Comparison of Broth Micro Dilution and Disk Diffusion Methods for Susceptibility Testing of Dermatophytes

Thesis

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List of Abbreviations

ABTS	**:**	2,2′-azino-di-3-ethyl-benzthiazoline sulfonate
$BaCl_2$	**:**	Barium chloride
BCP	**:**	Bromocresol purple
BLA	**:**	Borelli's lactrimel agar
BMD	**:**	Broth micro-dilution
C	**:**	Chloramphenicol
CHX	**:**	Cycloheximide
DD	**:**	Disk diffusion
DLSO	**:**	Distal lateral subungual onychomycosis
DMSO	**:**	Dimethyl sulfoxide
DTM	**:**	Dermatophyte test medium
E	**:**	Epidermophyton
ELISA	**:**	Enzyme linked immunosorbent assay
EO	**:**	Endonyx onychomycosis
FLC	**:**	Fluconazole
FTIR-S	**:**	Fourier transform infrared spectroscopy
GMS	**:**	Gomori methenamine silver
GRI	**:**	Griseofulvin
HIV	**:**	**Human Immunodeficiency Virus**
ITR	**:**	Itraconazole
LPCB	**:**	lactophenol cotton blue
M	**:**	Microsporum
MALDTI-TOF MS	**:**	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MHA	**:**	Mueller-Hinton Agar
MIC	**:**	Minimum inhibitory concentration
MPO	**:**	Mixed pattern onychomycosis
PAS	**:**	Periodic acid Schiff
PCR	**:**	Polymerase chain reaction
PDA	**:**	Potato dextrose agar

PSO	**:**	Proximal subungual onychomycosis
SDA	**:**	Sabaroud dextrose agar
SEM	**:**	Scanning Electron microscopy
SO	**:**	Superficial onychomycosis
T	**:**	Trichophyton
TDO	**:**	Total dystrophic onychomycosis
TER	**:**	Terbinafine
Vitek MS	**:**	Vitek Mass Spectrometry

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ABSTRACT

Background: Dermatophytes are responsible for the majority of the fungal infections involving skin, hair and nails. There has been a remarkable increase in the number of fungal infections especially in those people whose immune system is compromised by aging, HIV infection, organ transplantation or cancer therapy.

Objective: The aim of this study was to compare both broth microdilution method & disk diffusion method for in-vitro activity of some antifungal drugs (Terbinafine, Fluconazole, Itraconazole) against different species of dermatophytes.

Patients and Method: This study was performed on 50 dermatophyte isolates recovered from various clinical specimens (skin, hair and nail) collected from dermatology outpatient clinic of Ain Shams University Hospital. All samples were cultured on sabarouds. Isolates recovered from SDA were subcultured on Potato Dextrose Agar (PDA) & incubated at 28°C for 7 days to enhance sporulation. The growth was harvested in sterile saline & the conidial and hyphal suspension was adjusted to 0.5 macfarland. Then antifungal susceptibility was done using: Disk diffusion (DD) method and Broth micro dilution (BMD) method.

Results: There was **a highly significant** agreement between the antifungal susceptibility testing of fluconazole, itraconazole and terbinafine by disk diffusion method and Broth micro-dilution method. In our study agreement between both methods for itraconazole was 1.00 (kappa), for terbinafine was 0.947, and for fluconazole was 0.878. The factors that may affect the results of BMD or DD are type and size of inoculum, composition of the media, temperature and duration of incubation and disc strength.

Conclusion: There was **a highly significant** agreement between the antifungal susceptibility testing of fluconazole, itraconazole and terbinafine by disk diffusion method and Broth micro-dilution method.

Keywords: Dermatophytes, Disk diffusion, Broth micro-dilution

Introduction

Dermatophytes are responsible for the majority of the fungal infections involving skin, hair and nails ***(Chinelli et al., 2003).***

There has been a remarkable increase in the number of fungal infections especially in those people whose immune system is compromised by aging, HIV infection, organ transplantation or cancer therapy ***(Kannan et al., 2006).***

The organisms are transmitted either by direct contact with infected host (human or animal) or by direct or indirect contact with infected exfoliated skin or hair in combs, hair brushes, clothing, furniture, theatre seats, caps, bed linens, towels, hotel rugs, and locker room floors. Depending on the species the organism may be viable in the environment for up to 15 months. There is an increased susceptibility to infection when there is a pre-existing injury to the skin such as scars, burns, marching, excessive temperature and humidity ***(Bokhari, 2009).***

Clinically, dermatophytes (ringworm) can be classified depending on the site involved. These include Tinea capitis (scalp), Tinea corporis (non-hairy skin of the body), Tinea cruris (groin), Tinea pedis (foot) or athlete's foot and Tinea barbae or barber's itch (bearded areas of the face and neck). Favus is a chronic type of ringworm

involving the hair follicles ***(Ananthanarayan and Paniker, 2009)***.

According to the genera, dermatophytes can be classified into Trichophyton which affect mainly the skin and nails, Microsporum which affect mainly the hair and Epidermophyton which affect mainly the skin ***(El-Gohary et al., 2014).***

Trichophyton rubrum, Trichophyton tonsurans and Trichophyton mentagrophytes are the most common dermatophytes. Trichophyton rubrum affect face, trunk, beard area, nails, feet and groin area infection. Trichophyton mentagrophytes affect the surface of the hair (large spore ectothrix) which manifest clinically as kerion. Trichophyton tonsurans invade the hair shaft (endothrix) which manifest clinically as black dot infection. Another common dermatophyte is Microsporum canis, which is transmitted from animals such as cats and dogs to humans causing small spore ectothrix which manifest clinically as scally ringworm ***(Parija, 2011)***.

Though there are several antifungal drugs used to treat dermatophytosis, some infections respond well to topical antifungal therapy, whereas others like tinea capitis, tinea unguium (nail infection), and more extensive or severe types may require systemic therapy ***(Pakshir et al., 2009).***

The concurrent increase in fungal infections with increase in the use of antifungal drugs mostly for prolonged

periods has led to development of resistance to antifungal drugs ***(Jain et al., 2008).***

Antifungal susceptibility testing is performed to provide information for clinicians to select appropriate antifungal agents useful for treating a particular fungal infection. For a definitive therapy also, it is essential to evaluate the resistant dermatophytes using a standardized, simple and reproducible in vitro assay to determine the antifungal activity of drugs against isolates. In vitro antifungal susceptibility tests are now mainly used for epidemiological surveys, determination of the degree of antifungal activity, and the prediction of clinical outcome based upon an optimization of antifungal therapy ***(Pakshir et al., 2009).***

Various methods, such as broth macro and microdilutions, agar dilution, E-test, Sensititre colorimetric microdilution panels and disk diffusion have been used for determining the susceptibility of dermatophytes to antifungal agents ***(Perrins et al., 2005)***.