ABSTRACT

A significant challenge with controlling *C. auris* infections is its resistance to multiple antifungal agents, including azoles. Alternative strategies are required to combat the problem of antifungal resistance. Some of the suggested approaches include combination therapy and anti-virulence treatment strategies. There is growing interest in combining proton pump inhibitors (PPIs) with available antifungal drugs for better treatment outcomes. In this study, we investigated the antifungal activity of PPIs omeprazole and pantoprazole alone and in combination with fluconazole against resistant *C. auris* isolates. The anti-virulence activity of the synergistic combination against resistant *C. auris* isolates was determined. Lastly, the effect of the synergistic combination on *C. auris* energy-dependent efflux-activity was established.

The CLSI broth micro-dilution method was used to determine the antifungal susceptibility of 25 *C. auris* isolates against antifungal agents (fluconazole and amphotericin B) and PPIs (pantoprazole and omeprazole). The combination interaction of fluconazole and PPIs was established by determining the fractional inhibitory concentration index (FICI) of each combination. Time-kill curves were used to determine the antifungal activity of the synergistic combination over time and to confirm the combination interaction. All 25 *C. auris* isolates were screened for their ability to adhere to epithelial cells and proteinase secretion using microscopy and culture technique respectively. The effect of the synergistic combination on isolates with positive pathogenicity markers was determined.

Most of the *C. auris* isolates were resistant to fluconazole (92%) and all were sensitive to amphotericin B (100%). Omeprazole and pantoprazole had antifungal activity against *C. auris* at MIC values of 3125 µg/ml and 15625 µg/ml, respectively. The combination of fluconazole and pantoprazole reduced fluconazole MIC values by 4-fold, from 125 µg/ml to 31.2 µg/ml. The combination had synergistic effect against most *C. auris* isolates (56%), additive effect in 36% and indifference in 8%. There was no synergism in the fluconazole and omeprazole

combination. However, the combination had antagonist effect against most *C. auris* isolates (56%). All *C. auris* isolates displayed some degree of adherence to epithelial cells and 96% produced proteinases. Fluconazole and pantoprazole combination significantly reduced adherence (p values < 0.05) and proteinase secretion (p values< 0.001) at inhibitory and sub-inhibitory concentrations. Pantoprazole inhibited fluconazole efflux at inhibitory and sub-inhibitory concentrations.

The study showed that *C. auris* isolates express virulence factors such as adherence and proteinase production. The combination of pantoprazole and fluconazole has antifungal and anti-virulence activity against resistant *C. auris* isolates. In addition, it was shown that pantoprazole can attenuate fluconazole resistance by inhibiting the activity of energy-dependent efflux-pumps and it has synergistic effect with fluconazole. Therefore, pantoprazole has the potential to improve therapy outcomes when azoles are used.