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Letter to Editor

Microbiology Section

Phenomenon of Dip Effect in Antifungal Susceptibility Testing of *Candida tropicalis* by using MIC Testing Strips against Echinocandins

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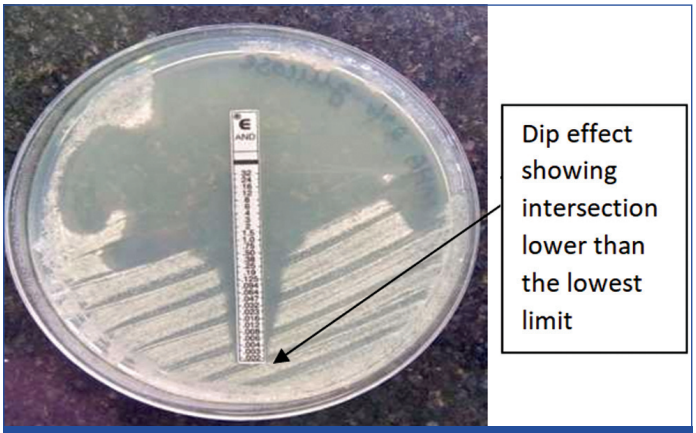
To the Editor,

Minimum Inhibitory Concentration (MIC) of an antifungal is the minimum concentration that completely inhibits the visible growth of fungi under regulated, uniform and consistent in-vitro conditions. Reporting of antifungal susceptibility testing in Clinical Microbiology laboratories through standardised broth dilution methods by Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility testing (EUCAST) can produce reproducibility within ± one to two-fold dilution, but are labour intensive and resource demanding procedures, requiring accurate training of the concerned personnel for performing and reading of the results. Hence, it becomes difficult to perform them regularly in high throughput laboratories and also in absence of enough trained manpower [1,2]. In such settings the Etest concentration gradient MIC Testing Strips (MTS) provide a valuable and convenient alternative solution, combining the principles of agar based diffusion methods and dilution methods. MTS comes as a ready to use impermeable plastic or paper reagent strips having embedded continual concentrations of antifungal in a predetermined gradient manner, covering a continuous concentration range generally across 15 two-fold dilutions. After application of MTS onto an inoculated agar surface, there is diffusion of preformed antifungal agent into the agar for over an hour. There is a formation of ellipse shaped area of inhibition which is symmetrical and has central part along the strip after 16-20 hours of incubation. The point of intersection of edge of ellipse with the strip is read as MIC in terms of µg/mL. Although convenient to use, it is not a reference method and still lacks the objectivity of reading and interpretation. One of the inhibition patterns posing as a reading challenge and leading to variability in reading is the "dip" effect, corresponding to a narrow inhibition zone at sub-MIC values i.e. ellipse is below the strip (does not intersect the strip). Although manufacturer's instructions suggest to read below the dip and report the MIC less than the lowest value on the MIC scale, such phenomenon have been scarcely reported in the published literature [3,4].

Isolate 1: Here we would like to illustrate the "dip" effect observed in a *Candida tropicalis* isolate from percutaneous nephrostomy urine sample using commercially available anidulafungin MTS paper impregnated porous HiMedia® Ezy MIC™ strip; demonstrating narrow, more elongated elliptical inhibition zones and smaller intersection angles at low concentrations next to the strip [Table/Fig-1] [5]. MTS was also performed for caspofungin and micafungin which showed an MIC of 0.125 for both without any "dip" effect. Sensititre yeast one broth [6] (SYO; Trek Diagnostic systems, Cleveland, OH, USA) was also performed for the same isolate, which is a commercially prepared 96 well microbroth dilution system, comprising of increasing concentrations of antifungals. It relies on colorimetric change of alamar blue as an indicator of fungal growth. With Sensititre testing, the MIC for anidulafungin was 0.12. The isolate was also tested on Vitek 2 compact with Vitek 2 –YS08 Antifungal susceptibility card. The MIC values of echinocandins performed by three different methods on isolate one are summarised in [Table/Fig-2]. The isolate was interpreted as sensitive by all the three methods. Anidulafungin is not included in the Vitek card.

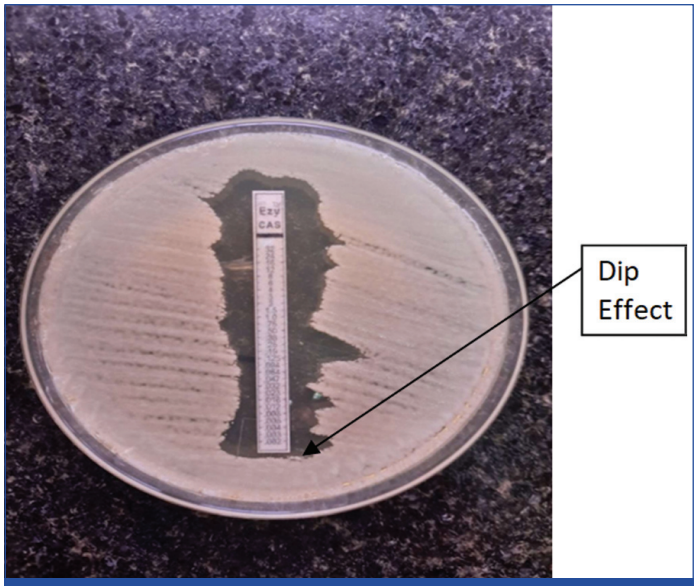


[Table/Fig-1]: Isolate one-Dip effect of *Candida tropicalis* with anidulafungin showing smaller intersection angles at low concentrations next to the strip [5].

Isolate 2: In another isolate of *Candida tropicalis* retrieved from stool culture (stool culture surveillance is done at our institution every fortnightly for bone marrow transplant patients and haematolymphoid carcinoma patients to predict empirical treatment in case of gut translocation of gut microbiota leading to sepsis), caspofungin HiMedia® Ezy MIC™ strip showed the "dip" effect with the intersection way beyond the lowest point of 0.002 [Table/Fig-3]. On testing with Vitek 2 compact, the MIC was ≤0.12. It was Vitek-2 compact ≤0.06, which was also sensitive. Sensititre Yeast One broth could not be performed in this isolate due to non availability of the kit and reagents at that time. Micafungin and anidulafungin MIC E strips were not done for this isolate as the treating physician

Methods	Caspofungin	Interpretation	Micafungin	Interpretation	Anidulafungin	Interpretation
Sensititre	0.06	Susceptible	0.03	Susceptible	0.12	Susceptible
Vitek	<0.12	Susceptible	<0.06	Susceptible	-	Susceptible
Strip	0.125	Susceptible	0.125	Susceptible	0.002	Susceptible

[Table/Fig-2]: MICs of first *Candida tropicalis* isolate with three different assays.



[Table/Fig-3]: Isolate two -"dip" effect of *Candida tropicalis* against caspofungin showing intersection lower than the lowest value.

requested only for caspofungin testing in view of suspect of impending fungal sepsis. Both the isolates demonstrated the result as sensitive. Siopi M et al., using MTS (Liofilchem, Roseto delgi Abruzzi, Italy) and Etest gradient concentration strips (bioMerieux, Marcy l'Etoile, France) in a review have indicated a drug and MIC dependence of this phenomenon and stated its occurrence to be inversely related to the MIC of the isolate as it was observed only with caspofungin. Etest MICs of the isolate with "dip" effect were higher than the Etest MICs of the isolates without the "dip" effect [7,8]. We would like to highlight the occurrence of this phenomenon in our laboratory so that clinical microbiologists can have an observant eye for this phenomenon at their setups as well and hence more number of such isolates will be identified and tested, which will help deducing its objective interpretation and remove the subjective error. Inability to confirm MIC results for isolate 2 by Sensititre testing will remain as the limitation of the study.

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