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Single-Step PCR Using $(GACA)_4$ Primer: Utility for Rapid Identification of Dermatophyte Species and Strains▽

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Dermatophytes are fungi that belong to three genera: *Epidermophyton*, *Microsporum*, and *Trichophyton*. Identification of dermatophyte species is essential for appropriate diagnosis and treatment of dermatophytosis. Routine identification depends on macroscopic and microscopic morphology, which is time-consuming and does not identify dermatophyte strains. In this study, two PCR-based methods were compared for their abilities to identify 21 dermatophyte isolates obtained from Egyptian patients to the species and strain levels. The first method employed a two-step method: PCR amplification, using ITS1 and ITS4 as primers, followed by restriction enzyme digestion using the endonuclease MvaI. The second method employed a one-step approach employing the repetitive oligonucleotide $(GACA)_4$ as a primer. Dermatophyte strains were also identified using a conventional culture method. Our results showed that the conventional culture method identified four species: *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, and *Trichophyton violaceum*. Moreover, both PCR methods agreed with the diagnosis made using the conventional approach. Furthermore, ITS1/ITS4-based PCR provided no strain differentiation, while $(GACA)_4$-based PCR identified different varieties among the *T. mentagrophytes* isolates. Taken together, our results suggest that $(GACA)_4$-based PCR has utility as a simple and rapid method for identification of dermatophyte species as well as utility for differentiation of *T. mentagrophytes* variants.

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Comparison between the Standardized Clinical and Laboratory Standards Institute M38-A2 Method and a 2,3-Bis(2-Methoxy-4-Nitro-5-[(Sulphenylamino)Carbonyl]-2*H*-Tetrazolium Hydroxide-Based Method for Testing Antifungal Susceptibility of Dermatophytes▽

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In this study, we determined the utility of a 2,3-bis(2-methoxy-4-nitro-5-[(sulfenylamino)carbonyl]-2*H*-tetrazolium hydroxide (XTT)-based assay for determining antifungal susceptibilities of dermatophytes to terbinafine, ciclopirox, and voriconazole in comparison to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 method. Forty-eight dermatophyte isolates, including *Trichophyton rubrum* (n = 15), *Trichophyton mentagrophytes* (n = 7), *Trichophyton tonsurans* (n = 11), and *Epidermophyton floccosum* (n = 13), and two quality control strains, were tested. In the XTT-based method, MICs were determined spectrophotometrically at 490 nm after addition of XTT and menadione. For the CLSI method, the MICs were determined visually. With *T. rubrum*, the XTT assay revealed MIC ranges of 0.004 to >64 μg/ml, 0.125 to 0.25 μg/ml, and 0.008 to 0.025 μg/ml for terbinafine, ciclopirox, and voriconazole, respectively. Similar MIC ranges were obtained against *T. rubrum* by using the CLSI method. Additionally, when tested with *T. mentagrophytes*, *T. tonsurans*, and *E. floccosum* isolates, the XTT and CLSI methods resulted in comparable MIC ranges. Both methods revealed similar lowest drug concentrations that inhibited 90% of the isolates for the majority of tested drug-dermatophyte combinations. The levels of agreement within 1 dilution between both methods were as follows: 100% with terbinafine, 97.8% with ciclopirox, and 89.1% with voriconazole. However, the agreement within 2 dilutions between these two methods was 100% for all tested drugs. Our results revealed that the XTT assay can be a useful tool for antifungal susceptibility testing of dermatophytes.

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