In Vitro Interaction between Fluconazole and Triclosan against Clinical Isolates of Fluconazole-Resistant Candida albicans Determined by Different Methods

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The in vitro interaction between triclosan and fluconazole against 24 azole-resistant clinical isolates of Candida albicans was evaluated by the microdilution checkerboard technique. The synergisms were verified by time-killing curves and agar diffusion tests in selected strains. Antagonistic activity was not detected.

Candida albicans is the primary cause of opportunistic fungal disease in humans. It is predominantly found at low levels among the normal oral flora but can thrive in immuno-compromised individuals (16, 25). Fluconazole has been used successfully as a prophylactic and a first-line therapeutic antifungal agent (5, 6, 19). However, the increase inazole use has precipitated a rise in drug resistance in clinical isolates. Triclosan, a chlorinated aromatic compound, has antimicrobial (4, 8, 20), antiparasitic (26), and anti-inflammatory (1, 24) activities. It has been used in personal care products (2). Combination therapy can improve the efficacy of antimicrobial therapy for infections recalcitrant to most treatments. Therefore, we aimed to assess the presence of combination effects with triclosan and fluconazole in C. albicans.

A total of 24 clinical isolates of fluconazole-resistant C. albicans were used in this study, and C. albicans ATCC 10231, 103 CFU/ml. The plates were incubated at 35°C, and the optical density (OD) value was determined at 492 nm after 48 h, a modification to the CLSI reference method. All experiments were conducted in triplicate, and the median MIC-1 endpoint value, which represents a 80% reduction in turbidity, was calculated (3). The drug MICs were determined by broth microdilution according to CLSI method M27-A (3) with an inoculum of 2.5 × 10^5 CFU/ml. The plates were incubated at 35°C, and the optical density (OD) value was determined at 492 nm after 48 h, a modification to the CLSI reference method. All experiments were conducted in triplicate, and the median MIC-1 endpoint value, which represents an 80% reduction in turbidity, and MIC-2 endpoint value, which represents a 50% reduction in turbidity, were calculated (3). The drug interactions were analyzed using the fractional inhibitory concentration index (FICI) and ΔE models based on the concentration index (FICI) and interactions were analyzed using the fractional inhibitory 50% reduction in turbidity, were calculated (3). The drug

<table>
<thead>
<tr>
<th>Drug</th>
<th>Median MIC-2 endpoint (range) of drug (µg/ml)</th>
<th>Median MIC-1 endpoint (range) of drug (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLC</td>
<td>16 (4–32)</td>
<td>256 (64–&gt;512)</td>
</tr>
<tr>
<td></td>
<td>1 (1–2)</td>
<td>2 (1–4)</td>
</tr>
<tr>
<td>TLC</td>
<td>32 (32–64)</td>
<td>64 (32–64)</td>
</tr>
<tr>
<td></td>
<td>8 (4–8)</td>
<td>8 (8–16)</td>
</tr>
</tbody>
</table>

a TCL, triclosan; FLC, fluconazole.

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The synergism and antagonism were defined as respective increases or decreases of $\geq 2 \log_{10}$ CFU/ml in antifungal activity produced by the drug combination compared with the more active agent alone after 24 h (10, 12).

The checkerboard results are summarized in Table 1. The MIC-2 endpoint values for fluconazole and triclosan in \textit{C. albicans} ranged from 4 to 32 $\mu$g/ml and from 32 to 64 $\mu$g/ml, respectively. The drug combination markedly reduced the MIC-2 endpoints of fluconazole and triclosan to 1 to 2 $\mu$g/ml and 4 to 8 $\mu$g/ml, respectively. A previous report has stated that the \textit{in vivo} triclosan concentration in saliva was about 13 $\mu$g/ml at 10 min after brushing with toothpaste, and the duration of activity of triclosan at a concentration of 10 $\mu$g/ml in saliva was about 0.7 h (13). From our data, the

### TABLE 2. FICI and $\Delta E$ analyses of in vitro interaction between TCL and FLC against 24 clinical isolates of \textit{C. albicans}$^a$

<table>
<thead>
<tr>
<th>Endpoint type</th>
<th>FICI model</th>
<th>Result according to nonparametric method$^b$</th>
<th>$\Delta E$ model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FICI, median (range)</td>
<td>$\Sigma$SYN % ($n$)</td>
<td>$\Sigma$ANT % ($n$)</td>
</tr>
<tr>
<td>MIC-2</td>
<td>0.313 (0.125 to 0.375)</td>
<td>SYN (all isolates)</td>
<td>116.8 (15) to 589.2 (22)</td>
</tr>
<tr>
<td>MIC-1</td>
<td>0.25 (0.125 to 0.25)</td>
<td>SYN (all isolates)</td>
<td>50.1 (8) to 686.8 (24)</td>
</tr>
</tbody>
</table>

$^a$ TCL, triclosan; FLC, fluconazole.

$^b$ INT, interpretation; SYN, synergism; ANT, antagonism; IND, indifference; $n$, number of interactions.

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**FIG. 1.** Agar disk diffusion assay for FLC combined with TCL in \textit{C. albicans} YL345. Panel B describes the image for panel A, and panel D describes the image for panel C.
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INTERACTION BETWEEN TCL AND FLC AGAINST IN VITRO ITS AN ANTIFUNGAL EFFECT

The inhibition zones increased to 19.2, 18, 15.6, and 10.8 mm than ones produced by single-drug treatments. The sizes of produced by the combination were predominantly larger confirmed by agar diffusion tests (Fig. 1). The halo diameters antagonisms were not observed.

The corresponding median FICI and ΔE values are shown in Table 2. The MICs ranged from 0.125 to 0.375 and from 0.125 to 0.25 when analyzed using the MIC-2 and the MIC-1 endpoints, respectively. The ΔE values ranged from 116.8% to 589.2% when calculated using the MIC-2 endpoint. Antagonisms were not observed.

The synergism between fluconazole and triclosan was confirmed by agar diffusion tests (Fig. 1). The halo diameters produced by the combination were predominantly larger than those produced by single-drug treatments. The sizes of the inhibition zones increased to 19.2, 18, 15.6, and 10.8 mm when 16 μg/ml fluconazole was combined with 16, 8, 4, and 2 μg/ml of triclosan, respectively. The time-kill curves verified the synergistic combinations (Fig. 2). Triclosan and fluconazole did not significantly affect isolate growth when the drugs were used alone at 16 μg/ml and 4 μg/ml, respectively. The combination therapy yielded a 3.0-log_{10}CFU/ml decrease compared with triclosan alone after 24 h, wherein there was a significant difference (P < 0.01).

Taken together, our findings indicate that triclosan exhibits an antifungal effect in vitro against azole-resistant C. albicans when combined with fluconazole. In the checkerboard assay, the FICI model has been frequently used to determine the interaction between antifungal drugs (7, 9, 12, 17, 21, 23). The ΔE model is a useful method for characterizing drug interactions. We verified the positive interactions using the agar diffusion test and time-kill curves, which were able to detect differences in the rate and degree of antifungal activity over time (11). An agar diffusion test can provide more visually convincing results. A combination treatment with triclosan has been previously demonstrated to significantly enhance the efficacy of triclosan against microbes (20, 22). In contrast to various previous reports (20, 22), triclosan is a better synergist to fluconazole against C. albicans.

In conclusion, the combination treatment of fluconazole and triclosan effectively synergizes against C. albicans. Our findings may provide an alternative approach to overcoming antifungal drug resistance. However, the mechanisms underlying the synergy must be further elucidated.

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REFERENCES


