Title: ULTRASTRUCTURAL IMAGE BANK OF CLINICAL AND ENVIRONMENTAL FUNGAL SPECIES AS TEACHING METHODOLOGY.

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Abstract:

Fungal pathogens are receiving attention of scientific community, especially with increasing incidence of fungal infections, mainly in immunocompromised patients. Ultrastructural diversity of fungi observed by scanning electron microscopy (SEM) enables identification of pathogens in a more precise way, unlike other analysis methods. In this context, this study aimed to create a image bank as resource for the teaching and learning of medical mycology. SEM images were made using the following fungi: *Candida albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *Cryptococcus neoformans*, *C. gattii*, *Rhodotorula* sp., *Trichosporon asahii*; *Microsporum canis*, *M. gypseum*, *Trichophyton rubrum*, *T. mentagrophytes*, *T. tonsurans*, *Fusarium solani*, *Penicillium* sp., *Rhizopus* sp. and *Histoplasma capsulatum* var. *capsulatum*. For yeasts, thermanox slides were performed in two different methods, as first with microcultive in Thermanox slides, up to 4 days of incubation in corn meal agar for *Candida*, potato agar for *Cryptococcus* and *Rhodotorula* and malt agar for *Trichosporon*. In the second, previously grown cultures as described above were placed in direct contact with Thermanox® slides for 1 hour. For filamentous fungi, microcultives were performed with Thermanox slides in Lactrimel agar, to stimulate conidia formation, up to 15 days at room temperature. All slides were fixed with glutaraldehyde alone or supplemented with 0.1% Alcian Blue or 0.1% Congo Red, during 24 h at 4°C. Slides were washed with sodium cacodilate buffer twice, followed by serial alcoholic dehydration (ethanol at 50%, 70%, 80%, 95% and 100% - twice each). Samples were dried at 37°C and then treated with hexamethyldisilazane for 30 minutes. Slides were coated with a gold layer of 20 nm and photographed in SEM Inspect 50. Better preservation of fungal structures was achieved at glutaraldehyde with Alcian Blue. The following structures were observed: blastoconidia and budding cells in *Candida* spp., *Cryptococcus* spp. and *Rhodotorula* sp.; pseudohyphae and hyphae in *C. albicans*; arthroconidia in *T. asahii;* macroconidia of *Microsporum* spp. and *F. solani*; microconidia of *Trichophyton* spp.; spiral-shaped hyphae of *T. mentagrophytes*; sporangia, sporangiospores and rhizoids from *Rhizopus* sp.; conidiophores, phialides, and conidia of *Aspergillus* sp.; and stalagmospores of *H. capsulatum*. Construction of

the image bank with morpho-structural aspects of these pathogens is a powerful tool for teaching medical mycology.

Keywords: Scanning electron microscopy; fungal pathogens; medical micology.

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