Original Article

To determine antifungal susceptibility for the isolates using agar dilution method: A prospective cohort study

M. Suganthi, V. Dillirani, P. R. Thenmozhivalli, R. Selvi, B. J. Anand

Department of Microbiology, Government Kilpauk Medical College, Chennai, Tamil Nadu, India

Correspondence:
Dr. M. Suganthi M, Department of Microbiology, Government Kilpauk Medical College, Chennai, Tamil Nadu, India. E-mail: suganlalith@gmail.com

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ABSTRACT

The aim of the study was to standardize *in vitro* antifungal susceptibility testing by agar dilution method to find out the minimum inhibitory concentration (MIC) of fluconazole, ketoconazole, itraconazole, terbinafine, and griseofulvin fungal isolates of skin, hair, and nail. Various samples were collected from patients with clinically diagnosed dermatophytosis. Skin scrapings, hair, and nail were collected from one hundred and seventy patients. The antifungal drugs such as fluconazole (64–0.0625 µl/ml), ketoconazole (32–0.0313 µl/ml), itraconazole (32–0.0313 µl/ml), terbinafine (32–0.0313 µl/ml), and griseofulvin (32–0.0313 µl/ml) these water-insoluble drugs were incorporated in dissolved in dimethyl sulfoxide. The MIC was determined as the lowest concentration of the antifungal drug preventing the growth of macroscopically visible colonies on drug-containing plates when there was visible growth on the drug-free control plates. The MIC range, MIC 50, and MIC 90 for the drug griseofulvin were found to be 0.06–0.1, 0.25, and 1, respectively, for ketoconazole were found to be 0.06–0.1, 0.5, and 1, respectively, for the drug fluconazole were found to be 1–32, 8, and 16, respectively, itraconazole were found to be 0.06–4, 0.5, and 1, respectively, and for terbinafine were found to be 0.03–0.12, 0.06, and 0.12, respectively. This technique was found to be reliable, cost-effective and easy to perform with consistent results. Further results concluded that the fluconazole showed a higher MIC value when compared to other antifungal drugs.

Keywords: Agar dilution method, antifungal susceptibility testing, fluconazole, itraconazole, ketoconazole, minimum inhibitory concentration, terbinafine and griseofulvin

Introduction

The cutaneous infections of man include a wide variety of diseases in which the integuments and its appendages the hair and the nail are involved.[1] Infection is generally restricted to the non-lining cornified layer, but a variety of changes occur in the host due to the presence of the infectious agent and its metabolic products.[2] Majority of the infections are caused by a homogenous group of keratinophilic fungus called the dermatophytes. An overwhelming number of dermatophytes and species have been implicated as a cause of skin, hair, and nails and this number is steadily increasing.[3] Dermatophytosis remains a significant public health problem. Numerous antifungal agents have been developed since griseofulvin became available through a breakthrough experimental work of gentles in Guinea pig in 1958.[4]

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The need for antifungal susceptibility testing increases beyond testing dermatophytes species because resistance to antifungal drugs has been demonstrated against such diverse fungi as *Trichophyton rubrum*, *Trichophyton interdigitale*, *Trichophyton tonsurans*, *Trichophyton verrucosum*, *Tricholosporum violaceum*, *Trichophyton schoenleini*, *Epidermophyton floccosum*, *Microsporum gypseum*, and *Microsporum audouinii* species. Hence, it becomes evident that the need for meaningful susceptibility test result is very important for fungi as it is for bacteria.[5,6]

Although antifungal susceptibility testing remains less well-established and utilized than antibacterial testing, the scientific support for its validity has benefited greatly by extrapolation from antibacterial testing.[7] The methods for antifungal sensitivity testing include National Committee for Clinical Laboratory Standards new name Clinical Laboratory Standards Institute (CLSI), broth-based methodology (M 27-A), CLSI methodology for molds,[8] E-test agar based testing methods, and flow cytometry and use of viability dyes. The above methods are time-consuming and labor intensive; hence, a more economical method such as agar dilution have been described.[9] There are only a limited number of antifungal susceptibility testing reports on ocular fungal isolates from India.[9] The