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Rohan Prakash Kumar

JSS Academy of Higher Education and Research

R Gowtham

JSS Academy of Higher Education and Research

Sowmya G Shivappa

`sowmyags@jssuni.edu.in`

JSS Academy of Higher Education and Research

Mahadevaiah Neelambike Sumana

JSS Academy of Higher Education and Research

Diya Sukumaran

JSS Academy of Higher Education and Research

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In Vitro Evaluation of the Interaction between Fluconazole and Linezolid against Clinical *Candida* Isolates by Checkerboard Assay

Rohan Prakash Kumar¹, Gowtham R², Sowmya G Shivappa^{3*}, Mahadevaiah Neelambike Sumana⁴, Diya Sukumaran⁵

^{1,5} Post Graduate Student, Department of Microbiology, JSS Medical College and Hospital, JSS AHER, Mysuru, Karnataka, India.

² Ph. D Scholar, Department of Microbiology, JSS Medical College and Hospital, JSS AHER, Mysuru, Karnataka, India.

^{3,4} Professor of Microbiology, JSS Medical College and Hospital, JSS AHER, Mysuru, Karnataka, India.

Corresponding author:

Sowmya G Shivappa

Professor of Microbiology, JSS Medical College and Hospital, JSS AHER, Mysuru.

Email: sowmyags@jssuni.edu.in

Abstract

Background: Antifungal resistance among *Candida* species presents a challenge to effective treatment, creating interest in combination therapy and antimicrobial drug repurposing. Linezolid, an oxazolidinone antibacterial agent, has demonstrated potential for enhancing azole activity in previous in vitro studies. This study evaluated the interaction between fluconazole and linezolid combination against clinical *Candida* isolates using the checkerboard microbroth dilution method.

Methodology: A total of 82 clinically significant *Candida* isolates were identified and speciated using the VITEK 2 Compact automated system. The minimum inhibitory concentrations (MICs) of fluconazole and linezolid were determined by broth microdilution, followed by a two-dimensional checkerboard assay. Fluconazole concentrations ranged from 0.125 to 64 µg/mL, while linezolid concentrations ranged from 4 to 256 µg/mL. Drug interactions were assessed using the fractional inhibitory concentration index (FICI) and classified as synergistic, additive, indifferent, or antagonistic. *Candida parapsilosis* ATCC 22019 was used as the quality control strain.

Results: Indifferent interactions were observed in 48 (58.5%) isolates, additive interactions in 27 (32.9%), and synergistic interactions in 7 (8.5%). Overall, additive or synergistic activity was detected in 34 (41.5%) isolates, while no antagonistic interactions were observed. Interaction

patterns varied among *Candida* species, indicating heterogeneity in the response to the drug combination.

Conclusion: The fluconazole–linezolid combination demonstrated variable in vitro interactions, with additive and synergistic effects observed in a subset of clinical *Candida* isolates. These findings support further investigation of antimicrobial drug repurposing and combination strategies through laboratory studies, experimental models, and clinical research.

Keywords: *Candida* species, Checkerboard method, Drug Synergism, Fluconazole, Linezolid.

Introduction

Candida species are ubiquitous yeasts that form part of the normal human microbiota and commonly colonize mucosal surfaces and the skin [1, 2]. Changes in host defenses or disruption of the normal microbiota may enable *Candida* species to shift from commensal organisms to opportunistic pathogens [2, 3]. *Candida* infections range from superficial mucosal infections to invasive disease, with invasive candidiasis associated with considerable morbidity and mortality [4]. Antifungal resistance has emerged as a major challenge in the treatment of candidiasis [4, 5].

Fluconazole is widely used for the treatment of candidiasis because of its favorable pharmacokinetic properties, good oral bioavailability, and established clinical efficacy [4, 6]. It inhibits lanosterol 14- α -demethylase, encoded by *ERG11*, thereby interfering with ergosterol biosynthesis [7]. Fluconazole resistance has been reported in both *C. albicans* and non-*albicans* *Candida* species. Increased expression of drug efflux pumps, alterations in the target enzyme, and changes in ergosterol biosynthesis are among the mechanisms associated with resistance [5, 8]. The presence of fluconazole-resistant isolates has increased interest in approaches that can enhance the activity of existing antifungal agents.

Combination therapy and drug repurposing have been investigated as approaches to improve antifungal activity [9, 10]. Linezolid is an oxazolidinone antibacterial agent used mainly for infections caused by Gram-positive bacteria [11, 12]. Lu et al. reported synergistic activity between linezolid and azole antifungal agents against *C. albicans*, including enhanced activity of

fluconazole against resistant isolates in vitro [13]. These findings provide a basis for investigating the interaction between linezolid and fluconazole against clinical *Candida* isolates.

The checkerboard microdilution method is widely used to evaluate interactions between antimicrobial agents, with the fractional inhibitory concentration index (FICI) used to quantify the combined effect [14]. In the present study, FICI values were interpreted as synergistic at ≤ 0.5 , additive at $>0.5-1$, indifferent at $>1-4$, and antagonistic at >4 , based on previously published antifungal combination studies [15, 16]. The present study aimed to evaluate the in vitro interaction between linezolid and fluconazole against *Candida* species using the checkerboard microdilution method.

METHODOLOGY

Clinically significant isolates of *Candida* isolated from various samples received in the microbiology department laboratory were identified and speciated by using automated identification system Vitek Compact2.

Broth Microdilution and Checkerboard Assay

The minimum inhibitory concentrations (MICs) of fluconazole and linezolid against clinical *Candida* isolates were determined using a broth microdilution technique in sterile 96-well microtiter plates, with reference to the Clinical and Laboratory Standards Institute (CLSI) M27 methodology for yeast antifungal susceptibility testing [17]. Fluconazole and linezolid were tested individually at concentrations ranging from 0.125 to 64 $\mu\text{g/mL}$ and 4 to 256 $\mu\text{g/mL}$, respectively. The interaction between the two agents was investigated using a two-dimensional checkerboard micro broth dilution assay. Fluconazole was dispensed along the columns, whereas linezolid was arranged across the rows, generating a matrix of different drug combinations (**Figure 1**).

For the checkerboard combination assay, 50 μL of the respective fluconazole dilution and 50 μL of the corresponding linezolid dilution were added to the designated wells, followed by 100 μL of standardized *Candida* inoculum, resulting in a final volume of 200 μL per well. The yeast suspension was adjusted to a turbidity equivalent to a 0.5 McFarland standard and serially diluted 1:50, followed by a further 1:20 dilution in RPMI 1640 broth (HI media, India). The resulting working inoculum was added to the wells at a 1:1 ratio with the drug mixture to achieve a final

inoculum concentration of 0.5×10^3 – 2.5×10^3 CFU/mL. Inoculum preparation was performed according to the CLSI M27, 4th edition (2017) [17].

This resulted in a final assay volume of 200 μ L per well. The checkerboard design enabled the assessment of growth inhibition across varying concentrations of both antimicrobial agents. Individual drug controls, a growth control containing inoculum without antimicrobial agents, and a sterility control containing drug-free medium without inoculum were included. *Candida parapsilosis* ATCC 22019 was used as the quality control strain for antifungal susceptibility testing, with reference to the applicable CLSI quality control standards [17, 18].

The plates were incubated aerobically at $35 \pm 2^\circ\text{C}$ for 18 to 24 hours. Minimum inhibitory concentration (MIC) were determined by visual assessment of growth and recorded as the lowest concentration of the individual drug or combination that produced complete inhibition of visible growth relative to the growth control. The interaction between fluconazole and linezolid was evaluated using the fractional inhibitory concentration index (FICI). The FICI was calculated by summing the fractional inhibitory concentrations of the two agents, and the resulting values were classified according to the predefined interaction criteria used in the study [15, 16].

A1 empty well		FLUCONAZOLE										12 Growth control
		2 64 $\mu\text{g/ml}$	3 32 $\mu\text{g/ml}$	4 16 $\mu\text{g/ml}$	5 8 $\mu\text{g/ml}$	6 4 $\mu\text{g/ml}$	7 2 $\mu\text{g/ml}$	8 1 $\mu\text{g/ml}$	9 0.5 $\mu\text{g/ml}$	10 0.25 $\mu\text{g/ml}$	11 0.125 $\mu\text{g/ml}$	
L I N E Z O L I D	B 256 $\mu\text{g/ml}$	64+256 $\mu\text{g/ml}$	32+256 $\mu\text{g/ml}$	16+256 $\mu\text{g/ml}$	8+256 $\mu\text{g/ml}$	4+256 $\mu\text{g/ml}$	2+256 $\mu\text{g/ml}$	1+256 $\mu\text{g/ml}$	0.5+256 $\mu\text{g/ml}$	0.25+256 $\mu\text{g/ml}$	0.125+256 $\mu\text{g/ml}$	<i>C.parapsilosis</i> ATCC 22019 0.5 $\mu\text{g/ml}$
	C 128 $\mu\text{g/ml}$	64+128 $\mu\text{g/ml}$	32+128 $\mu\text{g/ml}$	16+128 $\mu\text{g/ml}$	8+128 $\mu\text{g/ml}$	4+128 $\mu\text{g/ml}$	2+128 $\mu\text{g/ml}$	1+128 $\mu\text{g/ml}$	0.5+128 $\mu\text{g/ml}$	0.25+128 $\mu\text{g/ml}$	0.125+128 $\mu\text{g/ml}$	1 $\mu\text{g/ml}$
	D 64 $\mu\text{g/ml}$	64+64 $\mu\text{g/ml}$	32+64 $\mu\text{g/ml}$	16+64 $\mu\text{g/ml}$	8+64 $\mu\text{g/ml}$	4+64 $\mu\text{g/ml}$	2+64 $\mu\text{g/ml}$	1+64 $\mu\text{g/ml}$	0.5+64 $\mu\text{g/ml}$	0.25+64 $\mu\text{g/ml}$	0.125+64 $\mu\text{g/ml}$	2 $\mu\text{g/ml}$
	E 32 $\mu\text{g/ml}$	64+32 $\mu\text{g/ml}$	32+32 $\mu\text{g/ml}$	16+32 $\mu\text{g/ml}$	8+32 $\mu\text{g/ml}$	4+32 $\mu\text{g/ml}$	2+32 $\mu\text{g/ml}$	1+32 $\mu\text{g/ml}$	0.5+32 $\mu\text{g/ml}$	0.25+32 $\mu\text{g/ml}$	0.125+32 $\mu\text{g/ml}$	4 $\mu\text{g/ml}$
	F 16 $\mu\text{g/ml}$	64+16 $\mu\text{g/ml}$	32+16 $\mu\text{g/ml}$	16+16 $\mu\text{g/ml}$	8+16 $\mu\text{g/ml}$	4+16 $\mu\text{g/ml}$	2+16 $\mu\text{g/ml}$	1+16 $\mu\text{g/ml}$	0.5+16 $\mu\text{g/ml}$	0.25+16 $\mu\text{g/ml}$	0.125+16 $\mu\text{g/ml}$	Sterility control (media control)
	G 8 $\mu\text{g/ml}$	64+8 $\mu\text{g/ml}$	32+8 $\mu\text{g/ml}$	16+8 $\mu\text{g/ml}$	8+8 $\mu\text{g/ml}$	4+8 $\mu\text{g/ml}$	2+8 $\mu\text{g/ml}$	1+8 $\mu\text{g/ml}$	0.5+8 $\mu\text{g/ml}$	0.25+8 $\mu\text{g/ml}$	0.125+8 $\mu\text{g/ml}$	

	H 4 μg/ml	64+4 μg/ml	32+4 μg/ml	16+4 μg/ml	8+4 μg/ml	4+4 μg/ml	2+4 μg/ml	1+4 μg/ml	0.5+4 μg/ml	0.25+4 μg/ml	0.125+4 μg/ml	
100μl of each drug alone (FIZ: A2-A11; LZ: B1-H1), 50 μl Fluconazole+ 50μl Linezolid (other wells) +100 μl <i>Candida</i> inoculum Final volume: 200 μl												

Figure 1: Concentration of Fluconazole and Linezolid for Checkerboard assay

Interpretation and Synergy Testing:

The Fractional Inhibitory Concentration (FIC) index for each combination was calculated by:

$$\text{FIC} = \frac{\text{MIC of drug linezolid in concentration}}{\text{MIC of drug linezolid alone}} + \frac{\text{MIC of drug fluconazole in combination}}{\text{MIC of drug fluconazole alone}}$$

Interpretation of FIC Index:

- i. $\text{FIC} \leq 0.5$: Synergy — the drugs are more effective together than alone.
- ii. $\text{FICI} > 0.5\text{--}1.0$: Additive or Partial Synergy
- iii. $\text{FICI} > 1.0\text{--}4.0$: Indifference
- iv. $\text{FIC} > 4$: Antagonism — the drugs are less effective together than alone.

RESULTS

Among 82 *Candida* isolates from various clinical, urine was most common sample, accounting for 76 of the 82 isolates, while vaginal swab, pus and ear together yielded only 6 isolates. *Candida albicans* was the predominant species isolated with 30 isolates and was the only species obtained from non-urine specimens. *Candida tropicalis* was other Non*Candida* Species (NAC), with 29 isolates, followed by *C. guilliermondii* and *C. glabrata* with nine and seven isolates respectively. The remaining species, namely *C. parapsilosis*, *C. auris*, *C. krusei*, and *C. norvegensis*, were isolated in lesser numbers and exclusively from urine samples, reflecting the predominantly urinary origin of the study isolates (Table 1).

Table 1: Distribution of *candida* species isolated across clinical specimens

Species	Urine	Vaginal swab	Pus	Ear	Total
<i>C.albicans</i>	24	3	2	1	30
<i>C.tropicalis</i>	29	-	-	-	29
<i>C.guilliermondii</i>	9	-	-	-	9

<i>C.glabrata</i>	7	-	-	-	7
<i>C.parapsilosis</i>	3	-	-	-	3
<i>C.auris</i>	2	-	-	-	2
<i>C.norvegenesis</i>	1	-	-	-	1
<i>C.krusei</i>	1	-	-	-	1
Total	76	3	2	1	82

Synergistic activity for fluconazole with linezolid was detected by Checkerboard method all 82 clinical *Candida* isolates. *Candida* species (*C.glabrata*, *C.auris*, *C.krusei*) with intrinsic or reduced susceptibility to fluconazole were also included to evaluate whether the addition of linezolid could enhance azole activity against inherently resistant isolates. Their inclusion enabled a more comprehensive assessment of the synergistic potential of the drug combination across different *Candida* species with varying susceptibility profiles. Alone MIC of fluconazole and alone MIC of linezolid was obtained along combination MIC (Figure 2). Along with clinical isolates ATCC *Candida parapsilosis* (ATCC 22019) strain was tested as quality control strain with MIC of 1 µg/mL as per CLSI guidelines (M27M44S). The interaction between fluconazole and linezolid against all 82 *Candida* isolates was evaluated using the fractional inhibitory concentration index (FICI) (table 2). Indifferent interaction was the most observed in 48 (58.52%) isolates. An additive interaction was demonstrated in 27 (32.92%) isolates, while synergistic interaction ($FICI \leq 0.5$) was observed in 7 (8.54%) isolates (Figure 3). No antagonistic interaction was detected among the isolates tested. Overall, the combination of fluconazole and linezolid exhibited either additive or synergistic activity in 34 (41.46%) isolates, indicating that the combination did not adversely affect the antifungal activity of fluconazole

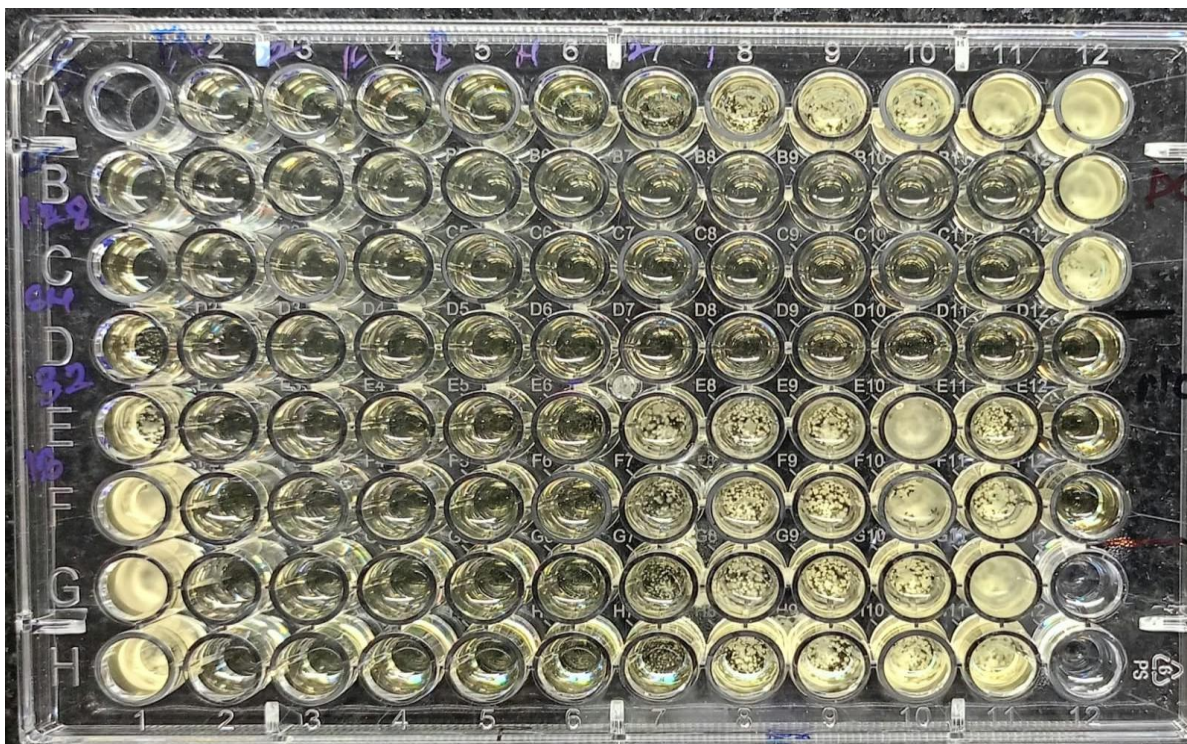


Figure 2: Checkerboard assay showing the interaction between Fluconazole and Linezolid against *Candida* isolates

Table 2: Determination of Synergistic Activity and FICI by the Checkerboard Method

Sample number	Isolate	Flu MIC Alone	Lz MIC Alone	Flu+ Lz combination	FICI	Interpretation
1.	<i>C.tropicalis</i>	8	64	2+32	0.75	Additive
2.	<i>C.tropicalis</i>	2	64	0.5+16	0.5	Synergy
3.	<i>C.albicans</i>	1	32	0.5+32	1.5	Indifferent
4.	<i>C.albicans</i>	1	64	0.5+32	1	Additive
5.	<i>C.tropicalis</i>	2	64	0.5+16	0.5	Synergy
6.	<i>C.tropicalis</i>	2	32	1+32	1.5	Indifferent
7.	<i>C.parapsilosis</i>	16	128	4+64	0.75	Additive
8.	<i>C.albicans</i>	1	128	0.25+32	0.5	Synergy
9.	<i>C.tropicalis</i>	2	32	0.5+32	1.25	Indifferent
10.	<i>C.parapsilosis</i>	2	64	0.5+32	0.75	Additive
11.	<i>C.albicans</i>	1	32	0.5+32	1.50	Indifferent
12.	<i>C.albicans</i>	0.5	64	0.25+32	1	Additive
13.	<i>C.tropicalis</i>	0.5	64	0.5+32	1.5	Indifferent

14.	<i>C.albicans</i>	2	32	0.5+32	1.25	Indifferent
15.	<i>C.albicans</i>	4	64	1+32	0.75	Additive
16.	<i>C.albicans</i>	2	64	1+32	1	Additive
17.	<i>C.guilliermondii</i>	4	64	2+32	1	Additive
18.	<i>C.tropicalis</i>	2	64	2+32	1.5	Indifferent
19.	<i>C.albicans</i>	1	64	1+32	1.5	Indifferent
20.	<i>C.albicans</i>	1	64	1+32	1.5	Indifferent
21.	<i>C.tropicalis</i>	8	32	2+16	0.75	Additive
22.	<i>C.parapsilosis</i>	2	32	0.5+16	0.75	Additive
23.	<i>C.norvegensis</i>	32	64	16+32	1	Additive
24.	<i>C.auris</i>	2	8	1+8	1.5	Indifferent
25.	<i>C.albicans</i>	0.5	32	0.25+16	1	Additive
26.	<i>C.albicans</i>	1	32	0.5+32	1.5	Indifferent
27.	<i>C.tropicalis</i>	2	16	0.5+8	0.75	Additive
28.	<i>C.tropicalis</i>	16	16	4+16	1.25	Indifferent
29.	<i>C.tropicalis</i>	0.5	32	0.5+32	1,50	Indifferent
30.	<i>C.albicans</i>	2	64	0.5+32	0.75	Additive
31.	<i>C.tropicalis</i>	1	32	0.5+16	1	Additive
32.	<i>C.tropicalis</i>	0.5	32	0.25+16	1	Additive
33.	<i>C.auris</i>	0.5	16	0.25+16	1.5	Indifferent
34.	<i>C.tropicalis</i>	64	32	16+32	1.25	Indifferent
35.	<i>C.albicans</i>	8	64	2+16	0.5	Synergy
36.	<i>C.tropicalis</i>	0.5	16	0.5+16	2	Indifferent
37.	<i>C.glabrata</i>	1	32	0.25+8	0.5	Synergy
38.	<i>C.guilliermondii</i>	2	32	0.5+8	0.5	Synergy
39.	<i>C.tropicalis</i>	1	32	0.5+16	1	Additive
40.	<i>C.guilliermondii</i>	2	32	1+16	1	Additive
41.	<i>C.albicans</i>	1	32	1+32	2	Indifferent
42.	<i>C.tropicalis</i>	8	32	4+32	1.5	Indifferent
43.	<i>C.glabrata</i>	2	32	1+32	1.5	Indifferent
44.	<i>C.tropicalis</i>	2	16	1+16	2	Indifferent
45.	<i>C.guilliermondii</i>	1	16	0.5+16	1.5	Indifferent
46.	<i>C.albicans</i>	8	16	4+16	1.5	Indifferent
47.	<i>C.albicans</i>	1	32	0.5+32	1.5	Indifferent
48.	<i>C.albicans</i>	1	32	0.5+16	1	Additive

49.	<i>C.tropicalis</i>	0.5	16	0.5+16	2	Indifferent
50.	<i>C.albicans</i>	0.5	32	0.5+16	2	Indifferent
51.	<i>C.glabrata</i>	2	16	1+16	1.5	Indifferent
52.	<i>C.tropicalis</i>	16	32	4+32	1.5	Indifferent
53.	<i>C.glabrata</i>	0.5	16	0.5+16	2	Indifferent
54.	<i>C.tropicalis</i>	1	16	1+16	2	Indifferent
55.	<i>C.guilliermondii</i>	2	32	1+32	2	Indifferent
56.	<i>C.tropicalis</i>	1	32	1+32	2	Indifferent
57.	<i>C.krusei</i>	8	32	4+16	1	Additive
58.	<i>C.albicans</i>	1	16	0.5+16	1.5	Indifferent
59.	<i>C.albicans</i>	4	32	2+16	1	Additive
60.	<i>C.albicans</i>	1	32	1+32	2	Indifferent
61.	<i>C.albicans</i>	0.25	16	0.25+16	2	Indifferent
62.	<i>C.albicans</i>	2	32	1+16	1	Additive
63.	<i>C.albicans</i>	1	32	1+32	2	Indifferent
64.	<i>C.albicans</i>	8	64	4+32	1	Additive
65.	<i>C.tropicalis</i>	4	32	2+32	1.5	Indifferent
66.	<i>C.tropicalis</i>	2	32	1+16	1	Additive
67.	<i>C.glabrata</i>	1	32	1+16	2	Indifferent
68.	<i>C.guilliermondii</i>	1	32	0.5+32	1.5	Indifferent
69.	<i>C.albicans</i>	2	16	1+16	2	Indifferent
70.	<i>C.glabrata</i>	0.5	16	0.5+16	2	Indifferent
71.	<i>C.guilliermondii</i>	1	32	0.25+8	0.5	Synergy
72.	<i>C.tropicalis</i>	1	16	0.5+16	2	Indifferent
73.	<i>C.tropicalis</i>	1	32	0.25+32	1.25	Indifferent
74.	<i>C.albicans</i>	0.5	32	0.5+16	1.5	Indifferent
75.	<i>C.tropicalis</i>	32	64	16+16	0.75	Additive
76.	<i>C.tropicalis</i>	0.25	16	0.25+16	2	Indifferent
77.	<i>C.guilliermondii</i>	0.5	16	0.25+8	1	Additive
78.	<i>C.tropicalis</i>	2	32	1+32	1.5	Indifferent
79.	<i>C.tropicalis</i>	2	32	1+32	1.5	Indifferent
80.	<i>C.albicans</i>	0.5	16	0.25+8	1	Additive
81.	<i>C.guilliermondii</i>	0.5	16	0.5+16	2	Indifferent
82.	<i>C.albicans</i>	1	32	1+16	1.5	Indifferent

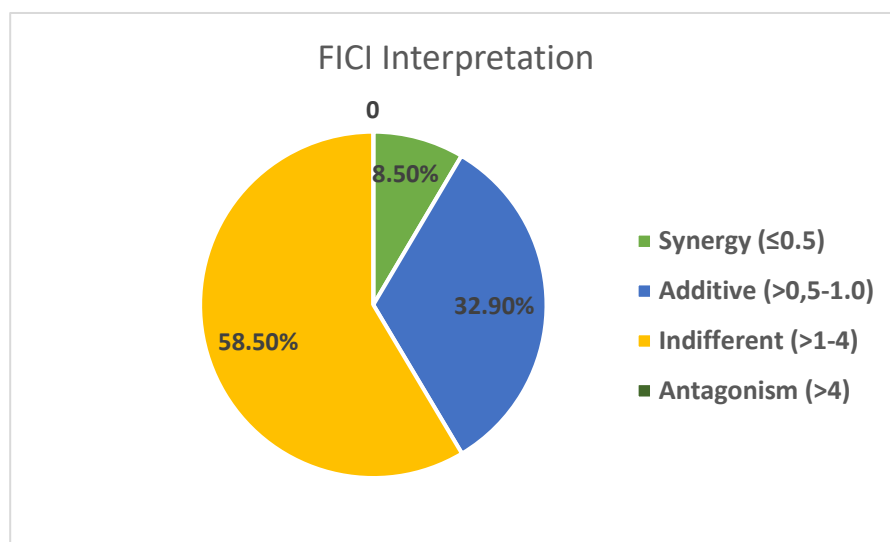


Figure 3: FICI interpretation of fluconazole with linezolid for synergism

Species-wise analysis of the checkerboard assay showed variable interactions between fluconazole and linezolid among the clinical *Candida* isolates. *Candida albicans* (n = 30) exhibited synergistic, additive, and indifferent interactions in 2 (6.7%), 11 (36.7%), and 17 (56.7%) isolates, respectively, while *Candida tropicalis* (n = 29) showed corresponding interactions in 2 (6.8%), 8 (27.5%), and 19 (65.5%) isolates. All *Candida parapsilosis* isolates (n = 3) demonstrated additive interaction, whereas *Candida guilliermondii* (n = 9) exhibited synergy in 2 (22.2%) isolate and additive in 3(33.3%) and indifferent interactions in 4 (44.4%) isolates each. The single isolate of *Candida norvegensis* showed an additive interaction. Among *Candida auris* (n = 2), both showed indifferent interaction, while *Candida glabrata* (n = 7) demonstrated synergy in 1 (14.3%) isolate and indifferent interaction in 6 (85.7%) isolates. Whereas the single *Candida krusei* isolate demonstrated an additive interaction. No antagonistic interaction was observed in any of the *Candida* species tested (table 4).

Table 4: Species-wise Distribution of Synergistic Activity by the Checkerboard Method

Species	Synergy	Additive	Indifferent	Antagonism
<i>C.albicans</i> (n=30)	2(6.7%)	11(36.7%)	17(56.7%)	0
<i>C.tropicalis</i> (n=29)	2(6.9%)	8(27.6%)	19(65.5%)	0
<i>C.guilliermondii</i> (n=9)	2(22.2%)	3(33.3%)	4(44.4%)	0
<i>C.glabrata</i> (n=7)	1(14.3%)	-	6(85.7%)	0

<i>C.parapsilosis</i> (n=3)	-	3(100%)	-	0
<i>C.auris</i> (n=2)	-	-	2(100%)	0
<i>C.norvegenesis</i> (n=1)	-	1(100%)	-	0
<i>C.krusei</i> (n=1)	-	1(100%)	-	0
Total(n=82)	7(8.5%)	27(32.9%)	48(58.5%)	0

DISCUSSION

The present study evaluated the in vitro interaction between fluconazole and linezolid against 82 clinical *Candida* isolates using the checkerboard micro broth dilution method. The study included *Candida* isolates with heterogeneous fluconazole susceptibility profiles, including species recognised to exhibit intrinsic or reduced susceptibility to azoles. This design allowed the interaction to be examined across multiple *Candida* species rather than being restricted to *C. albicans* alone. The principal finding was that indifferent interaction predominated, while additive and synergistic interactions were observed in a subset of isolates. Antagonism was not detected.

Indifferent interaction was observed in 48(58.5%) isolates, additive interaction in 27(32.9%) and synergism in 7(8.5%). Thus, additive or synergistic activity was found in 34 (41.5%) isolates. These results indicated that the interaction patterns of fluconazole and linezolid combination showed variability among the tested *Candida* isolates. Variability could be attributed to differences in species-level biology, baseline fluconazole susceptibility, cell stress responses and the degree to which linezolid impacts pathways contributing to azole activity. The dominance of indifference is consistent with the general antifungal-combination literature, in which interactions are often organism-, drug pair-, experimental endpoint- and analytical model-specific rather than consistently synergistic across all isolates [19, 20].

The findings can be compared with the study by Lu et al., which reported marked synergy between linezolid and azoles against *C. albicans*, including fluconazole-resistant isolates. In that investigation, linezolid at selected concentrations substantially reduced azole MICs, and synergistic activity was supported using both FICI analysis and a Bliss-independence model. The same study also reported protection in a *Galleria mellonella* model, providing evidence beyond a single in vitro endpoint [13]. The lower overall frequency of synergy in the present study may be related to differences in isolate composition, inclusion of multiple *Candida* species, baseline MIC

distributions, drug concentration ranges, endpoint definitions, and interaction criteria. Therefore, the results of the present study should be regarded as complementary to, rather than directly equivalent to, the findings of Lu et al.

Species-specific analysis revealed that the interaction pattern was not uniform. *C. albicans* and *C. tropicalis* displayed mostly indifferent interactions, with additive and synergistic effects being present in smaller proportions. Additive interaction was observed for all the three *C. parapsilosis* isolates while the *C. auris* isolates showed indifference. The small number of isolates in several species limits species-specific inference, particularly for *C. parapsilosis*, *C. auris*, *C. krusei*, and *C. norvegensis*. The observation of synergy in one *Candida glabrata* isolate is notable, given the species recognized for intrinsic resistance to fluconazole. Despite this finding, the observation is limited to a single isolate and does not establish a consistent or reproducible species-specific interaction. These findings support larger, stratified studies examining interaction according to species, resistance phenotype, and individual MIC values.

The inclusion of isolates with intrinsic or reduced fluconazole susceptibility was an important feature of the study. Combination strategies are frequently investigated as a means of improving activity against organisms with limited susceptibility to established agents. Importantly, the presence of additive or synergistic FICI values does not by itself demonstrate reversal of resistance. A resistance-modifying effect requires documentation of the fluconazole MIC alone, the MIC in combination, the magnitude of MIC reduction, and reproducibility across independent experiments. The present findings suggest that the combination warrants further investigation in selected isolates, but they do not establish linezolid as an antifungal or confirm clinical efficacy against fluconazole-resistant *Candida*.

The biological basis of the interaction remains to be established. Fluconazole inhibits Erg11-mediated ergosterol biosynthesis, and resistance may involve efflux-pump overexpression, target alterations, and changes in sterol pathways [5,8]. Linezolid primarily inhibits bacterial protein synthesis through binding to the 50S ribosomal subunit; its activity against *Candida* is therefore considered a repurposing phenomenon rather than a conventional antifungal mechanism [11,12]. Lu et al. proposed that linezolid may influence fungal cellular processes in a manner that enhances azole activity, but the precise molecular mechanism requires further confirmation [13].

The checkerboard assay provided a practical approach for evaluating multiple concentration combinations. The use of individual-drug controls, growth control, sterility control, and *Candida parapsilosis* ATCC 22019 as a quality-control strain strengthened the internal validity of the susceptibility testing. The present results should therefore be interpreted as evidence of phenotypic interaction under the specified assay conditions. The study was performed in vitro and did not evaluate drug exposure, toxicity, pharmacokinetic compatibility, or therapeutic efficacy in humans.

Conclusion

Fluconazole and linezolid exhibited heterogeneous in vitro interactions against the *Candida* isolates evaluated. Indifferent interactions were predominant, followed by additive effects, while synergy was observed in a smaller proportion of isolates, with no antagonistic interactions detected. The synergistic and additive interactions observed support antimicrobial drug repurposing as a potential strategy for enhancing antifungal activity. These in vitro findings provide a basis for further molecular, animal, and clinical studies to evaluate combination therapy for improving fluconazole efficacy against *Candida* infections.

Declarations

Abbreviations:

FICI: Fractional inhibitory concentration index

CLSI: Clinical and Laboratory Standards Institute

MIC: Minimum Inhibitory Concentration

Ethics approval and consent to participate:

The study was approved by the Institutional Ethics Committee of JSS Medical College, Mysuru (JSS/MC/PG/25MC/2024-25).

Consent for publication:

Not applicable.

Clinical Trial Registration:

Not applicable. This study was an in vitro laboratory-based investigation and did not involve human participants or clinical intervention.

Availability of data and materials:

The datasets generated and/or analyzed during the current study are available from the corresponding.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

R.P.K: collected the clinical isolates, performed the test, and reviewed the manuscript. G.R: collected the clinical isolates, conducted the study, and original manuscript draft. S.G.S: supervised the project, guided methodology, provided critical revisions, and reviewing the manuscript. S.M.N: supervised the project, guided methodology, and reviewing the manuscript. D.S: performed the literature review and assisted in tests, and manuscript drafting. All authors contributed to the interpretation of results and approved the final manuscript.

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