

EFFECT OF CONCENTRATION EXCEEDING MICs OF ANTIFUNGAL AGENTS ON THE SELECTION OF RESISTANT MUTANTS OF CANDIDA SPP. AND CRYPTOCOCCUS NEOFORMANS.

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While naturally occurring anti-fungal resistance appears to be rare, stepwise development of resistance to fluconazole among *Candida* strains has been well documented in HIV-infected patients receiving repeated therapy for oropharyngeal candidiasis (OPC). This observation has led to concerns about the potential emergence of anti-fungal resistance in other settings, especially in the face of long-term or recurrent therapy among immunocompromised patients. Over the past years, 2 promising new anti-fungal agents have been introduced: a second generation, broad-spectrum azole, voriconazole, and an echinocandin, caspofungin. Both agents have proven to be effective in the treatment of mucosal and disseminated candidiasis, including infections caused by fluconazole-resistant *Candida* spp. The potential for emergence of resistance to these agents, however, has not been well characterized. The purpose of this study is to evaluate the frequency of emergence of resistance to these agents *in vitro*. Four strains each of *C. albicans*, *C. parapsilosis*, *C. lusitanae* and *C. krusei* were tested against both caspofungin and voriconazole. We determined that MICs exhibited by each strain were ≤ 1.0 $\mu\text{g}/\text{mL}$. The strains were cultured overnight, and washed and re-constituted in normal saline solution at $1 \times 10^6/\text{mL}$. Next, 0.1 ml inocula of this suspension and serial ten-fold dilutions were spread onto RPMI1640 plates containing 4 $\mu\text{g}/\text{mL}$ of voriconazole or caspofungin. The inocula were felt to reflect physiologically relevant concentrations, since we have found most oral OPC samples from our patients contain 10^3 to 10^5 CFU. In addition, the drug concentrations were chosen to be $\geq 4 \times$ MIC, since this is often the level desired in blood or tissue. The plates were incubated up to 2 weeks, and were observed daily for breakthrough colonies. If breakthrough colonies were found, they were picked and checked for true resistance by re-growing onto RPMI 1640 plates containing the respective drug. In addition to *Candida* spp., four *Cryptococcus neoformans* strains were tested against voriconazole using the same methods, although the starting inoculum was $1 \times 10^9/\text{mL}$. Our results are summarized in the following table:

Organism	Vori MICs (ug/ml)	Frequency of Vori breakthrough	Caspo MICs (ug/ml)	Frequency of Caspo breakthrough
<i>C. albicans</i>	0.03-0.13	10^{-1}	0.125	$10^{-1} - 10^{-4}$
<i>C. parapsilosis</i>	0.02-0.37	$\leq 10^{-5}$	0.25-1.0	$10^{-1} - \leq 10^{-5}$
<i>C. lusitanae</i>	0.01-0.02	$10^{-4} - \leq 10^{-5}$	0.125-0.25	$10^{-4} - \leq 10^{-5}$
<i>C. krusei</i>	0.29	$\leq 10^{-5}$	0.125-0.5	$\leq 10^{-5}$
<i>C. neoformans</i>	0.16-0.5	$\leq 10^{-8}$		

For voriconazole, significant breakthrough rates were noted among all *C. albicans* strains tested. The emergence of breakthrough resistant mutants can occur in any strain regardless of the initial MICs. Selected *C. lusitanae* strains demonstrated breakthrough at $> 10^{-5}$. Of note, none of the *C. parapsilosis* and *C. krusei* strains demonstrated any breakthrough; nor did any *C. neoformans* strains, even at inocula of 10^8 CFU. Based on these findings, we would anticipate accumulation of resistance among *C. albicans* strains, similar to that observed with fluconazole in the treatment of OPC. As with fluconazole, the emergence of voriconazole resistance among *C. neoformans* isolates should be rare. The emergence of resistance among *C. krusei* strains would also appear to be rare, unlike the intrinsic resistance to fluconazole exhibited by this species. For caspofungin, breakthrough was notable for selected strains of *C. albicans* and *C. parapsilosis*, but was low for all strains of *C. lusitanae* and *C. krusei*. The initial MICs of *C. parapsilosis* predicted the emergence of breakthrough resistant mutants: mutants develop only among strains with MICs ≥ 0.5 $\mu\text{g}/\text{mL}$. Based on these observations, we would anticipate emergence of resistance among *C. albicans* and *C. parapsilosis* strains clinically. The conclusions from this study will require clinical validation.