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# "Evaluation of the "dip-effect" in *in vitro* antifungal susceptibility testing of *Candida* spp. against echinocandins using gradient concentration strips"

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**Objectives**: Gradient concentrations strips are widely used for antifungal susceptibility testing in clinical laboratory. Nevertheless, the "dip-effect" (a narrow inhibition zone at sub-MIC concentrations parallel to the strip) may hinder objective MIC determination of echinocandins. We therefore assess the performance of gradient concentration strips versus colorimetric broth microdilution method for *in vitro* susceptibility testing of echinocandins against *Candida* spp. using a practical formula that improves MIC determination when "dip-effect" occurs.

**Methods**: A total of 58 clinical isolates (12 *C.albicans*, 3 *C.dubliniensis*, 10 *C.glabrata*, 3 *C.krusei*, 12 *C.parapsilosis*, 11 *C.tropicalis*, 1 each of *C.inconspicua*, *C.kefyr*, *C.lipolytica*, *C.lusitaniae*, *C.rugosa* and 2 quality control *C.krusei* ATCC6258 and *C.parapsilosis* ATCC22019) were tested against caspofungin, micafungin and anidulafungin using the Liofilchem® MIC test strips (MTS) and Sensititre YeastOne (YO) panels according to the manufacturer's guidelines. MTS MICs were the lowest drug concentrations at which the border of the elliptical inhibition intercepted the strip scale ignoring trailing growth. However, when "dip-effect" appeared MICs were determined at the bottom of the dip following the recommendations of the company's reading guide and with a new approach consisting of a linear regression analysis of inhibition zones at supra MIC concentrations (y scale) over the corresponding concentrations (x scale) after subtracting the inhibition zone at sub-MIC concentrations ("dip-effect"). The MIC was then defined as the x intercept of the linear regression analysis. The agreement within ±1 and ±2 twofold dilutions and the twofold differences between MTS and YO endpoints were calculated, after rounding up MTS results to next upper two-fold values.

**Results:** No "dip-effect" was found with anidulafungin and micafungin whereas "dip-effect" was observed with caspofungin for 90%-100% of strains of all species except *C. parapsilosis*, *C. krusei* and *C. lipolytica* strains for which <15% of the strains demonstrated the "dip-effect". The agreement within ±1 and ±2 $\log_2$ dilutions between MTS and YO MICs was 89% and 96%, respectively for anidulafungin with a median (range) difference of 0(-4−1) two-fold, and 71% and 96%, respectively for micafungin with a median (range) difference of -1(-3−1) two-fold. For caspofungin, the agreement within ±1 and ±2 $\log_2$ dilutions between MTS and YO methods was 41% and 67%, respectively as a result of 2 (-1-4) two-fold differences when MICs determined according to MTS manufacturer's guidelines and increase to 66% and 88%, respectively, with 1 (-1-3) two-fold differences when MTS MICs were determined with the new approach.

**Conclusion**: The "dip-effect" restricts correct caspofungin MIC determination for a variety of *Candida* species. The formula implemented here is easy-to-perform and may improve MIC determination with gradient concentration strips.