isavuconazole, created a synergistic effect in isolates exhibiting the MDR phenotype and may yield higher success in the treatment of infections.[24,25] Other studies have reported that combining echinocandin with amphotericin B may be a promising therapeutic approach in the treatment of invasive candidiasis caused by *C. auris*.[13,26] Cabellero et al reported that the combination of amphotericin B with echinocandins exhibited a synergistic effect, resulting in a greater fungicidal effect per lower amphotericin B dose compared to monotherapy.[13] In a study featuring the *Caenorhabditis elegans* candidiasis model, it was reported that the combination of amphotericin B and echinocandins was the most promising drug combination in terms of in vivo activity. Although we observed high partial synergy in anidulafungin and azole antifungal combinations, the high rates of indifferent interaction in

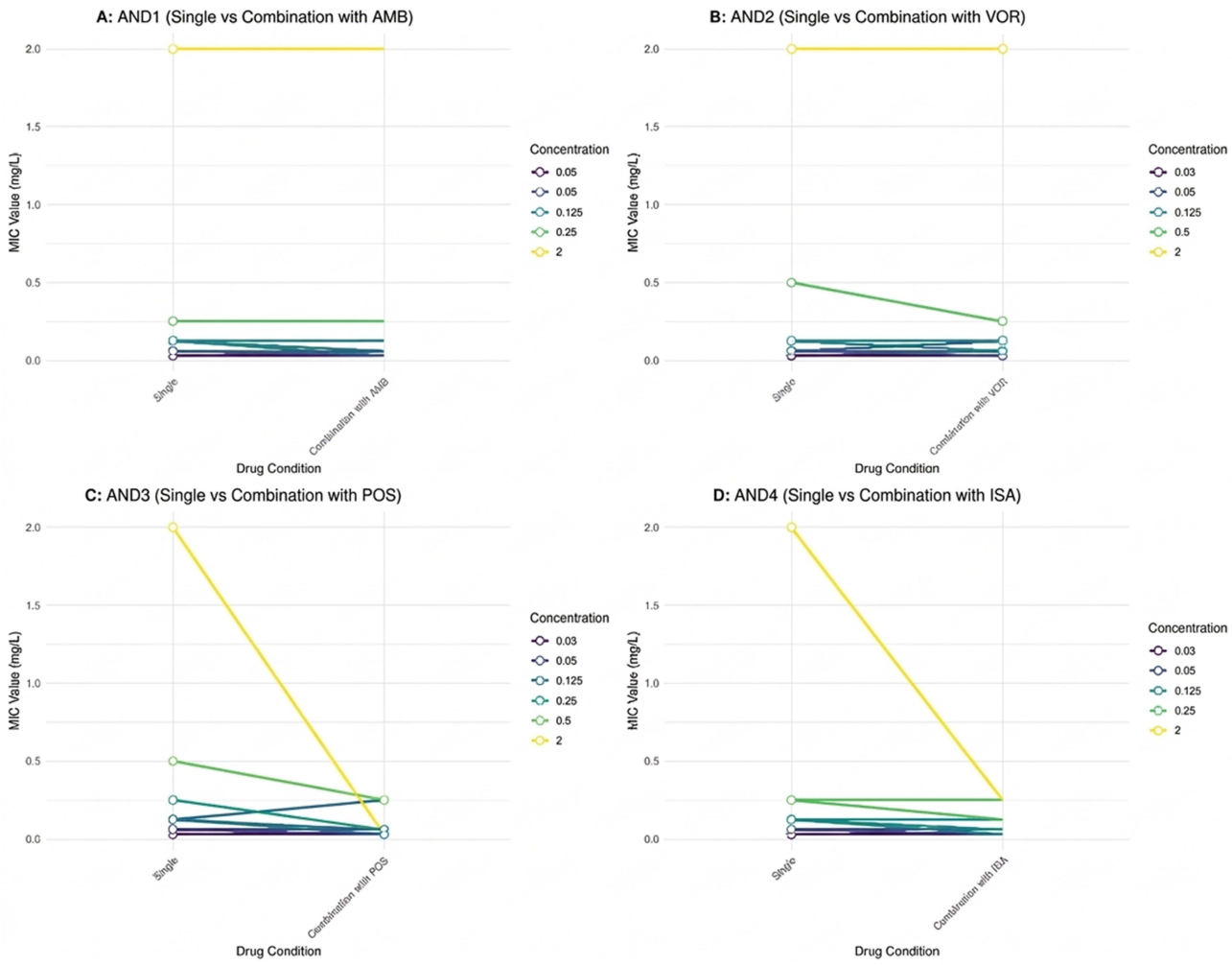

Figure 2 Comparison of anidulafungin (AND) MIC values alone and in antifungal combinations. (**A**): AND (Alone vs in combination with AMB); (**B**): AND (Alone vs in combination with VOR); (**C**): AND (Alone vs in combination with POS); (**D**): AND (Alone vs in combination with ISA). The graphs illustrate the MIC value changes of anidulafungin at different baseline concentrations (0.03–2.0 mg/L) following combination with various antifungal agents.
Abbreviations: AND, Anidulafungin; AMB, Amphotericin B; VOR, Voriconazole; POS, Posaconazole; ISA, Isavuconazole.

anidulafungin and amphotericin B combinations suggested that the treatment efficacy of this specific combination may be limited.[26] The efficacy of anidulafungin when combined with different antifungal drugs can vary across different genetic subtypes. Therefore, the observed variability in MIC values and the isolate-specific responses to combination therapy should be taken into account when selecting an appropriate treatment.[16]

At the same time, resistance was primarily limited to fluconazole, with a key finding being the absence of antagonism and the presence of partial synergy or indifferent interactions in the majority of isolates. In our study, the higher prevalence of indifferent interactions compared to other studies supports the idea that these variations could alter treatment outcomes.Although combinations of antifungal drugs are frequently used in combination therapy applications, studies have indicated that some other drug groups may also be preferred in combination applications.[27]

In one of these studies, reported that the antiemetic drug rolapitant strongly increased the antifungal effect of amphotericin B on fungal agents, especially *C. auris*.[28]

In another study, the use of HIV protease inhibitors lopinavir and ritonavir together with azole antifungal drugs was shown to be effective in the treatment of *C. auris* infections, both in vitro and in vivo.[29]

Xin et al[30] demonstrated the effectiveness of intravenous human immunoglobulin (IVIG) in protecting against disseminated infections with *C. auris* and *C. albicans* through in vivo experiments. Researchers also linked the protective

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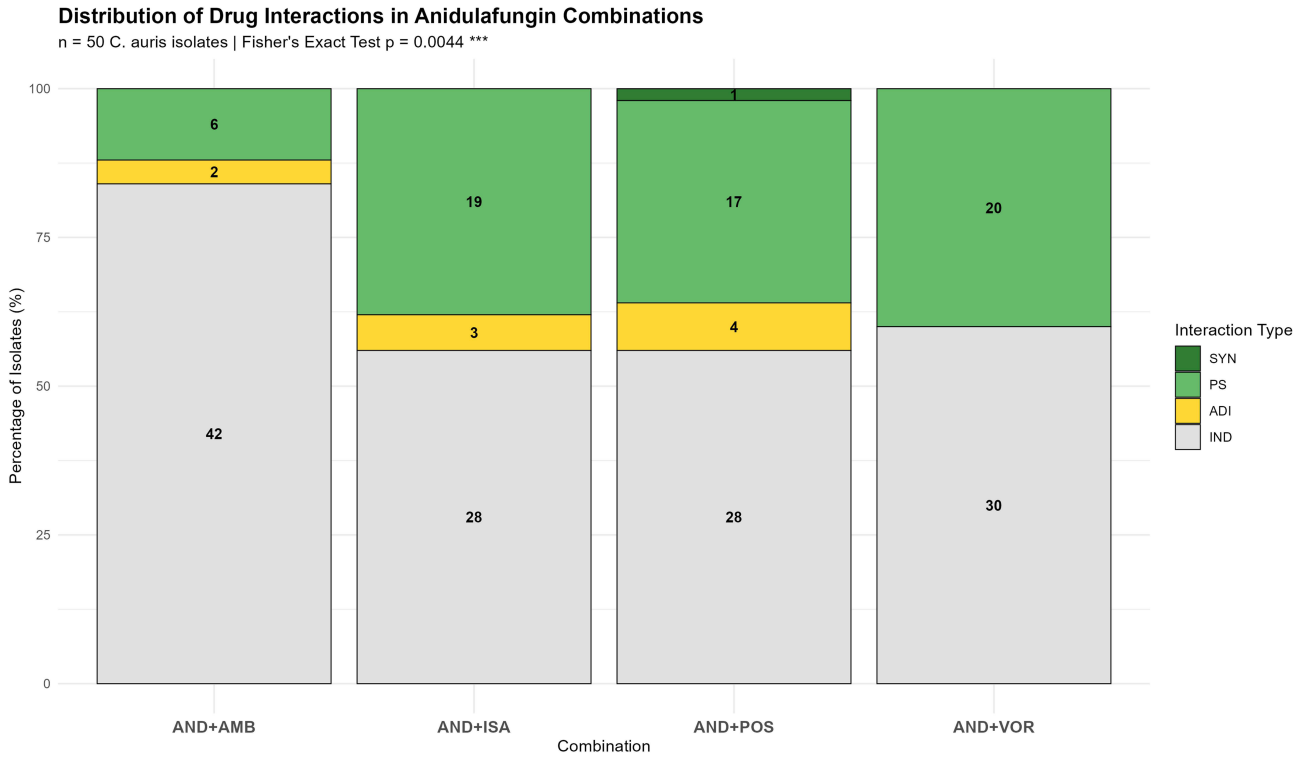

Figure 3 Distribution of drug interactions in anidulafungin-based combinations against 50 *C. auris* clinical isolates. Numbers within bars indicate the count of isolates for each interaction type. Fisher's exact test revealed significant differences in synergy rates between combinations (p = 0.0044).
Abbreviations: AND, anidulafungin; AMB, amphotericin B; ISA, isavuconazole; VOR, voriconazole; POS, posaconazole; SYN: Synergy, PS: Partial Synergy, ADI: Additive, IND: Indifferent.

efficacy of IVIG to the prolonged survival of mice with invasive candidiasis treated with the combination of Amphotericin B and IVIG.

It has been reported that the combination of herbal products such as essential oils (EOs), which have been used for centuries due to their therapeutic and aromatic properties, with antifungal drugs may be a promising therapeutic approach in the treatment of resistant fungal infections. Cavallo et al reported synergistic activity in the combination of EOs composed primarily of thyme, cinnamon, geranium, clove bud, lemongrass, and mentha of Pancalieri with antifungal drugs such as micafungin, fluconazole, and 5-flucytosine against resistant *C. auris* isolates. They suggested that these EOs may help overcome the challenging resistance problem in *C. auris*.[31]

Although the above-mentioned treatment approaches are promising in the treatment of multidrug resistant *C. auris* strains, their potential effects need to be supported by in vivo studies.

Table 3 Comparison of Synergy Rates Across Anidulafungin-Based Combinations

Combination	Synergy (S)	Partial Synergy (PS)	Additive (ADI)	Indifference (IND)	Total Synergy (S+PS)	Synergy Rate (%)
AND+VOR	0	20	0	30	20/50	40.0
AND+ISA	0	19	3	28	19/50	38.0
AND+POS	1	17	4	28	18/50	36.0
AND+AMB	0	6	2	42	6/50	12.0
p-value	—	—	—	—	**0.0044***	—

Notes: Fisher's exact test; pairwise comparisons: AND+AMB vs AND+ISA (p=0.030), AND+AMB vs AND+VOR (p=0.016) after Bonferroni correction.
Abbreviations: AND, anidulafungin; AMB, amphotericin B; ISA, isavuconazole; VOR, voriconazole; POS, posaconazole; S, synergy; PS, partial synergy; ADI, additive; IND, indifference.

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One of the significant reasons *C. auris* poses a growing threat to human health is its intrinsic resistance to one or more classes of antifungal drugs currently used in treatment.[32]

Molecular investigations have reported that resistance can develop as a result of genetic modifications leading to target site alterations, overexpression of efflux pumps, or changes in metabolism.[33]

Although resistance to fluconazole and amphotericin B is common among *C. auris* isolates, echinocandin resistance (for example, to caspofungin) remains relatively rare.[32]

Studies investigating antifungal resistance in *C. auris* have found that two point mutations in the ERG11 gene and the overexpression of the ABC transporter Cdr1 are associated with reduced fluconazole susceptibility.[33]

In studies where researchers identified and analyzed the expression of genome-wide ABC proteins in *C. auris*, they reported that the expression of various ABC transporters changed significantly in the presence of different antifungal drugs, suggesting a potential role in the drug resistance of *C. auris*.[34]

Furthermore, studies have reported that mutations in the FKS1 gene, which encodes the catalytic subunit of 1,3-beta-D-glucan synthase—an enzyme critical for cell wall synthesis in *Candida* species—are responsible for caspofungin resistance in *C. auris* isolates. In contrast, other isolates with the wild-type FKS1 gene were found to be susceptible to caspofungin at human therapeutic doses.[32]

Our study focused solely on phenotypic susceptibility and synergy testing, and therefore, it does not provide data on the molecular causes of resistance.While this study provided noteworthy findings that could contribute to the literature on antifungal combination therapies, it also had several limitations. First, the study's findings, based solely on in vitro analyses, do not fully reflect the complexity of in vivo environments. Interactions observed under controlled laboratory conditions may differ in clinical settings for a variety of reasons, including differences in drug pharmacokinetics, host immune responses, and pathogen behavior.[35] Secondly, the fact that we did not analyze the genetic diversity of *C. auris* isolates can be considered another limitation of the study, considering that genetic and phenotypic variations can significantly affect antifungal susceptibility and combination efficacy.[36] Therefore, future studies should include genomic analyses to better understand the relationship between isolate diversity and treatment outcomes. Thirdly, we tested a limited number of antifungal drugs and combinations. While the antifungal drugs and combinations we tested are clinically relevant, additional drug combinations incorporating new antifungal drugs should be investigated to address a broader range of therapeutic options. Lastly, the commonly used checkerboard microdilution method has some limitations, such as variability in determining outcomes and potential bias in FICI interpretation. Therefore, complementary methods such as timekill experiments or dynamic models should be employed to corroborate the results obtained using the checkerboard microdilution method and provide a more comprehensive assessment of drug interactions.[35,37] Extended research and in vivo studies are needed to address these limitations to fully understand the therapeutic potential of antifungal combinations in combating multidrug resistant *C. auris.*

Conclusion

In conclusion, the findings of this study evaluating the in vitro synergistic potential of anidulafungin based antifungal combinations against *C. auris* isolates highlighted the potential of these combinations to improve therapeutic outcomes in the treatment of multidrug-resistant infections. Among the combinations tested, anidulafungin-azole combinations showed significant partial synergy in a significant rate of isolates, with low MIC values and favorable FICIs, indicating that the drug combinations with different mechanisms of action can effectively overcome drug resistance problems and increase treatment efficacy. On the other hand, the fact that indifferent interactions were observed with anidulafungin with amphotericin B combination in a very high rate of isolates suggests that antifungal combinations should be determined based on specific fungalgenotypes. The fact that we did not observe an antagonistic interaction with any combination supports the safety of the therapeutic strategies we tested, although further validation is required. The consistency of the partial synergistic effect we observed across several antifungal combinations highlights the importance of continuing research on combination therapies against resistant fungal pathogens. Clinical trials to assess the relevance of this study's findings to real-world clinical practice may further contribute to more effective and personalized management of *C. auris* infections.

Ethics Approval and Informed Consent

The study was conducted ethically, adhering to the principles of the Declaration of Helsinki, and received approval from the Haydarpasa Numune Training and Research Hospital Ethics Committee (Protocol No: 2024/141 – 4624, dated November 5, 2024). Since the research relied solely on microbial isolates obtained from specimens routinely processed by our Mycology laboratory for diagnostic purposes, the need for patient informed consent was formally exempted.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The author(s) report no conflicts of interest in this work.

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